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STUDY OF FUNGICIDE PROTHIOCONAZOLE RESIDUES IN CROPS UNDER FIELD CONDITIONS IN UKRAINE

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Summary. *Objectives. The determination and assessment of new pesticides active ingredient — prothioconazole residues after crops treatment.*
Methods. Prothioconazole-desthio (the prothioconazole main metabolite) residue decline studies were conducted in two main agro-climatic zones of Ukraine during of prothioconazole-based fungicides field trials in 2002–2013. The recommended by manufacturer application rates were used: for seed treatment up to 270 g a.i./1000 kg seed (1 application) and for foliar spray up to 225 g a.i./ha (3 applications). Residues of prothioconazole-desthio in treated crops were determined by gas-liquid chromatography and high-performance liquid chromatography.
Results. The obtained data of field trials showed that prothioconazole-desthio residue in the harvest of treated crops were below limits of quantitation. The half-life (DT_{50}) values of prothioconazole degradation in cereals, rape and sunflower were calculated. The possible daily intake of prothioconazole-desthio were lower than allowable level.
Conclusion. Prothioconazole in terms of degradation rate of in the crops belongs to the pesticides of hazard class 3 in accordance (moderately persistent) with the Hygienic classification of pesticides by hazard. Dietary intake of prothioconazole-desthio residues is unlikely to present public health concern. MRLs for prothioconazole (prothioconazole—desthio) and values of pre-harvest interval (PHI) for safety use were recommended.
Key words: pesticide, prothioconazole, residues, field trials

Prothioconazole is a new active ingredient of pesticides produced by Bayer CropScience. This compound belongs to the new chemical class of triazolinthiones and has fungicidal properties. It is a broad-spectrum systemic pesticide to control fungal diseases of crops. It acts on ergosterol biosynthesis, formation, stability and functioning of fungal cell membrane.

There are a lot of prothioconazole-based fungicides have been registered in Ukraine: Lamardor 400 FS, Lamardor PRO FS 180, Yunta Quattro 373,4 FS, Fever 300 FS, Redigo M 120 FS and Scenic 80 FS, Soligor 425 EC, Tilmor 240 EC, Medison 263 SC, Propulse 250 SE, Aviator Xpro 225 EC according to the List of pesticides in Ukraine [1, 2].

These fungicides are used in Ukraine as fungicides for seed treatment and foliar spray application and provide protection to such crops as cereals (barley and wheat), soybean, rape, maize and sunflower.

The purpose of our study was to determine and estimate prothioconazole (prothioconazole-desthio) residues after crops treatment of prothioconazole containing pesticides.

Materials and methods

The different commercial prothioconazole formulation were used. All tested formulations and technical grades prothioconazole were obtained from manufacturer (tabl. 1).

The review and assessment of data concerning prothioconazole and prothioconazole-desthio toxicity was carried out before field trials according to requirement for pesticide state registration in Ukraine [3].

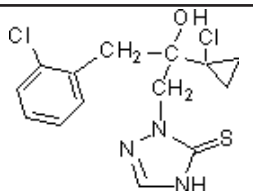
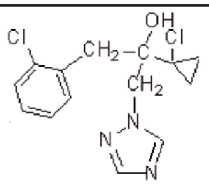
Field trials were conducted in Ukraine during 2002-2013 in two main agro-climatic zones (Steppe and Forest Steppe). Prothioconazole residue decline trials in treated crops were carried out in accordance with the basic principles outlined in the guidelines [4, 5].

Experimental plots received the standard agronomic practices through experimental period. The treatments were carried out by knapsack sprayer equipped with one nozzle. In each trial one application was made with different formulation. The plots were treated at recommended by manufacturer rates of formulations: for seed treatment up to 270 g/1000 kg seed and for foliar spray application from 53-160 g/ha (2 applications) to 225 g/ha (3 applications). Untreated plots were left to serve as control.

Crops sampling was performed from various places of the experimental plots according to the FAO/WHO recommendations and Ukrainian guidelines [4, 5]. Field trials design for each fungicide formulation included several sampling intervals. Samples of crops were taken starting from 1 hour after pesticide application (0 day) up to the harvest day. Within this period we took samples with equally time intervals after treatment. Terms of sampling depended on the timing of

Table 1

Chemistry properties of prothioconazole and prothioconazole-desthio

Common name	**Prothioconazole (JAU 6476)**	**Prothioconazole-desthio (JAU 6476-desthio)**
Chemical name	IUPAC:2-[(2RS)-2-(1-chlorocyclo-propyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2H-1,2,4-triazole-3(4H)-thione	CA: α-(1-chlorocyclopropyl)-α-o(2-chlorophenyl)methyl-1H-1,2,4-triazole-1-ethanol
CAS RN:	178928-70-6	120983-64-4
Structural formula		

application, characteristics of crops vegetation and agro-climatic conditions during the crops growth period. The control samples were taken in each sampling time. After collecting the samples were placed into polyethylene containers and frozen at — 18 °C [6].

Taking into consideration rapid degradation of prothioconazole in plant and the toxicological significance of the metabolites, residue determination of the active ingredient in the crops samples was carried out by prothioconazole-desthio (toxicologically relevant metabolite).

Residues of prothioconazole-desthio in treated crops has been determined using gas-liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) [7-8].

The calculation of half-life (DT_{50}) of prothioconazole in treated crops was conduct based on mathematical modeling using single first-order kinetics [9].

Results and discussion

Based on data analysis of prothioconazole and prothioconazole-desthio toxicological properties, acceptable daily intake (ADI) of 0.001 mg/kg bw was established and approved in Ukraine [10]. The toxicological and hygienic assessment of prothioconazole and its metabolites [3] indicate that the prothioconazole metabolite is prothioconazole-desthio determining the main toxicity properties of active ingredient, and also is the main component of its residues in agricultural commmodities.

Following brand-named formulation were used for crop seed treatment (tabl. 2):

The results of our trials indicated that in grain (seed) of crop grown after seed treatment on the harvest day (83th -116th day) prothioconazole-desthio residues were not determined.

Residues in crops treated by foliar spray application

Cereals: barley and wheat

The data of our experiments demonstrated that after two application of fungicide Soligor 425 EC at rate 53 g a.i./ha in wheat grain prothioconazole-desthio was not found in all studied samples including harvest day (41th days).

After two applications of fungicide Tilmor 240 EC at rate 120 g a.i./ha in barley green plant on the 0 day prothioconazole-desthio residues were 0,52 mg/kg; in barley ear on the 9th days after application active ingredient residues were 0,5 mg/kg. In grain barley on the 20th days after application and on the harvest day (30th days) prothioconazole-desthio was not detected.

The prothioconazole-desthio residues in wheat ear, treated by fungicide Medison 263 SC at rate 160 g a.i./ha (2 applications) were: 0,58 mg/kg 0 day; 0,69 mg/kg on the 7th day and 0,79 mg/kg on the 13th day after application. In wheat grain on the harvest day (30th days) prothioconazole-desthio was not found.

In green plant of wheat after two applications of fungicide Aviator Xpro 225 EC at rate 120 g a.i./ha on the 0 day prothioconazole-desthio residues were 0,68 mg/kg; in wheat ear on the 13th days were 0,11 mg/kg, in wheat grain on the 30th days (harvest day) - were below the LOQ (<0,1 mg/kg). In the second trials with the same condition on the 32th days post-application (harvest day) in wheat grain prothioconazole-desthio residues were also below LOQ.

In wheat grain after three treatments of fungicide Aviator Xpro 225 EC at rate 225 g a.i./ha on the 0 day prothioconazole-desthio residues were 0,33 mg/kg, which were declining during growth period: on the 14th days after application and on the harvest day (28th days) prothioconazole-desthio residues were < LOQ.

Therefore prothioconazole-desthio residues in cereals grain on the 28^{th}-41^{th} days after multiple application were from not detected to <0,1 mg/kg (LOQ).

Table 2

Fungicides used for seed treatment

Pesticides formulation name	Application rate	Treated seed (crop)
Lamardor 400 FS	37,5 g a.i./1000 kg seed	Barley, wheat, maize
Lamardor PRO FS 180	50 g a.i./1000 kg seed	Barley
Lamardor 400 FS	50 g a.i./1000 kg seed	Soybean
Yunta Quattro 373,4 FS	53 g a.i./1000 kg seed	Barley, wheat
Scenic 80 FS	60 g a.i./1000 kg seed	Barley, wheat
Lamardor 400 FS	75g a.i./1000 kg seed	Wheat
Redigo M 120 FS	180 g a.i./1000 kg seed	Maize
Feuver 300 FS	270 g a.i./1000 kg seed	Maize, soybean

The calculated half-life (DT_{50}) values for prothioconazole in cereals ranged from 4,7 to 9,5 days (hazard class 3 according to the Hygienic classification of pesticides by hazard [11]).

Rape

In rape green plant and pods after two applications fungicide Tilmor 240 EC at rate 80 g a.i./ha X 2 on the 20th days after application prothioconazole-desthio residues were 0,1 mg/kg; in pods on the 30th days and in seed on the 50th days after application prothioconazole-desthio was not found.

In green plant rape treated by fungicide Propulse 250 SE at rate 125 g a.i./ha (2 applications) on the 0 day and on the 7th day after application prothioconazole-desthio residues were 0,17 mg/kg and 0,15 mg/kg respectively. In stems and pods rape on the 14th day after application prothioconazole-desthio was not detected. In seed rape on the harvest day (30th days) prothioconazole-desthio residues were 0,08 mg/kg (<LOQ).

Therefore prothioconazole-desthio residues in rape seed on the 30th days after application were from not detected to <0,1 mg/kg (LOQ).

The calculated half-life (DT_{50}) value for prothioconazole in rape was 13 day (hazard class 3 according to the Hygienic classification of pesticides by hazard [11]).

Sunflower

In sunflower green plant on the 0 day treated by fungicide Propulse 250 SE at rate125 g a.i./ha (2 applications) prothioconazole-desthio residues were 0,58 mg/kg; on the 14th day after application prothioconazole-desthio residues were 0,11 mg/kg. In sunflower seed on the 30th day after application prothioconazole-desthio residues were 0,054 mg/kg (<LOQ) and on the harvest day (on the 52th days) active ingredient was not detected.

In plant of sunflower after two applications fungicide Propulse 250 SE at rate 125 g a.i./ha on the 0 day prothioconazole-desthio residues were 0,23 mg/kg, which were declining during growth period: on the 7th and 14th day after — were below LOQ (<0,1 mg/kg) and on the 28th days after application (on the harvest day) — was not detected. In sunflower seed on the 63th days after application (harvest day) active ingredient was not found.

After aviation applications fungicide Propulse 250 SE at rate 125 g a.i./ha (2 applications) in seed of sunflower on the 0 day and on the 14th days after application prothioconazole-desthio residues were below LOQ. On the harvest day (after 28th days application) in sunflower seed prothioconazole-desthio was not found.

Therefore prothioconazole-desthio residues in sunflower seed on the 28th-33th days after application ranged from not detected to <0,1 mg/kg (LOQ).

The calculated DT_{50} values for prothioconazole in sunflower ranged from 4.5 to 14.4 days (hazard class 3 according to the Hygienic classification of pesticides by hazard [11]).

Regulation and control of prothioconazole-desthio residues in soybean, maize, rape and sunflower oils are not required due to fast disappearance of residues and negligible residues on the harvest day.

Based on obtained results of our investigations we recommended and approved [12-14] following maximum residue limits (MRLs) for prothioconazole (prothioconazole-desthio), mg/kg: Cereals — 0,1 (LOQ by HPLC — 0,1).

- Soybean — 0,05 (LOQ by GLC — 0,05);
- Maize — 0,1 (LOQ by HPLC — 0,1);
- Rape — 0,1 (LOQ by GLC — 0,1)
- Sunflower — 0,1 (GLC — 0,1)
- Soybean, maize, rape and sunflower oils — not required

The values of pre-harvest interval (PHI) were rec-

ommended: cereals — 30-35 days, rape — 30 days and sunflower — 50 days.

The possible daily intake of prothioconazole calculated using ADI value and significance in the Ukrainian regional diet of treated crops was lower than allowable level. So use of prothioconazole-desthio fungicides for provide protection to cereals (barley and wheat), soybean, rape, maize and sunflower will not result in a consumer exposure exceeding the toxicological reference value.

Conclusions

1. The obtained data of field trials, which were conducted in Ukraine during 2002-2013 in two main agroclimatic zone, using prothioconazole containing fungicides at the recommended by manufacturer rate, showed that prothioconazole residue in the harvest of treated crops were below limits of quantitation.

2. Results of our own experiments revealed that prothioconazole in terms of degradation rate of in the crops belongs to the pesticides of hazard class 3 in accordance with the Hygienic classification of pesticides by hazard.

3. On the basis of conducted research were recommended MRLs for prothioconazole (prothioconazole-desthio) safety use: cereals — 0,1 mg/kg (HPLC — 0,1), soybean — 0,05 mg/kg (GLC — 0,05); maize — 0,1 mg/kg (HPLC — 0,1); rape — 0,1 mg/kg (GLC — 0,1), sunflower — 0,1 mg/kg (GLC — 0,1).

4. Dietary intake of prothioconazole residues is unlikely to present public health concern.

REFERENCES

1. The List of pesricides and agrochemicals permitted to use in Ukraine: catalog / [compilation by V.O.Yashchuk, D.V. Ivanov, R.M. Krivosheya et.al.] — Kyiv: Univest Media, 2012. –832p.
2. Addition to List of pesricides and agrochemicals permitted to use in Ukraine / [compilation by V.O. Yashchuk, A.P. Koretsky, V.E. Polosenko et.al.] Kyiv: Univest Media, 2013. –400 p.
3. Pesticide residues in food — 2009. — Rome: WHO, 2009. — P. 251–259.
4. Guidelines for hygienic evaluation of new pesticides: approved by MH USSR 13.03.87 №4263–87. — Kyiv, Ministry of Health USSR,1988. — 210 p.
5. Guidelines on Pesticide Residue Trials to provide Data for the Registration of Pesticides and the Establishment of Maximum Residue Limits. — Rome: FAO/WHO, 1986. — 48 p. [http://www.fao.org/AG/AGP/AGPP/Pesticid/].
6. Unified rules of sampling agricultural products, foodstuff and objects of environment for determination of trace amounts of pesticides: approved by MH USSR 21.08.1979 №2051-79 –Moscow: Ministry of Health USSR, 1980. — 40 p.
7. Guidelines for the determination of prothioconazole-desthio in grain cereals by HPLC (high-performance liquid chromatography) №664-2006 from 12.05.06./Grynko AP, Kuznetsova O.M., Shvets M.V. // guidelines for the determination of trace pesticides in food, feed and surrounding environment. — digest №64. –The Ministry of Ecology and Natural Resources of Ukraine, Kyiv, 2009. — P.19–32.
8. Guidelines for the determination of prothioconazole-desthio in corn grain and corn oil by high performance liquid chromatography number 872-2008 on 01.12.08. / [A.P. Grynko, O.M. Kuznetsova, N.V. Tolstova, N.P. Overchenko] // Guidelines for the determination of trace pesticides in food, feed and surrounding environment. — digest №75. –The Ministry of Ecology and Natural Resources of Ukraine, Kyiv, 2011. — P. 179–195.
9. Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration: report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0. –434 p.
10. Additions №46 to DSanPin 8.8.1.2.3.4-000-2001 «Permissible dose, concentration, number on levels of pesticides in agricultural raw, foodstuff, air of the working area, atmospheric air, water reservois, soil approved by Ministry of Health of Ukraine 20.09.01 №137» approved by resolution of main state sanitary doctor of Ukraine №4 from 02.03.2009 — Kyiv, 2009. — 24 p.
11. Hygienic classification of pesticides according to degree of danger: DSanPiN 8.8.1.2.002–98 approved by Ministry of Health of Ukraine 28.09.98 №2. — Kyiv, 1998. — 20 p.
12. Additions №47 to DSanPin 8.8.1.2.3.4-000-2001: Approved by resolution of main state sanitary doctor of Ukraine №7 from 13.03.2009 — Kyiv, 2009. — 14 p.
13. Additions №60 to DSanPin 8.8.1.2.3.4-000-2001: Approved by resolution of main state sanitary doctor of Ukraine №9 from 06.06.2011 — Kyiv, 2011. — 7 p.
14. Additions №61 to DSanPin 8.8.1.2.3.4-000-2001: Approved by resolution of main state sanitary doctor of Ukraine №22 from 15.11.11 — Kyiv, 2011. — 22 p.

Вивчення вмісту залишкових кількостей фунгіциду протіоконазолу в сільськогосподарській продукції

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Резюме. **Мета.** Дослідження та оцінка залишкових кількостей нової діючої речовини пестицидів — протіоконазолу в сільськогосподарській продукції.

Методи. Вивчення динаміки вмісту протіоконазолу-дестіо (основного метаболіту протіоконазолу) було проведено в 2 головних агрокліматичних зонах України протягом польових досліджень фунгіцидів на його основі. Сільськогосподарські культури оброблялись пестицидами з нормами витрат, рекомендованими виробником: для передпосівної обробки насіння — до 270 г д.р./т насіння, в умовах однократної обробки та для обробки рослин в період вегетації — до 225 г д.р./га в умовах трикратної обробки. Залишкові кількості протіоконазолу-дестіо в пробах сільськогосподарської продукції визначались за допомогою хіміко-аналітичних методів газорідинної хроматографії (ГРХ) та високоефективної рідинної хроматографії (ВЕРХ).

Результати. Результати проведених польових досліджень свідчать про достатньо швидке зниження залишкових кількостей діючої речовини. Виявлено, що в період збору врожаю вміст протіоконазолу-дестіо в зерні вивчених культур був нижчим за рівень його кількісного визначення. Проведено разрахунок періодів напіврозпаду досліджуваної сполуки в оброблених рослинах у період вегетації.

Висновки. Протіоконазол за показником деградації в рослинах відноситься до пестицидів 3 класу небезпечності (помірно стійких речовин). Рекомендовані величини МДР безпечного вмісту протіоконазолу в сільськогосподарській продукції.

Ключові слова: протіоконазол, залишкові кількості пестицидів, польові дослідження.

Изучение содержания остаточных количеств фунгицида протиоконазола в сельскохозяйственной продукции

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Резюме. **Цель.** Определение и оценка уровней содержания остаточных количеств нового действующего вещества пестицидов — протиоконазола в сельскохозяйственной продукции.

Методы. Изучение динамики содержания протиоконазола-дестио (основного метаболита протиоконазола) было проведено в 2 главных агроклиматических зонах Украины в 2002–2013 гг в ходе полевых испытаний фунгицидов на его основе. Сельскохозяйственные культуры обрабатывались пестицидами с нормами расхода, рекомендованными производителем: для предпосевной обработки семян — до 270 г д.в. /т семян, однократно и для обработки растений в период вегетации — до 225 г д.в. /га, трехкратно. Остаточные количества протиоконазола-дестио в исследуемых пробах сельскохозяйственной продукции определялись с помощью химико-аналитических методов газожидкостной хроматографии (ГЖХ) и высокоэффективной жидкостной хроматографии (ВЭЖХ).

Результаты. Результаты проведенных исследований свидетельствуют о достаточно быстром снижении остаточных количеств изучаемого вещества. Выявлено, что в период сбора урожая содержание протиоконазола-дестио в зерне изучаемых культур было ниже уровня его количественного определения. Рассчитаны периоды полураспада вещества в изучаемых сельскохозяйственных культурах.

Выводы. Протиоконазол по показателю деградации в растениях относится к пестицидам 3 класса опасности (умеренно стойким веществам). Рекомендованы величины МДУ безопасного содержания протиоконазола в сельскохозяйственной продукции.

Ключевые слова: протиоконазол, остаточные количества пестицидов, полевые испытания.

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