

The toxicological effects of the herbicide 2,4 – D.C.P.A. on progesterone levels and mortality in *Wistar* female rats

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ABSTRACT

The effect of the ester butylglucol of 2,4-Dichlorophenoxyacetic acid (herbicide) on the rate of fertilization and on progesterone levels during gestation in rats, has been studied. The experiments consisted of four groups; one receiving the drug in the drinking water at a concentration of 125 mg / kg / day (the agriculture dose), the second and the third group received per os doses of 50 and 100 mg / kg / day of the herbicide, while the fourth group served as controls. The obtained results show that all treatments lead to a decrease in both indexes of females' fertility and in that of newborn mortality. On the other hand, the index of fertilization has been highly affected by the drug application, with a clear delay in fertilization in animals treated with 100 mg / kg / day and 125 mg / kg / day. Progesterone levels have also been suppressed in individuals receiving the herbicide at 50 and 125 mg / kg / day.

Key words: Fertility, Reproduction, Herbicide, Rat

الملخص

تهدف هذه الدراسة إلى بحث تأثير المبيد العشبي (2,4-D) على معدل الإخصاب وعلى مستوى هرمون البروجسترون خلال فترة الحمل عند الفئران. تكونت هذه التجربة من أربع مجاميع من الفئران ، الأولى أعطيت المبيد في الماء الشروب بجرعة 125 ملغ / كلغ / يوم (الجرعة المستعملة في الفلاحة)، الثانية و الثالثة اعطيت جرعة 50 و 100 ملغ / كلغ / يوم على التوالي ، في حين استعملت الرابعة كشاهد. أوضحت النتائج المتحصل عليها أن كل المعاملات أدت إلى الإقلال في نسبة كل من إخصاب الإناث و موت الصغار. من جهة أخرى فان نسبة الإخصاب تأثرت كثيرا من جراء المعاملة بالمبيد، حيث ظهر تأخرا واضحا في إخصاب الحيوانات المعاملة بجرعة 100 و 125 ملغ / كلغ / يوم. لقد انخفض مستوى هرمون البروجسترون كذلك عند الأفراد المعاملين بجرعة 50 و 125 ملغ / كلغ / يوم.

Introduction

The use of pesticides , that dates to the early forties, as a mean of improving both food and agricultural products, has been shown to have a wide range of side effects on wild animals as well as on humans. Such a toxicity is due, basically, to the fact that most utilized pesticides are not, either, completely dissolved or wide spread in the air, leading to their incorporation in the food chain. Among the mostly cited side-effects of pesticides, respiratory complications, sensitivity and skin cancer (Hill & Carlisle, 1947; Shokry *et al.*, 1990; Lu & Regula, 1995). Besides, it has been reported that most pesticides affect, in one way or in another, the reproductive function. For instance, it has been shown that treatment of rats with carbamates induces, a partial sterility in females, a regression of testicules and increases the rate of mortality of new-borns (Shtenberg & Ozhovan, 1971). The present investigation has looked at the effect of 2,4-Dichlorophenoxyacetic acid on some of the reproductive parameters (the index of, fertility, fecondity and newborns mortality rate) and on levels of plasma progesterone in female rats.

Materials and methods

Thirty-two sexually mature females of *Wistar* rats (10-week -old), weighing 180-220 g, have been placed in plastic cages (42 x 27 x 15 cm). They have been divided into four groups of eight each, under a short photoperiod of 8L: 16D, ambient temperature (22 + 3 °C) and free access to food and water. After two weeks of acclimatization each female has been accommodated with an adult male, with a daily vaginal plug checking, and the day on which a vaginal plug was found was denoted as day one of pregnancy. Three groups of female rats were treated with the herbicide, while the fourth one (group a) served as controls. Individuals in group "b" received the herbicide at a dose of 125 mg / kg / day (a dose corresponding to the dose used in the field), those in group "c" received 100 mg / kg / day, while rats in group "d" had the herbicide at a dose of 50 mg / kg / day. Each of the treated individuals received 2 ml, *per os*, of the corresponding solution daily between 9-10 am, throughout the gestating period.

Blood samples (2 ml) were collected, from the tail, in heparinized tubes at days 1, 6, 12 and 18 of the gestation period. Samples were centrifuged for 10 minutes at 3000.r.p.m, plasma was redrawn and stored at - 4 °C till assayed for progesterone. The number of live and dead fetuses with their body weights was also recorded. The indexes of fertility, fecundity and fetal death ratio were calculated as follow (Vaissaire, 1977):

1) Fertility Index = $\frac{\text{Number of live new borns} \times 100}{\text{Number of fertilized females}}$.

2) Fecundity Index = $\frac{\text{Number of fertilized females} \times 100}{\text{Number of mated females}}$. 3) Fetal DIndex = $\frac{\text{Number of borns dead} \times 100}{\text{Number of new borns}}$.

Progesterone concentrations were estimated by an Enzyme-Linked Immunosorbant Assay (ELISA), as described by Siekmann & Breuer. Data were analyzed by Analysis of variance with repeated measures and Student *t*-test as appropriate.

3. Results

Indexes of both fertility and fecundity are illustrated in table 1. There was a significant difference in fertility index between those of females treated with 50 ($P < 0.05$), 100 mg / kg / day and 125 mg / kg / day ($P < 0.001$) and those of the controls. For fecundity index the difference was significant at doses of 100

mg / kg / day and 125 mg / kg / day ($P < 0.05$) compared with the control. Clear increases in foetal death index were only observed between the control and those of 100 and 125 mg / kg / day ($P < 0.05$). No significant changes have been recorded in the weights of live fetuses in all groups and that of the control. However, a significant decrease was observed in plasma progesterone concentration in female rats treated with 50 and 125 mg / kg / day ($P < 0.001$) in the 12th and 18th days of gestational period compared to the control.

Parameters	Control <i>n</i> = 8	50 mg <i>n</i> = 8	100 mg <i>n</i> = 8	125 mg <i>n</i> = 8
Fertility Index	225	172*	132**	83.33**
Fecundity Index	100	100	83.33*	66.66**
Fetal death Index	2.85	6.52	10.8*	26.83*
Weight of live fetuses	5.21 ± 0.21	5.5 ± 0.15	5.89 ± 0.27	4.88 ± 0.17

Table 1. Changes in reproductive parameters in female rats treated with 2,4-Dichlorophenoxyacetic acid (50, 100 & 125 mg / kg /day) during the gestation period. Each point represents the Mean $\pm$ SD. Significant differences from controls are given as: * $P < 0.05$ & ** $P < 0.001$.

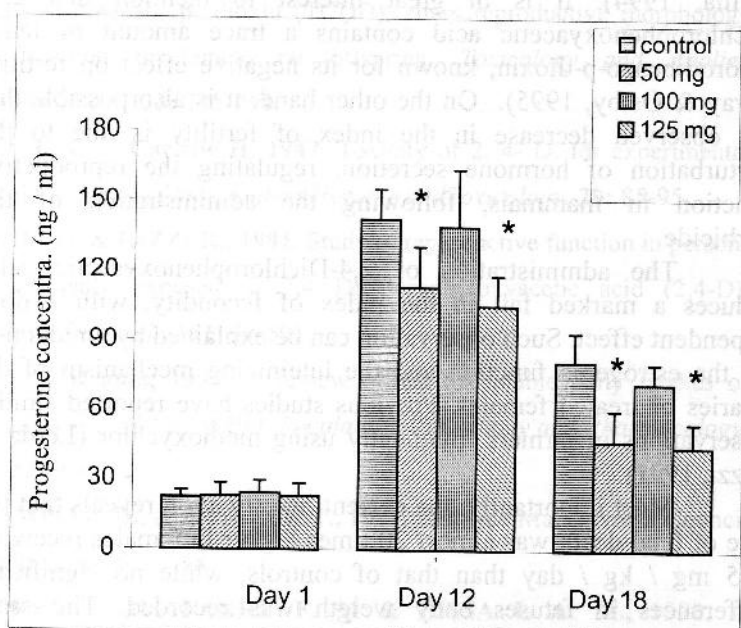


Figure legends

Figure 1. Changes in plasma progesterone concentrations (ng / ml) at days 1, 12 & 18 of the gestation period in control females or in those treated with 2,4-Dichlorophenoxyacetic acid. Significant differences from controls are given as: * $P < 0.001$. Each point represents the Mean $\pm$ SD.

Discussion

The present study demonstrates that 2,4-Dichlorophenoxyacetic acid affects most of the investigated parameters. The obtained data shows that the index of fertility was highly affected in rats treated with the herbicide, and that the effect was positively proportional to the dose (table1). The decrease in the fertility index might be due to either a reduction in the passage of spermatozoa via the female reproductive tracts or to the possible changes in the biochemical constituents of the uterine mucus. Similar studies conducted on male rats, treated with 16.67 mg / kg /

day, revealed a considerable reduction in the index of fertility that was attributed to a decrease in the motility of spermatozoa and to a marked decrease in the number of spermatozoa (Ocampo, & Avila, 1994). It is of great interest to mention that 2,4-Dichlorophenoxyacetic acid contains a trace amount of tetrachlorodibenzo-p-dioxin, known for its negative effect on fertility (Gray & Ostby, 1995). On the other hand, it is also possible that the observed decrease in the index of fertility is due to the perturbation of hormone secretion, regulating the reproductive function in mammals, following the administration of the herbicide.

The administration of 2,4-Dichlorophenoxyacetic acid, induces a marked fall in the index of fecundity, with a dose dependent effect. Such observation can be explained by a reduction in, the estrogenic function and the luteinizing mechanism of the ovaries of treated females. Previous studies have reported similar observations in farmers, frequently using methoxychlor (Lerda & Rizzi, 1991).

Most importantly, the current investigation reveals that the rate of fetal death was almost 20 times higher in females receiving 125 mg / kg / day than that of controls, while no significant differences in fetuses body weight was recorded. The same observations have been found in other investigations but using a much higher dose of the herbicide (Shtenberg & Ozhovan, 1971).

Finally, the use of 2,4-Dichlorophenoxyacetic acid appears to affect plasma progesterone levels, particularly in individuals treated with 50 & 125 mg /kg/ day, where levels were below those of controls at day 12 and day 18 of the gestation period. It is rather difficult to explain these fluctuations, notably where the fall in progesterone concentration was not associated with any spontaneous abortions. Nonetheless, the observed changes in plasma progesterone levels might be due to a fall in the secreting activity of the granulosa cells of the ovaries.

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RELATIONS IMMUNO-CORTICOTROPES DANS UN ENVIRONNEMENT NOCICEPTIF CHEZ LA RATTE GESTANTE (WISTAR)

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RESUME.

L'administration orale d'ammonitrate, le stress éther et la stimulation antigénique provoquent chez la ratte gestante des variations immunitaires, hormonales et altèrent les capacités reproductrices.

Une activation positive du système immunitaire est notée lors de la stimulation et l'administration du xénobiotique, et une immunodépression lors du stress.

La riposte corticotrope se situe au 6^{ème} jour de la gestation, elle est encore et de loin plus marquée à partir du 12^{ème} jour chez les animaux gavés à l'ammonitrate. En fin de gestation, ces taux semblent se stabiliser chez les animaux gavés. L'action de l'ammonitrate entraîne une toxicité majeure.

ABSTRACT.

In gestating rats, oral administration of ammonium-nitrate, stress with ether and antigenic stimulation were found to provoke immunological variations, hormonal changes and did alter the reproductive capacities.

In this aspects, a positive activation of the immune system and immuno-depression have both been recorded during xenobiotics administration and antigenic stimulation, and during stress, respectively.

Corticotrope response occurred at day 6 of gestation and was been more pronounced at day 12 and onwards in animals receiving ammonium-nitrate. at the end of the gestation period, however, there was a stabilization in corticotrope response in treated animals. Ammonium-nitrate administration seemed to entraine a major toxicity effects.

Mots clés Stress, immunité, reproduction, ACTH, PRL, rat