UDC 615.9:632.952:612.6:591.16:591.46 DOI: 10.33273/2663-9726-2021-54-1-52-61

REPRODUCTIVE TOXICITY OF TRIAZOLE FUNGICIDES CYPROCONAZOLE AND EPOXICONAZOLE WHEN EXPOSED TO MALE AND FEMALE WISTAR RATS DURING GAMETOGENESIS

N. Shepelskaia, Ya. Kolianchuk
L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Ukraine (State Enterprise), Kyiv, Ukraine

ABSTRACT. Aim. *Studying the effect of generic pesticides cyproconazole (98 %) and two samples of epoxiconazole (epoxiconazole 1- 95,75 % and epoxiconazole 2 - 98,7 %) on the reproductive system of male and female Wistar Han rats at the level of the organism when exposed during gametogenesis, identification and characterization of their hazard, as well as assessment of the risk of reproductive toxicity of these compounds.*
Materials and Methods. *The test samples were administered daily (5 days a week) by oral gavage at doses of 0.2 and 2.0 mg/kg for cyproconazole and 0.5 and 2.0 mg/kg for epoxiconazoles during 11 weeks for males, and 10 weeks for females. Also, there were kept intact males and females, intended for crossover mating with experimental animals. After the end of the exposure, functional indicators of the state of the gonads and the ability of animals to reproduce offspring were studied. The duration and the frequency of each stage of the estrous cycle in female rats and the number of motile sperm, the total amount of sperm and the number of abnormal forms of germ cells of the male rats were studied. The reproductive function state in females was evaluated on day 20th of pregnancy. Thereby the number of corpora lutea in the ovaries, number of alive, dead and resorbed foetuses and embryos, the foetus weight, total weight of litters were registered. The studies were carried out in accordance with the recommendations of the Bioethics Commission and the Centre's standard operating procedures, developed in accordance with the recommendations and requirements of Good Laboratory Practice (GLP).*
Conclusions. *Test substances at a maximum dose of 2.0 mg/kg of body weight have reproductive toxicity and endocrine-disruptive effect, exerting a significant antiandrogenic effect on males and antiestrogenic effect on female rats. No-observed-adverse-effect-level (NOAEL) for gonadal and reproductive toxicity for male and female Wistar Han rats were established. They are 0.2 mg/kg body weight for cyproconazole and 0.5 mg/kg body weight for epoxiconazole.*
Key Words: *azole fungicides, cyproconazole, epoxiconazole, reproductive toxicity, antiandrogenic and antiestrogenic effects, Wistar Han rats.*

Introduction. The work was carried out within the framework of the research of the State Enterprise "Scientific Centre of Preventive Toxicology, Food and Chemical Safety named after Academician L.I. Medved of the Ministry of Health of Ukraine" on the topic "Scientific substantiation of modern regulatory requirements for the use of pesticides and agrochemicals: predicting long-term effects of action (carcinogenic, mutagenic, teratogenic activity, reproductive toxicity, chronic intoxication)"; State registration number 0108U007458.

Triazoles are the largest group of fungicides belonging to the azole class. These preparations can be used for spraying plants in the early phases of disease development or for preventive treatments. They are used against pathogens of various diseases of fruit and vegetable crops.

Triadimefon was one of the first fungicides from the triazole group that have got application in the USSR since the 1970s [1]. Fungicides of the triazole group have replaced the aging benzimidazoles and, thanks to their unique mechanism of action and a wide spectrum of activity, have become the most widely used fungicides. Difenoconazole, tebuconazole, cyproconazole and epoxiconazole are the most popular among them [2].

The fundamental mechanism of action of triazoles is inhibition of sterol biosynthesis. In many species of fungi, it exists mainly in the form of ergosterol and plays an important role in the stabilization and functioning of cell membranes, and affects the processes of cell division, stimulation of growth and sexual reproduction [3]. Different representatives of triazole derivatives affect different stages of sterol biosynthesis. As a result, the spectrum of activity of substances of this chemical class is somewhat different. For example, cyproconazole also has specific activity against rust fungi [1].

However, azole compounds play a key role not only as an antifungal agent in agriculture, but are also used for human mycoses, as well as non-steroidal antiestrogens in the treatment of estrogen-dependent breast tumours in post-menopausal women. This widespread use of azoles is based on their universal mechanism of action for both target and non-target organisms, i.e. inhibition of certain pathways of steroidogenesis by binding with high affinity to sterol 14-alpha-demethylase enzymes and aromatase enzymes.

As it is known, sterol 14-alpha-demethylase is critical to produce sterols that activate meiosis, which modulate the development of germ cells in mammals of both sexes. Aromatase is responsible for the physiological balance of androgens and estrogens. In high doses, azole fungicides and other azole compounds affect reproductive organs, fertility, and development in many species [4].

Aim. The aim of these studies is to research the effect of pesticides cyproconazole and epoxiconazole on the reproductive system of male and female Wistar Han rats at the level of the organism when exposed during gametogenesis, to identify and characterize their hazard, and to assess the risk of reproductive toxicity of these compounds.

Materials and Methods. Research on animals was carried out in accordance with the requirements and provisions of the "European Convention for the Protection of Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 03/18/1986) ETS No. 123, "Guide for the Care and Use of Laboratory Animals" (National Academies Press, USA, 2011) [5-6].

The experiment investigated generic active ingredients from different manufacturers, namely cyproconazole (cyproconazole – 98% purity) and two samples of epoxiconazole (epoxiconazole 1 – 95.75% and epoxiconazole 2 – 98.7% purity).

The experiments were carried out on male and female Wistar Han rats obtained from the SPF breeding station of the State Enterprise "Scientific Centre of Preventive Toxicology, Food and Chemical Safety named after Academician L.I. Medved of the Ministry of Health of Ukraine" at the age of 5-6 weeks and weighing 80-100 g. The adaptation period lasted five days.

The animals were housed in a "clean" zone of a conventional barrier-type vivarium. The room was provided with forced ventilation (12 volumes per hour) with prepared air. Temperature and relative humidity were recorded daily. The lighting was artificial (12 hours light, 12 hours dark). The animals received ad libitum deionized filtered drinking water, which was disinfected by UV irradiation and purified by reverse osmosis, as well as a balanced granulated hypo-phytoestrogenic food produced by Altromin (Germany).

When studying each test substance, the animals were divided into three groups of 20 males and 20 females each. The test substances were administered to experimental animals in the form of a freshly prepared aqueous emulsion daily, except Saturday and Sunday, intragastrically at doses of 0.2 mg/kg and 2.0 mg/kg of body weight (bw) for cyproconazole and 0.5 mg/kg and 2.0 mg/kg bw for epoxiconazole 1 and epoxiconazole 2. Control animals also received an equivalent amount of a solvent (distilled water with an OP-10 emulsifier) intragastrically using a probe.

In parallel with the control and experimental animals, intact males and females intended for mating were kept (10 males and 20 females for each experimental group of animals).

Each animal was assigned an individual number and marked by marking on the tail. The cage was labelled with the study number, animal numbers, sex, group number, and the dose of the test substance.

The animals were examined daily throughout the experiment to register any visible signs of reaction to the effect of the compound under study.

Test males were weighed weekly during the entire exposure period. The experimental females were weighed weekly until the mating period and at 0, 6, 13 and 20 days post coitum.

During the last 2 weeks of the exposure period, the experimental females received daily vaginal

swabs to determine the duration of the entire estrous cycle, the frequency and duration of its individual stages. Smears were also prepared from the first day of adding intact males to experimental females and experimental males to intact females to determine the moment of mating.

After finishing the exposition, the functional indicators of the state of the gonads and the ability of animals to reproduce offspring were studied. From the experimental groups, 10 males were randomly selected, which were subjected to the study of the morpho-functional state of the gonads. The number of motile spermatozoa, their total number, the number and percentage of abnormal forms of germ cells, as well as the absolute and relative weight of the testes and epididymis were evaluated. The rest of the experimental males were mated with intact females, and the experimental females were mated with intact males (in a ratio of 1 male to 2 females). Every morning during the mating period, vaginal smears were prepared for each female and examined for the presence of sperm. The day of detection of sperm in the vaginal contents of the female was taken as day 0 of pregnancy. The duration of the mating period did not exceed 3 weeks.

The state of reproductive function was considered on the 20th day of pregnancy in experimental females who became pregnant from intact males, as well as intact females mated with experimental males. At the same time, the number of corpora lutea in the ovaries, the number of live, dead and resorbed foetuses and embryos, the body weight of the foetuses, the litter weight, and the presence of gross developmental anomalies were recorded. The indices of mating, conception, fertility, pregnancy were determined, and the duration of the pre-coital interval was considered.

All data obtained in the experiment were processed statistically. Data were presented as group means (M) with standard error of the mean (m). The statistical significance of the intergroup differences ($P < 0.05$) was assessed by the Student's t-test.

Studies were conducted in accordance with the guidelines and requirements of Good Laboratory Practice (GLP).

Results and Discussion. None of the test substances influenced the general condition and behaviour of the animals, there were no clinical signs of intoxication, and no animal mortality was recorded.

However, cyproconazole at the maximum studied dose (2.0 mg/kg) had a general toxic effect characterized by a significant decrease in the body weight of males from the seventh to the eleventh week of the experiment (Fig. 1).

The data obtained on the morpho-functional parameters of the experimental male rats indicate that all the test substances under study at a dose of 2.0 mg/kg bw showed antiandrogenic

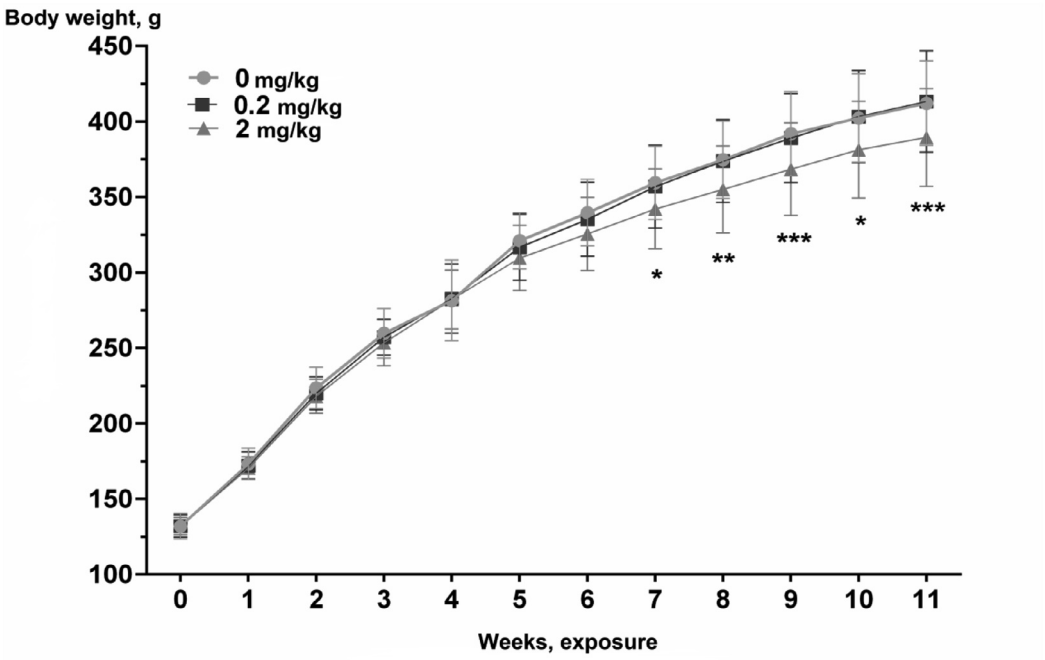

Fig. 1. Dynamics of body weight of male rats during the period of exposure to cyproconazole
Note: * - P≤0.05; ** - P≤0.03; *** - P≤0.02 relative to control.

effect to one degree or another. It was established a significant decrease in the total number of spermatozoa (epoxiconazole 1), in the number of motile spermatozoa (cyproconazole, epoxiconazole 1 and epoxiconazole 2), in the percentage of motile sperm (cyproconazole and epoxiconazole 1), in the absolute and relative weight of the testes (epoxiconazole 2), as well as an increase in the percentage of pathological forms of germ cells (epoxiconazole 2) (Table 1).

As can be seen from the above data, under the influence of the maximum dose of all three tested compounds, there is a tendency to a decrease in the total number of spermatozoa in the epididymis of the test males, reaching statistical significance when exposed to the epoxiconazole 1 sample. As for the sperm motility, all three substances at a dose of 2.0 mg/kg bw reduce the absolute and relative value of this indicator. The tendency to an increase in pathological forms of spermatozoa is also induced by all studied test substances, however, this tendency reaches a statistically significant value under the influence of cyproconazole. The absolute and relative weight of the testes decreases with a high degree of reliability in the group of experimental males who received the epoxiconazole 2 sample at the maximum dose, which allows to conclude that this generic has the most significant antiandrogenic effect among the tested compounds.

However, it should be noted that the detected antiandrogenic activity of this sample did not have a noticeable effect on the ability of animals of this group (2.0 mg/kg bw) to reproduce offspring, which was judged by the reproductive parameters of the intact females that became pregnant from them (Table 2). There are trends towards a decrease in the number of live foetuses in the litter, the total weight of the litter, and an increase in the absolute and relative pre-implantation death of embryos. However, these tendencies do not reach a statistically significant level, in contrast to the results of exposure to the epoxiconazole 1 sample (2.0 mg/kg bw), which induces a significant decrease in foetus weight in intact females, and in this group, there is also a tendency to a decrease in the conception and fertility indices.

The greatest changes in male reproductive capacity are found under the influence of the maximum dose of cyproconazole. There is a significant, although statistically equivocal, decrease in the number of live foetuses in a litter (by 26.7%), a statistically significant decrease in the average weight of foetuses and the total weight of an offspring, statistically significant decrease in the indices of conception and fertility. All three test substances did not have a damaging effect on the studied parameters of males when exposed to minimal doses (0.2 mg/kg bw for cyproconazole and 0.5 mg/kg bw for epoxiconazole).

In the process of analysing the data concerning the effect of the tested compounds on the reproductive system of females, their toxic effect was also found at a dose of 2.0 mg/kg bw, but of an antiestrogenic nature.

Thus, a 2-week observation of the estrous cycle in females receiving cyproconazole at a dose of 2.0 mg/kg bw showed a statistically significant increase in the duration of the estrous cycle, and when exposed to epoxiconazole 1, an increase in the progesterone-dependent stage of dioestrus in relation to the control (Fig. 2).

The most significant changes in the reproductive function in experimental females are

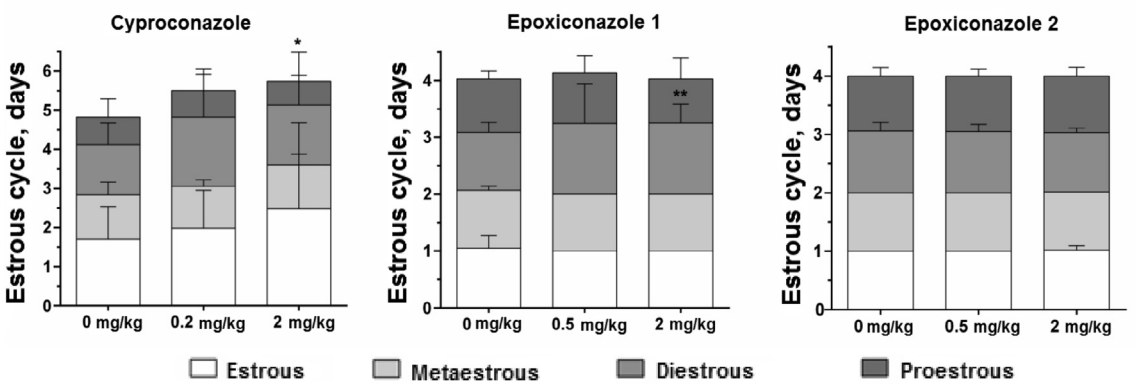

Fig. 2. Estrous cycle of experimental females
Note: * - P≤0.05; ** - P≤0.01 relative to control.

Table 1

Changes in morpho-functional indicators of the state of the gonads in male rats

Test substances	Doses, mg/kg	Total sperm amount	Motile sperm		Pathological forms of sperm (%)	Testes weight	
			Number	%		Absolute, g	Relative
		(M ± m)	(M ± m)	(M ± m)	(M ± m)	(M ± m)	(M ± m)
Cyproconazole	0	113,30±2,80	46,90±2,40	41,19±1,38	1,17±0,25	3,61±0,15	9,01±0,3
	0.2	111,60±5,66	43,50±2,92	38,74±1,35	1,92±0,54	3,73±0,12	9,45±0,31
	2.0	106,30±5,07	39,00±2,17[2)]	36,66±1,05[3)]	2,56±0,56[1)]	3,45±0,23	8,72±0,60
Epoxiconazole 1	0	116,60±2,26	54,00±1,82	46,32±1,25	0,35±0,14	3,90±0,10	10,28±0,21
	0.5	111,90±2,42	48,50±2,15	43,20±1,20	0,93±0,31	3,72±0,04	9,81±0,12
	2.0	102,60±4,61[4)]	37,70±2,72[6)]	36,43±1,56[6)]	0,43±0,19	3,72±0,08	9,92±0,21
Epoxiconazole 2	0	121,40±3,78	49,20±2,37	40,45±1,20	0,57±0,17	3,78±0,08	9,53±0,21
	0.5	126,50±3,18	51,90±1,93	40,95±0,74	0,94±0,19	3,64±0,06	9,29±0,15
	2.0	111,60±3,94	41,80±1,04[4)]	37,60±0,72	1,16±0,30	3,42±0,07[5)]	8,57±0,21[5)]

Notes: [1)] — P≤0,05; [2)] — P≤0,04; [3)] — P≤0,03; [4)] — P≤0,01; [5)] — P≤0,004; [6)] — P≤0,0001 in relation to control

observed when exposed to cyproconazole and epoxiconazole 2.

Cyproconazole induces, with a high degree of statistical significance, a decrease in the number of live foetuses in the litter, a decrease in the average weight of foetuses and the total weight of the litter, a decrease in the conception and fertility indices. There is a tendency towards an increase in pre-implantation death of embryos.

Exposure to epoxiconazole 2 also leads to a decrease in the number of live foetuses in the litter, to a decrease, albeit not statistically significant, in the average weight of foetuses and, consequently, to a significant decrease in the total weight of offspring. Pre-implantation death of embryos is statistically significant.

The results we obtained eloquently indicate the endocrine-disruptive nature of the toxic effect of cyproconazole and epoxiconazole on the reproductive system of female and male rats.

As already mentioned, azole fungicides affect the construction of cell membranes of fungi and yeast, blocking the synthesis of the main membrane component, namely ergosterol. They do this by competitively inhibiting the enzyme of lanosterol 14a-demethylase (CYP51A1), which depletes ergosterol and accumulates lanosterol and other 14-methylated sterols. As a result, the structure of the plasma membrane is disrupted and becomes more susceptible to further damage [7-9]. These changes also affect the activity of several membrane-bound enzymes, including those involved in nutrient transportation and chitin synthesis [10].

It is also known that sterol 14a-demethylase plays an important role in the synthesis of cholesterol in mammals [11]. However, many azole compounds are nonspecific in binding to the haem component exclusively of CYP51, they can also inhibit many different CYPs [4,12,13] and, in particular, CYP19 (aromatase), which in the context of this communication is of particular interest considering its importance in biosynthesis of estrogen [14]. Influence on CYP19

Table 2

Changes in the reproductive parameters of experimental females and intact females mated with experimental males

Test substances and groups ♀♀	Doses, mg/kg	Number of live foetuses	Total litter weight	Average weight of foetuses	Pre-implantation loss		Indices	
					Number	%	of conception	of fertility
		(M ± m)	(M ± m)	(M ± m)	(M ± m)	(M ± m)	(M ± m)	(M ± m)
Cyproconazole, Experimental ♀♀	0	9,06±0,99	37,39±3,47	3,89±0,06	4,94±1,02	33,85±6,98	85	85
	0.2	9,63±0,78	34,90±2,70	3,67±0,11	2,81±0,60	21,60±4,81	80	80
	2.0	5,11±1,10[4)]	16,57±3,57[7)]	3,23±0,26[3)]	7,89±1,73	51,94±7,86	45[4)]	45[4)]
Cyproconazole, Intact ♀♀	0.2	7,56±0,94	28,82±3,73	3,77±0,06	3,94±0,85	28,87±6,49	80	80
	2.0	6,62±1,08	21,16±3,12[5)]	3,35±0,16[6)]	6,69±1,03	46,77±7,49	65[2)]	65[2)]
Epoxiconazole 1, Experimental ♀♀	0	10,16±0,74	38,79±2,83	3,84±0,05	1,58±0,45	13,81±4,14	95	95
	0.5	10,89±0,52	40,72±2,02	3,74±0,04	0,78±0,22	5,40±1,38	90	90
	2.0	10,65±0,50	39,96±1,86	3,77±0,07	1,47±0,38	11,71±3,45	85	85
Epoxiconazole 1, Intact ♀♀	0.5	11,00±0,58	40,78±2,07	3,72±0,06	1,21±0,46	9,75±3,77	95	95
	2.0	10,24±0,70	37,72±2,56	3,69±0,04[2)]	1,65±0,49	13,41±3,83	85	85
Epoxiconazole 2, Experimental ♀♀	0	12,72±0,48	44,80±1,51	3,54±0,05	0,83±0,25	6,06±1,96	95	95
	0.5	12,95±0,46	44,42±1,74	3,43±0,04	0,70±0,16	4,91±1,18	85	85
	2.0	10,63±0,76[2)]	37,16±2,64[3)]	3,49±0,04	2,11±0,58[1)]	16,62±4,84[1)]	90	90
Epoxiconazole 2, Intact ♀♀	0.5	11,18±0,77	38,84±2,64	3,51±0,05	2,00±0,62	15,28±5,21	100	100
	2.0	11,94±0,56	42,15±2,13	3,52±0,05	1,00±0,30	6,35±1,69	95	95

Notes: [1)] — P≤0,05; [2)] — P≤0,03; [3)] — P≤0,02; [4)] — P≤0,01; [5)] — P≤0,004; [6)] — P≤0,002; [7)] — P≤0,0004 in relation to control

is not limited to mammals. It has been proven that azoles inhibit the expression and / or activity of aromatase in birds, reptiles, amphibians, and fish [15-20].

However, the endocrine-disruptive effect of azole compounds on the reproductive function of most species is not limited to inhibition of aromatase production. Many studies have shown that triazoles, which include both cyproconazole and epoxiconazole, are also antagonists of estrogen and androgen receptors, in addition, they also exhibit weak agonistic affinity for the aryl-carbohydrate receptor [13, 21-25].

Thus, test compounds exhibit their endocrine-mediated toxicity by acting through several mechanisms of endocrine chain disruption. Their ability to interfere with the biosynthesis of steroid hormones leads to increased production of progesterone and a decrease in the production of testosterone and oestradiol, which also indicates inhibition of enzymes involved in the conversion of progesterone to testosterone [26].

In conclusion, it should be noted that, despite a fairly extensive study of representatives of azole compounds on the reproductive system of various species of the animal world, there are no data in the available literature on the study of the effect of triazoles on the whole organism of mammals under conditions of exposure during gametogenesis. And, as our studies have shown, most of the pathological changes in the reproductive function under the influence of endocrine disruptors (violation of sexual behaviour, a decrease in the indices of conception and fertilization, a decrease in fertility, deterioration of sperm parameters, up to azoospermia, reduced viability of a fertilized egg, increased intrauterine death of embryo and foetuses of F1 generation) is due to the influence of toxic compounds during the pre-embryonic stage of gametogenesis [27].

Conclusions

1. All studied samples of triazole fungicides had a toxic effect on the reproductive system of females and males when exposed to a higher dose.

2. Analysis of the observed changes indicates the endocrine-disruptive action of the tested compounds of antiandrogenic and antiestrogenic nature.

3. In males, the processes of spermatogenesis suffer, i.e. a decrease in the total number (epoxiconazole 1) and motor activity of spermatozoa (cyproconazole, epoxiconazole 1, epoxiconazole 2), an increase in pathological forms of germ cells (cyproconazole), a decrease in the absolute and relative mass of testes (epoxiconazole 2). The ability to reproduce F1 offspring is also impaired, which was judged by the reproductive parameters of intact females who became pregnant from them, i.e. a decrease in the average weight of foetuses (cyproconazole, epoxiconazole 1), a decrease conception and fertility indices (cyproconazole).

4. In females, the normal duration of the estrous cycle (cyproconazole and epoxiconazole 1) is disrupted, the number of live foetuses in the litter decreases, their average weight and total litter weight decrease (cyproconazole and epoxiconazole 2), pre-implantation death of embryos (epoxiconazole 2) increases, the conception and fertility indices decrease (cyproconazole).

5. Cyproconazole at the maximum studied dose (2.0 mg/kg) also had a systemic toxic effect on males, characterized by a significant decrease in body weight from the seventh to the eleventh week of the experiment.

6. In the range of doses studied, the NOAEL of the generic test substances are 0.5 mg/kg bw for epoxiconazole and 0.2 mg/kg bw for cyproconazole, which, considering the 100-fold safety factor, corresponds to the permissible daily doses of original analogues existing in Ukraine.

REFERENCES

1. Popov SYa, Dorozhkina LA, Kalinin VA. Osnovy khimicheskoi zaschity rastenii: ucheb. posobie. M.: Art-Lion; 2003. 190, [1] s.
2. Ragulin V. Fungitsidy – proizvodnye triazola [Internet]. 2011 Okt 24 [tsitirovano 2020 Okt 15]. Dostupno: https://www.agroxxi.ru/stati/ fungicidy-proizvodnye-triazola.html
3. Andreeva EI, Zinchenko VA. Sistemnye fungitsidy – ingibitory biosinteza ergosterina. 2. Tebukonazol, tsiprokonazol, dinikonazol, penkonazol, difenokonazol, fenarimol, triflorin, prokhloraz, imazalil, fenpropimorf, fenpropidin. AgroXXI. 2002;(4):14-5.
4. Zarn JA, Bruschweiler BJ, Schlatter JR. Azole fungicides affect mammalian steroidogenesis by inhibiting sterol 14 alpha-demethylase and aromatase. Environ Health Perspect. 2003 Mar;111(3):255-61. doi: 10.1289/ehp.5785.

5. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. Guide for the care and use of laboratory animals. Washington, D.C.; [Great Britain]: National Academy Press; 1996. xii, 125 p.
6. OECD principles of good laboratory practice (as revised in 1997) [Internet]. Paris; 1998 [cited 2020 Oct 15]. Available from: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/mc/chem(98)17&doclanguage=en
7. Georgopapadakou NH. Antifungals: mechanism of action and resistance, established and novel drugs. Curr Opin Microbiol. 1998 Oct;1(5):547-57. doi: 10.1016/s1369-5274(98)80087-8.
8. Ji H, Zhang W, Zhou Y, Zhang M, Zhu J, Song Y et al. A three-dimensional model of lanosterol 14α-demethylase of Candida albicans and its interaction with azole antifungals. J Med Chem. 2000;43(13):2493-505. doi: 10.1021/jm990589g.
9. Sheehan TP, Cirrito J, Numan MJ, Numan M. Using c-Fos immunocytochemistry to identify forebrain regions that may inhibit maternal behavior in rats. Behav Neurosci. 2000 Apr;114(2):337-52. doi: 10.1037//0735-7044.114.2.337.
10. Georgopapadakou NH, Walsh TJ. Antifungal agents: chemotherapeutic targets and immunologic strategies. Antimicrob Agents Chemother. 1996 Feb;40(2):279-91. doi: 10.1128/AAC.40.2.279.
11. Koltin Y, Hitchcock CA. The search for new triazole antifungal agents. Curr Opin Chem Biol. 1997 Aug;1(2):176-82. doi: 10.1016/s1367-5931(97)80007-5.
12. Zhang X, Hecker M, Tompsett AR, Park JW, Jones PD, Newsted J et al. Responses of the medaka HPG axis PCR array and reproduction to prochloraz and ketoconazole. Environ Sci Technol. 2008 Sep 1;42(17):6762-9. doi: 10.1021/es800591t.
13. Kjэrstad MB, Taxvig C, Nellemann C, Vinggaard AM, Andersen HR. Endocrine disrupting effects in vitro of conazole antifungals used as pesticides and pharmaceuticals. Reprod Toxicol. 2010 Dec;30(4):573-82. doi: 10.1016/j.reprotox.2010.07.009.
14. Jaklova Dytrtova J, Belonoznнkova K, Jakl M, Ryslava H. Triazoles and aromatase: The impact of copper cocktails. Environ Pollut. 2020 Nov;266(Pt 1):115201. doi: 10.1016/j.envpol.2020.115201.
15. Chardard D, Dournon C. Sex reversal by aromatase inhibitor treatment in the newt Pleurodeles waltl. J Exp Zool. 1999 Jan 1;283(1):43-50. doi: 10.1002/(sici)1097-010x(19990101)283:1<43::aid-jez6>3.0.co;2-g.
16. Vaillant S, Dorizzi M, Pieau C, Richard-Mercier N. Sex reversal and aromatase in chicken. J Exp Zool. 2001 Dec 1;290(7):727-40. doi: 10.1002/jez.1123.
17. Villeneuve DL, Knoebl I, Kahl MD, Jensen KM, Hammermeister DE, Greene KJ, et al. Relationship between brain and ovary aromatase activity and isoform-specific aromatase mRNA expression in the fathead minnow (Pimephales promelas). Aquat Toxicol. 2006 Mar 10;76(3-4):353-68. doi: 10.1016/j.aquatox.2005.10.016.
18. Chambers JE, Greim H, Kendall RJ, Segner H, Sharpe RM, Van Der Kraak G. Human and ecological risk assessment of a crop protection chemical: a case study with the azole fungicide epoxiconazole. Crit Rev Toxicol. 2014 Feb;44(2):176-210. doi: 10.3109/10408444.2013.855163.
19. Li S, Sun Q, Wu Q, Gui W, Zhu G, Schlenk D. Endocrine disrupting effects of tebuconazole on different life stages of zebrafish (Danio rerio). Environ Pollut. 2019 Jun;249:1049-59. doi: 10.1016/j.envpol.2019.03.067.
20. Chen ZF, Ying GG. Occurrence, fate and ecological risk of five typical azole fungicides as therapeutic and personal care products in the environment: A review. Environ Int. 2015 Nov;84:142-53. doi: 10.1016/j.envint.2015.07.022.
21. Costa NO, Vieira ML, Sgarioni V, Pereira MR, Montagnini BG, Mesquita Sde F et al. Evaluation of the reproductive toxicity of fungicide propiconazole in male rats. Toxicology. 2015 Sep 1;335:55-61. doi: 10.1016/j.tox.2015.06.011.
22. Cremonese C, Piccoli C, Pasqualotto F, Clapauch R, Koifman RJ, Koifman S et al. Occupational exposure to pesticides, reproductive hormone levels and sperm quality in young Brazilian men. Reprod Toxicol. 2017 Jan;67:174-85. doi: 10.1016/j.reprotox.2017.01.001.
23. Trudeau VL, Thomson P, Zhang WS, Reynaud S, Navarro-Martin L, Langlois VS. Agrochemicals disrupt multiple endocrine axes in amphibians. Mol Cell Endocrinol. 2020 Aug 1;513:110861. doi: 10.1016/j.mce.2020.110861.

24. Vieira ML, Costa NO, Pereira MRF, de Fatima Paccola Mesquita S, Moreira EG, Gerardin DCC. Chronic exposure to the fungicide propiconazole: Behavioral and reproductive evaluation of F1 and F2 generations of male rats. Toxicology. 2017 Aug 15;389:85-93. doi: 10.1016/j.tox.2017.07.012.
25. Teng M, Chen X, Wang C, Song M, Zhang J, Bi S et al. Life cycle exposure to propiconazole reduces fecundity by disrupting the steroidogenic pathway and altering DNA methylation in zebrafish (Danio rerio). Environ Int. 2020 Feb;135:105384. doi: 10.1016/j.envint.2019.105384.
26. Kjжrstad MB, Andersen HR, Taxvig C, Hass U, Axelstad M, Metzdorff S et al. Effects of azole fungicides on the function of sex and thyroid hormones [Internet]. 2007 [cited 2020 Oct 15]. Available from: https://www2.mst.dk/udgiv/publications/2007/978-87-7052-538-1/pdf/978-87-7052-539-8.pdf
27. Shepel'skaya NR, Kolyanchuk YaV. Sravnitel'nyi analiz razlichnykh metodologicheskikh podkhodov k identifikatsii reproduktivnoi toksichnosti pestitsidov. Visn. problem biolohii i medytsyny. 2018;(3):238-46.

РЕПРОДУКТИВНА ТОКСИЧНІСТЬ ТРІАЗОЛОВИХ ФУНГІЦИДІВ ЦИПРОКОНАЗОЛУ І ЕПОКСИКОНАЗОЛУ ПРИ ДІЇ НА САМЦІВ І САМИЦЬ ЩУРІВ WISTAR У ПЕРІОД ГАМЕТОГЕНЕЗУ

Н.Р. Шепельська, Я.В. Колянчук
Державне підприємство «Науковий центр превентивної токсикології, харчової та хімічної безпеки імені академіка Л.І. Медведя Міністерства охорони здоров'я України», м. Київ, Україна

РЕЗЮМЕ. Мета. *Вивчити на організменому рівні вплив генеричних пестицидів ципроконазолу (98 %) і двох зразків епоксиконазолу (епоксиконазол 1 – 95,75 % і епоксиконазол 2 – 98,7 %) на репродуктивну систему самців і самиць щурів Wistar Han при впливі в період гаметогенезу, ідентифікувати і характеризувати їх небезпеку, а також оцінити ризик репродуктивної токсичності цих сполук.*
Матеріал та методи. *Досліджувані зразки вводилися щодня (5 днів на тиждень) внутрішньошлунково, в дозах 0,2 і 2,0 мг/кг для ципроконазолу і 0,5 і 2,0 мг/кг для епоксиконазолу протягом 11 тижнів для самців 10 тижнів для самиць. Також утримувались інтактні самці та самиці, призначені для кросоверного спаровування з експериментальними тваринами. Після закінчення експозиції вивчались функціональні показники стану гонад і здатність тварин до відтворення потомства. У самиць вивчали тривалість та частоту кожної стадії естрольного циклу, а у самців кількість рухливих сперматозоїдів, загальну кількість сперматозоїдів, кількість аномальних форм статевих клітин та реєстрували морфометричні показники сім'яників і придатків. Стан репродуктивної функції у самиць досліджували на 20 день вагітності. Оцінювали кількість жовтих тіл в яєчниках, кількість живих, мертвих та резорбованих плодів та ембріонів, масу плоду, загальну масу приплоду. Дослідження проводились відповідно до рекомендацій Комісії з біоетики та стандартних операційних процедур Центру, відповідно до вимог належної лабораторної практики (GLP).*
Висновки. *Всі досліджувані речовини у високій дозі 2,0 мг/кг маси тіла викликають репродуктивну токсичність та ендокрин-деструктивний ефект, справляючи виражену антиандрогенну дію на самців та антиестрогенну дію на самиць щурів. Встановлено недіючі дози (NOAEL) за гонадо- і репродуктивною токсичністю для самців і самиць щурів Wistar Han - 0,2 мг/кг маси тіла для ципроконазолу і 0,5 мг/кг маси тіла для епоксиконазолу.*
Ключові слова: *азольні фунгіциди, ципроконазол, епоксиконазол, репродуктивна токсичність, антиандрогенна та антиестрогенна дія, щури Wistar Han.*

РЕПРОДУКТИВНАЯ ТОКСИЧНОСТЬ ТРИАЗОЛОВЫХ ФУНГИЦИДОВ ЦИПРОКОНАЗОЛА И ЭПОКСИКОНАЗОЛА ПРИ ВОЗДЕЙСТВИИ НА САМЦОВ И САМОК КРЫС WISTAR В ПЕРИОД ГАМЕТОГЕНЕЗА

Н.Р. Шепельская, Я.В. Колянчук
Государственное предприятие «Научный центр превентивной токсикологии, пищевой и химической безопасности имени академика Л.И. Медведя Министерства здравоохранения Украины», г. Киев, Украина

РЕЗЮМЕ. Целью *работы было изучение на организменном уровне влияния генерических пестицидов ципроконазола (98 %) и двух образцов эпоксиконазола (эпоксиконазол 1 – 95,75 % и эпоксиконазол 2 – 98,7 %) на репродуктивную систему самцов и самок крыс Wistar Han при воздействии в период гаме-*

тогенеза, идентификация и характеристика их опасности, а также оценка риска репродуктивной токсичности этих соединений.

Материалы и методы. *Испытуемые образцы вводились ежедневно (5 дней в неделю) внутрижелудочно, в дозах 0,2 и 2,0 мг/кг для ципроконазола и 0,5 и 2,0 мг/кг для эпоксиконазолов в течение 11 недель для самцов 10 недель для самок. Также содержались интактные самцы и самки, предназначенные для кроссоверного спаривания с экспериментальными животными. После окончания экспозиции изучались функциональные показатели состояния гонад и способность животных к воспроизводству потомства. У самок изучали продолжительность и частоту каждой стадии эстрольного цикла, а у самцов количество подвижных сперматозоидов, общее количество сперматозоидов, количество аномальных форм половых клеток и регистрировали морфометрические показатели семенников и придатков. Состояние репродуктивной функции у самок исследовали на 20 день беременности. Оценивали количество желтых тел в яичниках, количество живых, мертвых и резорбированных плодов и эмбрионов, массу плода, общую массу приплода. Исследования проведены согласно рекомендациям комиссии биоэтики и стандартных операционных процедур Центра, разработанных в соответствии с рекомендациями и требованиями Надлежащей лабораторной практики (GLP).*

Выводы. *Тестовые субстанции в максимальной дозе 2,0 мг/кг массы тела обладают репродуктивной токсичностью и эндокрин-деструктивным эффектом, оказывая выраженное антиандрогенное действие на самцов и антиэстрогенное действие на самок крыс. Установлены недействующие дозы (NOAEL) по гонадо- и репродуктивной токсичности для самцов и самок крыс Wistar Han — 0,2 мг/кг массы тела для ципроконазола и 0,5 мг/кг массы тела для эпоксиконазола.*

Ключевые слова: *азольные фунгициды, ципроконазол, эпоксиконазол, репродуктивная токсичность, антиандрогенное и антиэстрогенное действие, крысы Wistar Han.*

Received November 16, 2020