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# Subchronic Imidacloprid Exposure Induces Testicular Morphological and Histological Alterations and Modifies Germ-Cell Ros Levels in Wistar Rats

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## Abstract

The neonicotinoid imidacloprid (IMI) is widely used in agriculture and gardening and it has been found to cause harmful effects on the reproductive health of non-target animals. The aim of this study was to evaluate the effects of IMI on the body mass, testes mass, blood vessels, histology, and reactive oxygen species (ROS) of the male Wistar rat testes. Rats were orally administered IMI at doses of 2 or 8 mg/kg body mass/day for 60 days. These doses were selected based on the NOAEL dose. The body mass of rats was recorded twice a week during treatment; after euthanasia, tests were collected and their mass was measured with a scale. We examined the macroscopic appearance of blood vessels in rat tests and analyzed histological changes. Levels of ROS were determined with diclorodihidrofluorescein diacetate (DCFH-DA). No significant changes in body mass or testes mass were observed in the Wistar rats compared with the control group. ROS levels were increased in control and 2 mg/body mass/day groups however, we found a decrease in the 8 mg/body mass/day group. We also observed severe damage to blood vessels and testicular tissue, with blood vessels exhibiting pathological dilation in experimental groups, contributing to altered ROS levels in spermatozoa and, consequently, their fertilizing capacity. The relationship between blood vessel damage, spermatogenesis, and ROS requires in-depth investigation through molecular studies to support stricter regulation of this insecticide's use, given the harm it causes to animal and human health, as well as the environment.

**Keywords:** Neonicotinoid; Imidacloprid; Blood Vessel; Reactive Oxygen Species; Testes, Spermatozoa; Toxicology

## Introduction

Neonicotinoid insecticides have been extensively employed for insect control in agriculture, horticulture, forestry, and household environments since their introduction in the 1990s; the neonicotinoids bind to the nicotinic acetylcholine receptor (nAChR) to kill targeted insects. The most widely used neonicotinoids include imidacloprid (IMI), thiamethoxam, clothianidin, thiacloprid, dinotefuran, acetamiprid, and nitenpyram (1). These were considered less toxic to non-target organisms than other insecticides like organophosphates or carbamates (2). IMI is widely used in agriculture and horticulture for pest control (3); however, there is growing concern about its potential impact on farm animals, humans and the environment. Recent studies have shown that exposure to IMI can negatively affect reproductive health, including histopathological changes on testicular and

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epididymis tissues, DNA damage and antioxidants of male rats decreased spermatozoa count and motility, and altered reproductive hormone concentration (4-7). Despite these findings, the mechanisms underlying the effects of IMI on male reproduction are poorly understood. Detailed research is needed to fill this gap in knowledge and provide a basis for risk assessment and policy formulation. Current regulations regarding the use of imidacloprid vary by country, with some imposing stricter restrictions, making it essential to understand these effects to develop strategies that mitigate the risks associated with its use. This study seeks to provide data to inform future regulatory decisions to ensure environmental and human safety. In this study, we investigated the effect of IMI exposure on the body mass, testes mass, testicular morphology and histology, and reactive oxygen species (ROS) in the spermatozoa of Wistar rats. The results of this study will underscore the need for safer use of IMI in agriculture to protect animal and human reproductive health. This is important because controversy still surrounds the harm caused by IMI and therefore it has not been possible to limit its use. This multidisciplinary approach provides new insights into toxicity mechanisms and contributes to more effective mitigation strategies.

## Materials and Methods

Unless otherwise specified, all reagents were purchased from Sigma-Aldrich® MX. Optical and fluorescence microscopy analyses were performed using a Laser scanning confocal microscope (LSM 780 NLO system, Carl Zeiss, Oberkochen, Germany). Image acquisition and analysis were carried out using AxioVision Zen software (Zeiss, USA).

### Animals and experimental design

Fifteen male Wistar rats housed at the vivarium at the Universidad Autónoma Metropolitana-Iztapalapa (UAM-I) were used, following the guidelines approved by the Institutional Committee for the Care and Use of Animals in the UAM-I vivarium, and the "Regulations of the General Health Law on Health Research" from the Mexican Health Ministry (NOM-062-ZOO-1999). Two months old rats, with a body mass of 340-370 g, were kept under a 12x12-hour light/dark cycle, a controlled temperature of 18-26 °C with an inverted photoperiod (12-h light, 12-h dark), and food and water ad libitum. They were fed with commercial New Purina Rodent Laboratory Chow 5001 (Lab Dyet; San Luis, Missouri, USA) (8).

### Treatments

Rats were randomly divided into three groups of 5. The control group (IMI 0) received water orally without IMI, while the other two groups were treated with IMI orally at a concentration of 2 mg/kg body mass/day (IMI 2) and 8 mg/kg body mass/day (IMI 8). These doses were selected based on the NOAEL dose (9). The treatment was administered orally once a day for 60 days. After 60 days of treatment, the animals were euthanized using a $CO_2$ chamber and dissected as previously described (8).

### Body and testes mass

The body mass of rats was recorded twice a week to determine the dose per animal. The rats were weighed at the end of the study. After euthanasia the tests were collected, and their mass was measured.

### Morphology and histology of rat tests

Testicular morphology was analyzed visually. The left testes were fixed in a 10% formalin solution, washed, and dehydrated for paraffin embedding. The sections were stained with hematoxylin and eosin stain. Slides were revised in an optic microscope at 100x and 400x magnification (10).

### Reactive oxygen species (ROS)

Spermatogenic cells were obtained by perfusing the testis with phosphate-buffered saline (PBS). The eosin-nigrosin technique was applied to assess membrane integrity and cell morphology (11). To identify Reactive Oxygen Species (ROS) we used diclorodihidrofluorescein diacetate (DCFH-DA) as reported by (12). The right tests were used for obtaining spermatozoa using PBS. The sample was incubated for 15 minutes at 37 °C with 6.5 µL of DCFH-DA/mL of spermatozoa sample. Subsequently, it was centrifuged during 2 minutes at 600 x g, and the liquid was removed by decantation. The samples were analyzed under a fluorescence microscope.

### Statistical analysis

Experiments were performed using at least five experimental animals. ANOVA was used to compare the means of different groups, followed by multiple comparisons using Tukey's test, with the significance level $p < 0.05$. All statistical analyses were performed with IBM SPSS Statistics for macOS, v. 20.0 (IBM Corp., Armonk, USA).

## Results

Body mass was recorded twice a week during the treatment period. No significant differences were observed between the control group and the groups treated with IMI at the beginning or end of the treatment period (Fig. 1).

After euthanasia, tests were collected, and their mass was recorded. The treatment did not result in a significant change in testes mass in either group (Fig. 2).

To go further into the assessment of the potential morphological effects of IMI on rat tests, we assessed their blood vessels. We found that the small vessels and the testicular arteries were larger in rats from both treated groups when compared to the control group, both doses (IMI

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2 and IMI 8) appeared to induce pathological dilation (Fig. 3). Furthermore, also for both doses, the tunica albuginea is thinner in the treated groups compared to the control group, allowing the seminiferous tubules to be clearly seen through it (Fig. 3).

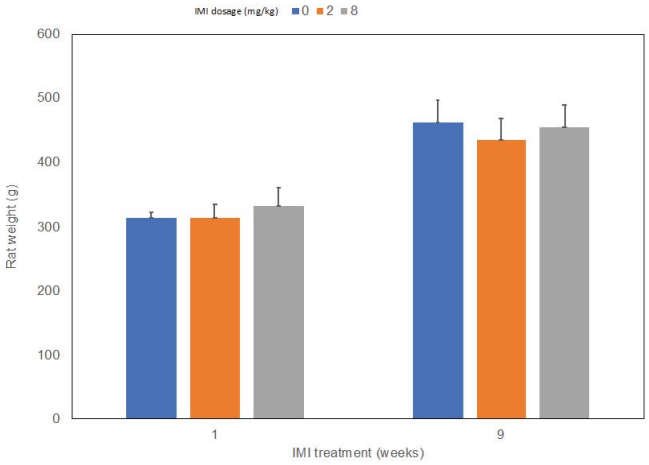


**Figure 1:** Imidacloprid does not affect rat weight during the treatment. Weight of the IMI 0 (blue), IMI 2 (orange) and IMI 8 (grey) groups at week 1 and week 9 of experimentation. Body mass was recorded after the first treatment and right before euthanasia. Data represents the meaning and SD of independent experiments. n=5 for each experimental group.

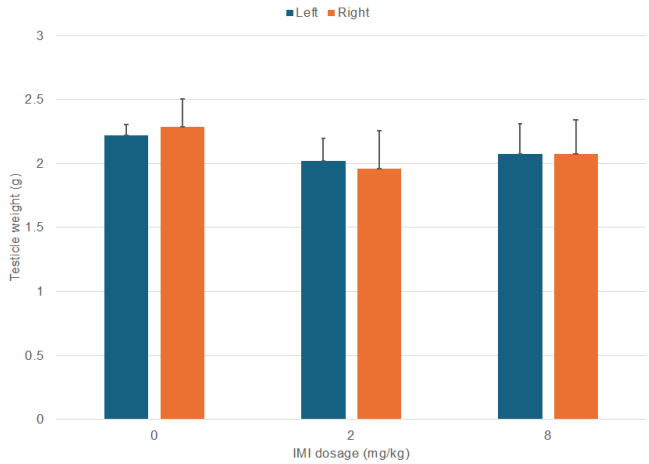


**Figure 2:** Imidacloprid does not affect the testes body mass of Wistar rats during of treatment. Weight of the right (blue) and left (orange) tests of IMI 0, IMI 2 and IMI 8 rats at week 9 of experimentation. Testes mass was recorded right before euthanasia. Data represents the meaning and SD of 3 independent experiments. n=5 for each experimental group.

To assess the integrity of the testicular tissue, we then did a histological study with hematoxylin and eosin staining. We found that the control group had a normal germinal epithelium with well-organized cells; in the IMI 2 group we observed an increased germinal epithelium area, and in the IMI 8 group we found a detachment of spermatogonia from the basal lamina (Fig. 4). We also found a dose-dependent decrease in the number of spermatogonia and spermatids in the rat's tests (Fig. 4). These results indicate clear damage to the integrity and maintenance of germ cell populations from treatment with IMI.

Seeing the morphological effect IMI had on the tests of rats, we then wondered what the repercussions in the metabolism of rat spermatozoa could be. To do so, we used the eosin-nigrosine technique to assess membrane integrity and cell morphology and to identify ROS we assessed fluorescence emitted by DCFH-DA. We found that fluorescence peaks

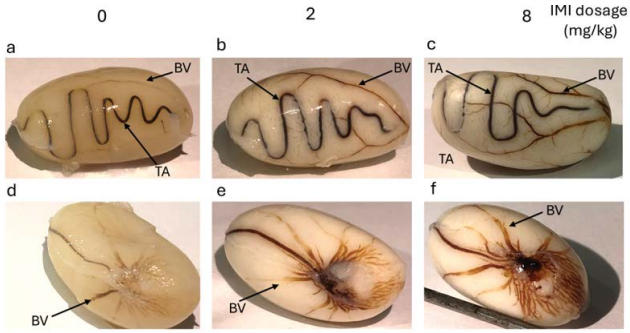


**Figure 3:** Testes blood vessels assessment of imidacloprid (IMI) treated male Wistar rats. (a,d) Control groups; (b,e) Testicular arteries and blood vessel (BV) are larger in rats treated with 2 mg/kg body mass/day of IMI compared to control rats; (c,f) Testicular arteries (TA) and BV are larger in rats exposed to 8 mg/kg body mass/day of IMI compared to the other groups.

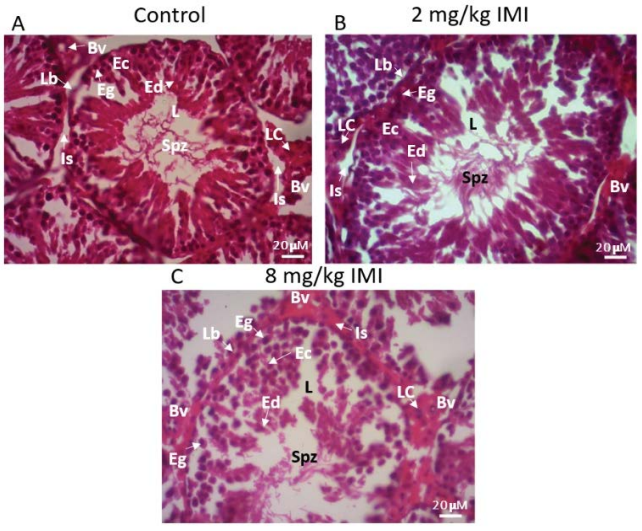


**Figure 4:** Testicular tissue from Wistar rats treated with imidacloprid (IMI). Testes were fixed in formalin solution, washed, and dehydrated for paraffin embedding. The sections were stained with hematoxylin and eosin. A) Control group (0 mg/kg/day). B) Group treated with 2 mg/kg/day of IMI. C) Group treated with 8 mg/kg/day of IMI. Ec = spermatocytes; Ed = elongated spermatids; Eg = spermatogonia, LB = basal lamina, L = Lumen; Spz = spermatozoa; Is = Interstitial space; Bv = Blood vessel; LC = Leydig cells.

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were similar in the IMI 0 group and the IMI 2 group. ROS levels decreased in the IMI 8 group. These results indicate that IMI altered the ROS levels at a high dose like IMI 8,

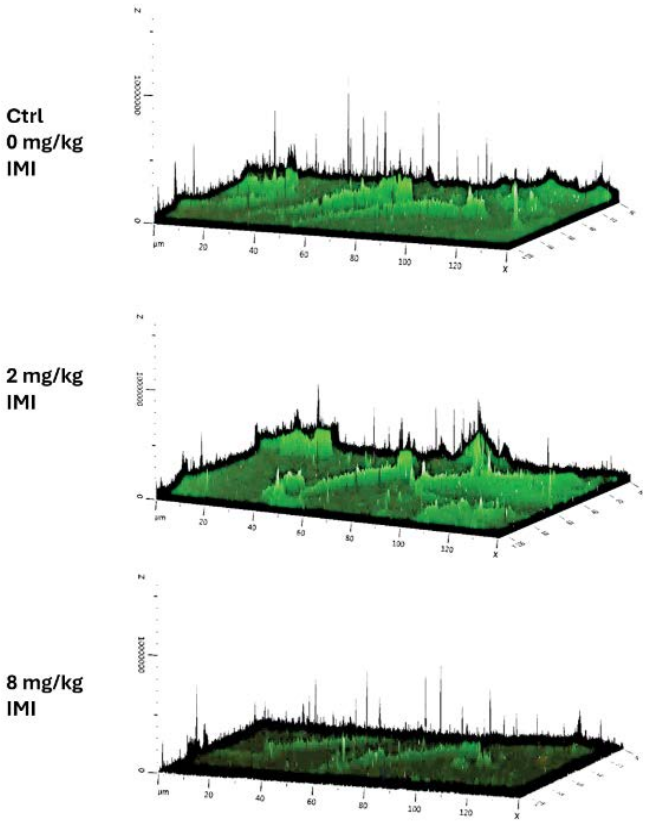


**Figure 5:** Effect of IMI in the levels of ROS in the spermatozoa from tests of Wistar rat. The fluorescence emitted by DCFH-DA indicates the ROS levels of the spermatozoa.

## Discussion

In the study, no significant changes in body mass were observed in Wistar rats treated with IMI. These findings are consistent with those reported by other authors (13), found no significant changes in body mass following administration of IMI at a dose of 0.06 mg/kg body weight/day. Similarly (14), reported no significant changes at a dose of 1 mg/kg body weight/day, although a significant increase in body mass was observed at 0.5 mg/kg body weight/day. In contrast (15), reported a reduction in body weight gain in male Sprague-Dawley rats orally exposed to 22.5 mg/kg body weight/day of IMI (1/20 LD50). Likewise (7), observed a notable reduction in body mass at a dose of 10 mg/kg body weight/day. These discrepancies may be related, at least in part, to the higher doses used in these studies compared with those administered in the present study. Regarding testicular mass, it has been reported that significant decreases in both absolute and relative testicular mass following exposure to 22.5 mg/kg body weight/day of IMI (7). In contrast, no significant changes in testicular mass were observed in the present study. This difference may be attributable to the substantially higher dose used by other authors. This suggests that alterations in testicular mass may occur primarily following exposure to higher doses of IMI. Tests of Wistar rat treated with IMI showed a pathological dilation. It is known that after chronic partial occlusion of the saphenous vein main branch of the rat, a rich collateral network develops, with morphological similarity to the reticular veins of the initial phase of the human leg varicosity disease (16). Varicose veins are caused by increased blood pressure in the veins, and it happens in the superficial veins. In the testes, it can raise the temperature in the scrotum, which may lower spermatozoa count and cause male infertility. Macroscopic findings of blood vessels of rat tests in this study are original and are in accordance with morphology changes in Wistar rat tests with arterial ischaemia and venous congestion reported by (17). Therefore, we hypothesize that treatment with IMI causes damage to the circulatory system, which in turn damages the tests and spermatozoa. The foregoing is consistent with the damage observed in the testicular tissue and the trend toward a decrease in the number of spermatogenic cells in the seminiferous tubules and with data of other studies that reported damage on the reproductive organs (9). This study suggests that IMI may affect the structure of the seminiferous epithelium and germ cell populations, especially at a dose of 8 mg/kg/day. It does not necessarily imply a generalized alteration of germ cell differentiation, but could instead reflect a reduction in their survival, proliferation, or maintenance within the seminiferous epithelium. Additionally, several studies have reported that IMI exposure can induce increased oxidative stress. ROS can trigger the intrinsic apoptotic pathway in which mitochondria release $H_2O_2$, a small chargeless molecule capable of penetrating the nucleus and contributing to DNA damage (18). Excessive ROS production can cause various types of cellular damage and is typically associated with impaired spermatozoa function; mitochondria are the primary source of ROS in spermatozoa. The high concentration of polyunsaturated fatty acids in mammalian spermatozoa, which are highly susceptible to free-radical attack, makes these cells particularly vulnerable to oxidative damage (18). In this study, ROS levels at the low dose (2 mg/kg/day) were similar with respect to the control group but were decreased at the higher dose (8 mg/kg/day); however, if this process alters spermatozoa physiology, an apoptotic process could be the cause of the decrease in ROS levels observed with the daily dose of 8 mg/kg of body weight because ROS levels have an impact beneficial and detrimental depending on the conditions (19). However, this interpretation remains speculative, and additional experiments, such as flow cytometric analysis of ROS production and apoptotic markers, would be necessary to confirm these findings and provide a better understanding of the mechanisms underlying the observed decrease in ROS levels.

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## Conclusion:

IMI is an insecticide that did not cause changes in the body mass or testicular mass of Wistar rats. However, it caused severe damage to blood vessels and testicular tissue, contributing to altered ROS levels in spermatozoa and, consequently, their fertilizing capacity. The relationship between blood vessel damage, spermatogenesis, and ROS requires in-depth investigation through molecular studies to support stricter regulation of this insecticide's use, given the harm it can cause to animal and human health as well as the environment.

### Declaration of Competing Interest

The authors declare the following financial interests/ personal relationships which may be considered as potential competing interests: Paola Berenice Ortiz-Sánchez (718726), Leslye Sámano-Hernández (267937), Merit Barrera-Mendoza (925356) and Karla Piña-de la Rosa (1167758) reports financial support was provided by Secretaría de Ciencia, Humanidades, Tecnología e Innovación (Secihti). Tony Lefebvre (M25S01-2025-74) reports financial support was provided by Ecos Nord and Secihti. Reyna Fierro (M25S01-2025-74 and 105961) reports financial support was provided by Ecos Nord and Secihti. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

1. Simon-Delso N, Amaral-Rogers V, Belzunces LP, et al. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environ Sci Pollut Res Int 22 (2015): 5-34.
2. Thompson DA, Lehmler HJ, Kolpin DW, et al. A critical review on the potential impacts of neonicotinoid insecticide use: current knowledge of environmental fate, toxicity, and implications for human health. Environ Sci Process Impacts 22 (2020): 1315-1346.
3. Hayssen V, Orr TJ. Introduction to "Reproduction: The Female Perspective from an Integrative and Comparative Framework". Integrative and Comparative Biology 60 (2020): 676-682.
4. Aloisi I, Piccini C, Cai G, et al. Male Fertility under Environmental Stress: Do Polyamines Act as Pollen Tube Growth Protectants? Int J Mol Sci 23 (2022).
5. Lahimer M, Abou Diwan M, Montjean D, et al. Endocrine disrupting chemicals and male fertility: from physiological to molecular effects. Front Public Health 11 (2023): 1232646.
6. Lovaković BT, Kašuba V, Sekovanić A, et al. Effects of Sub-Chronic Exposure to Imidacloprid on Reproductive Organs of Adult Male Rats: Antioxidant State, DNA Damage, and Levels of Essential Elements. Antioxidants 10 (2021): 1965.
7. Sardar A, David M, Jahan S, et al. Determination of biochemical and histopathological changes on testicular and epididymis tissues induced by exposure to insecticide Imidacloprid during postnatal development in rats. BMC Pharmacol Toxicol 24 (2023): 68.
8. Sámano-Hernández L, Fierro R, Marchal A, et al. Beneficial and deleterious effects of sitagliptin on a methionine/choline-deficient diet-induced steatohepatitis in rats. Biochimie 181 (2021): 240-248.
9. Bal R, Naziroğlu M, Türk G, et al. Insecticide imidacloprid induces morphological and DNA damage through oxidative toxicity on the reproductive organs of developing male rats. Cell Biochem Funct 30 (2012): 492-499
10. Hernández-Jardón N, Rojas-Castañeda JC, Landero-Huerta D, et al. Cryptorchidism: The dog as a study model. Front. Vet. Sci 9 (2022): 935307.
11. Ortiz-Sánchez PO, Roa-Espitia AL, Fierro R, et al. Perfluorooctane sulfonate and perfluorooctanoic acid induce plasma membrane dysfunction in boar spermatozoa during in vitro capacitation. Reprod Toxicol 110 (2022): 85-96.
12. Riley L, Ammar O, Mello T, et al. Novel methods to detect ROS in viable spermatozoa of native semen samples. Reprod Toxicol 106 (2021): 51-60.
13. Zhao GP, Wang XY, Li JW, et al. Imidacloprid increases intestinal permeability by disrupting tight junctions. Ecotoxicol Environ Saf 222 (2021): 112476.
14. Khalil SR, Awad A, Mohammed HH, et al. Imidacloprid insecticide exposure induces stress and disrupts glucose homeostasis in male rats. Environ Toxicol Pharmacol 55 (2017): 165-174.
15. Saber TM, Arisha AH, Abo-Elmaaty AMA, et al. Thymol alleviates imidacloprid-induced testicular toxicity by modulating oxidative stress and expression of steroidogenesis and apoptosis-related genes in adult male rats. Ecotoxicol Environ Saf 221 (2021): 112435.
16. Patai BB, Dornyei G, Anna-Maria Tokes AM, et al. Initiation of reticular and spider veins, incompetent

perforantes and varicose veins in the saphenous vein network of the rat. Nature Research 10 (2020): 15381.

17. Hirai S, Hatayama N, Naito M, Nagahori K, Kawata S et al. Pathological effect of arterial ischaemia and venous congestion on rat testes. Sci Rep 7 (2017): 5422.

18. Aitken RJ, Jones KT, and Robertson SA. Reactive oxygen species and sperm function--in sickness and in health. J Androl 33 (2012): 1096-1106.

19. de Lamirande E and Gagnon C. Impact of reactive oxygen species on spermatozoa: a balancing act between beneficial and detrimental effects. Hum Reprod 10 (1995): 15-21.



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