

Assessment of Maternal and Embryo-Fetal Toxicity of Copper Oxide Fungicide

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Abstract—The excessive use of agricultural pesticides and the resulting contamination of food and beds of rivers have been a recurring problem nowadays. Some of these substances can cause changes in endocrine balance and impair reproductive function of human and animal population. In the present study, we evaluated the possible effects of the fungicide cuprous copper oxide Sandoz® on pregnant Wistar rats. They received a daily oral administration of 10^3 or 3.10^3 mg/kg of the fungicide from the 6th to the 15th day of gestation. On day 21 of gestation, the maternal and fetal toxicity parameters and indices were determined. The administration of cuprous oxide (Copper Sandoz) in Wistar rats, the period of organogenesis, revealed no evidence of maternal toxicity or embryo at the studied doses.

Keywords—Reproductive toxicity, endocrine disrupter, copper Sandoz®, rodent

I. INTRODUCTION

THE use of pesticides in agriculture has been a great ally to control pests and increase crop productivity worldwide. However, due to indiscriminate and large-scale, and errors in handling the equipment for application xxx to human and animal population is directly vulnerable to pesticide exposure, mainly through residues in food and drinking water [2]. Many pesticides are suspected to alter and compromise endocrine hormone-dependent functions such as reproduction. Substances that interfere negatively with the hormonal regulation are known as endocrine disrupters. It can bind to hormone receptors and produce a response, acting as a mimic, or can bind to a receptor and block its interaction with the natural hormone, modify the number of receptors, interfering in the synthesis, metabolism and elimination of the hormone, deactivate enzymes that degrade hormones, among others [9]. There are currently associated with increased incidence of infertility, changes in libido, male urogenital malformations (cryptorchidism and hypospadias above) and changes in semen quality (in terms of concentration, motility and cell abnormalities) to human exposure to endocrine disruptors [8]. Copper Sandoz® is a fungicide used in the south of Bahia (Brazil) for the control of plant diseases associated with the culture of cacao, especially the witch's broom. The copper-based fungicides are suspected to act as endocrine disruptors in the body and can cause damage to reproductive function.

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In this study we sought to evaluate the effects of cuprous copper oxide, the major component of this fungicide on pregnancy in rodent, specifically investigating the potential embryo fetal-toxic and its influence on maternal health.

II. METHODOLOGICAL PROCEDURES

A. Animals and Breeding

Adult Wistar rats were used for the experiment. Eighteen primiparous females were maintained in the vivarium of Universidade Estadual de Santa Cruz, in rooms with constant temperature ($22 \pm 2^\circ\text{C}$), a light/dark cycle of 12 hours and receiving water and food *ad libitum*.

The adult female rats were mated with breeding males during the dark phase of their estrus. Vaginal wash was held the morning after mating for the verification and confirmation of copulation by the presence of spermatozooids in vaginal smear observed under light microscope or a vaginal plug. This day was considered as the beginning of pregnancy. The pairings were repeated for the non-inseminated females.

B. Treatment of the inseminated females

They were weighed, identified and randomly divided into two experimental groups ($n = 6$), receiving respectively 10^3 and 3.10^3 mg/kg of cuprous copper oxide (Copper Sandoz®) daily oral administration through the technique of gavage. The control group was treated with distilled water in equal volume (10 ml/kg). The treatment was performed in 6 (to avoid any influence of the pesticide in the implantation of the embryo in the womb) to the 15th day of gestation, covering the entire period of organogenesis. The rats were weighed on days 1, 6, 10 and 15-21 days of gestation.

C. Sacrifice of the pregnant females

The following parameters were evaluated in females:

- i. The body mass gain during pregnancy, - the relative mass of the collected organs and reproductive indices, - the number of fetuses per mother, - losses before and after deployment, calculated according to the following formulas.
- ii. Pre-implantation loss (number of corpora lutea - number of implantations/number of corpora lutea x100), and
- iii. Post-implantation loss (number of implantation sites - number of fetuses / number of implantation sites x 100).
- iv. Weight of the fetuses, the placenta the following indexes:
- v. The rate of vitality (number of live fetuses / number of fetal deaths), the sex ratio (number of males / number of females), and the placental Index (placental weight / fetal weight).

D. Sacrifice of the female progenitors

The pregnant females from each group were sacrificed on day 21 of gestation.

III. RESULTS AND DISCUSSION

The treatment of the pregnant females was from the 6th to the 15th day of gestation corresponding to the period of embryonic development called organogenesis. This is the most critical period of embryo formation, in which embryos are more susceptible to interference from external agents (teratogens) able to alter their development and increasing the incidence of congenital anomalies [11].

Fig. 1 shows the variation in body mass of females during pregnancy. According to the expected for this period, the animals showed increased mass over days of treatment. However, no significant difference was observed between the groups, noting that the treatment does not interfere in maternal weight gain.

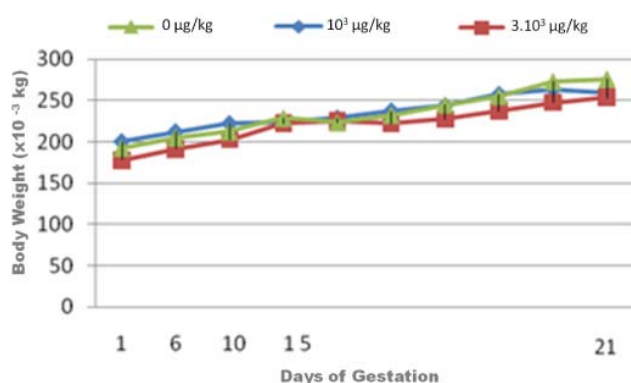


Fig. 1 Body weight of pregnant females treated with cuprous oxide-copper, from 6th to 15th day of gestation

Table I shows the mass of the right and left kidney, right and left adrenal, heart, lung, liver, brain, pituitary, spleen and right and left ovaries. There was no significant difference in mass of evaluated organs. According to Dallegrave (2003), in teratogenicity testing, treatment may not cause changes in organ weight possibly because the administration of the substances is carried out only in the second trimester of gestation (10 days) and also because the evaluation of organs occur 6 days after discontinuation, which would allow the organisms to reconstruct / renew tissues danificados.terço of gestation (10 days) and also because the evaluation of organs occurs 6 days after discontinuation, which would allow organisms to replenish / renew damaged tissue.

TABLE I
WEIGHT OF TREATED FEMALES ORGANS AT 21 DAYS OF GESTATION

	T0	T1	T2
Right kidney	0.784067 ± 0.073288	0.72242 ± 0.075106	0.747633 ± 0.025059
Left kidney	0.75885 ± 0.076346	0.6882 ± 0.069185	0.727867 ± 0.027975

Right adrenal	0.030183 ± 0.076346	0.02456 ± 0.00214	0.023183 ± 0.003325
Left adrenal	0.032717 ± 0.003647	0.02642 ± 0.00427	0.0264 ± 0.005237
Heart	0.649117 ± 0.057566	0.58478 ± 0.07513	0.628133 ± 0.049425
Lung	1.60605 ± 0.30225	1.80212 ± 1.46304	2.02605 ± 1.646776
Liver	9.0866 ± 2.583384	8.9998 ± 0.83550	8.924483 ± 1.777305
Brain	1.50595 ± 0.168072	1.53732 ± 0.087055	1.4709 ± 0.146885
Pituitary	0.011367 ± 0.001937	0.01068 ± 0.000545	0.008783 ± 0.00339
Spleen	0.408183 ± 0.108028	0.45552 ± 0.071907	0.516433 ± 0.128319
Right ovary	0.0562 ± 0.01495	0.04536 ± 0.008164	0.039467 ± 0.014649
Left ovary	0.041533 ± 0.010382	0.0381 ± 0.005317	0.035733 ± 0.009783

Fungicide daily oral administration doses: T0 = 0 µg/kg; T1 = 10³ µg/kg; T2 = 3.10³ µg/kg.

Table II shows the reproductive rates of the mother of three groups. There was no significant difference in the number of fetuses per mother between the groups. The difference in the percentage of pre-implantation loss observed between the groups cannot be attributed to treatment, since it was started from the 6th to the 15th day of gestation - during which the embryo is often implanted in the uterine endometrium (Grancia, 2007). The difference in the percentage of post-implantation losses cannot be attributed to treatment, because it was higher in the control group than in the treated groups.

TABLE II
REPRODUCTIVE INDICES OF THE FEMALES PROGENITORS

	T0	T1	T2
Reproductive Rate	8.25	8.6	8.5
Pre-implantation Loss	0.16	0.34	0.95
Post-implantation Loss	0.24	0.11	0.16

Mothers number (m): m₀=4, m₁=3; m₂=4.

Fetus number (f): f₀=33; f₁=26; f₂=34.

As noted in Table III, the fetal and placental weight, placental index and the rate of vitality did not differ significantly between groups. The proportion of males and females also did not differ significantly between the groups, showing that both sexes respond similarly to prenatal exposure of cuprous oxide.

TABLE III
PARAMETERS OF THE FETUSES

	<i>T0</i>	<i>T1</i>	<i>T2</i>
<i>Fetuses Weight</i>	4.6 ± 0.46	4.63 ± 0.24	4.58 ± 0.44
<i>Placentas Weight</i>	0.677 ± 0.08	0.626 ± 0.07	0.627 ± 0.15
<i>Placental Index</i>	0.147	0.135	0.136
<i>Vitality Rate</i>	1.0	1.0	1.0
<i>Sex Ratio (M:F)</i>	0.78	1.15	0.825

Fetus number (f): f₀=33; f₁=26; f₂=34

IV. CONCLUSION

Oral administration of copper oxide in pregnant rats during the fetal period of organogenesis, revealed no indices of effects on reproduction function with the studied doses, neither maternal nor embryo-fetal toxicity. Thus other studies would be carried out in order to better elucidate the role of this fungicide on gestation.

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