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TOXICOLOGY-HYGIENIC ASSESSMENT AND REGULATION OF NICOSULFURON CONTAINING HERBICIDES FOR MAIZE PROTECTION

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РЕЗЮМЕ. *Мета. Дослідження токсичності нікосульфурон вміщуючих гербіцидів та визначення рівнів залишкових кількостей нікосульфурону в зерні кукурудзи. Методи. Токсичність нікосульфурон вміщуючих формуляцій була вивчена відповідно до рекомендацій OECD. Вивчення динаміки залишкових кількостей нікосульфурону було проведено в 2 головних агрокліматичних зонах України з максимальною нормою витрат до 60 г д.р./га в фазі розвитку кукурудзи 12 — 20 ВВСН. Залишкові кількості (ЗК) нікосульфурону вивчені за допомогою методу ВЕРХ-УФ (межа кількісного визначення 0,01 мг/кг). Результати. Нікосульфурон вміщуючі гербіциди відносяться до малотоксичних речовин. Нікосульфурон небезпечний при потраплянні на слизові оболонки ока. Мутагенна, канцерогенна і тератогенна активність нікосульфурону не виявлені. Експериментальні польові випробування показали швидке зниження ЗК нікосульфурону в рослинах кукурудзи. ЗК гербіциду в зерні кукурудзи не визначались в період збору урожаю. Можливе надходження залишків нікосульфурону з харчовими продуктами не перевищує допустимий рівень. Висновки. Нікосульфурон та гербіциди на його основі відносяться до малотоксичних пестицидів. На базі проведених досліджень рекомендовано знизити величину існуючої МДР. Можливе надходження ЗК нікосульфурону з харчовими продуктами не несе небезпеки для населення.*
Ключові слова: нікосульфурон, оцінка, кукурудза, залишкові кількості.

РЕЗЮМЕ. *Цель. Исследование токсичности никосульфурон — содержащих гербицидов и определение уровней остаточных количеств никосульфурона в зерне кукурузы. Методы. Токсичность никосульфурон — содержащих формуляций была изучена в соответствии с требованиями OECD. Изучение динамики остаточных количеств было проведено в 2 главных агроклиматических зонах Украины с максимальной нормой расхода до 60 г д.в./га в фазу развития кукурузы 12 — 20 ВВСН. Остаточные количества (ОК) никосульфурона изучены с помощью метода ВЭЖХ-УФ (предел количественного определения 0,01 мг / кг). Результаты. Никосульфурон – содержащие гербициды относятся к малотоксичным веществам. Никосульфурон опасен при попадании на слизистые. Мутагенная, канцерогенная и тератогенная активность никосульфурона не выявлены. Экспериментальные полевые исследования показали быстрое снижение ОК никосульфурона в растениях кукурузы. ОК гербицида в зерне не определялись в период сбора урожая. Возможное поступление ОК никосульфурона с пищевыми продуктами не превышает допустимый уровень. Выводы. Никосульфурон и гербициды на его основе относятся к малотоксичным пестицидам. На базе проведенных исследований рекомендовано снизить величину существующей МДУ. Возможное поступление ОК никосульфурона с пищевыми продуктами не представляет опасности для населения.*
Ключевые слова: никосульфурон, оценка, кукуруза, остаточные количества.

SUMMARY. *Objectives. Investigation of nicosulfuron — containing herbicide toxicity and estimation nicosulfuron residue level in maize grain. Methods. Toxicity experiment were conducted on Wistar rats. Nicosulfuron toxicity in acute oral, dermal, inhalation test, dermal and eye irritation and sensitization tests has been studied according to OECD test guidelines. Nicosulfuron residue decline studies were conducted in two main agro-climatic zones of Ukraine at maximum doze rate up to 60 g a.i./ha at maize growth stages from 12 to 20 BBCH. Residues of nicosulfuron were analyzed by HPLC-UV method an LOQ of 0.01 mg/kg. Results. Nicosulfuron was found of low acute toxicity by the oral, dermal and inhalation. Harmful if contact to the mucous membranes of the eyes. Mutagenic, carcinogenic and teratogenic activity has not been revealed. Experimental field trials showed fast dissipation of residues. Herbicides residues in grain were not detectable at harvest time in all studied samples. The possible daily intake of nicosulfuron were significantly lower than allowable level. Conclusion. Nicosulfuron belongs to the substance of low toxicity. On the basis of conducted experiment has been recommended to reduce the existing MRL for nicosulfuron in maize. Dietary intake of nicosulfuron residues is unlikely to present public health concern.*
Key words: nicosulfuron, assessment, maize, residues.

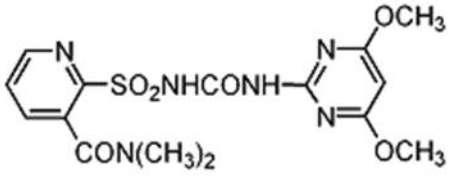
Nicosulfuron belongs to the group of sulfonylurea compounds which are widely used as herbicide. According to the list of pesticide in Ukraine [1], 35 nicosulfuron containing herbicide used for maize protection. It is a systemic substance absorbed by foliage and roots of broad leaved weeds and annual grasses. It inhibits the acetolactate synthase.

Chemical name nicosulfuron:

IUPAC: 2-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-N,N-dimethylnicotin-amide; 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-dimethylcarbamoyl-2-pyridylsulfonyl)urea;

CA: 2-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino] carbonyl]amino]sulfonyl]-N,N-dimethyl-3-pyridinecarboxamide.

Chemical structure:



Molecular formula $C_{15}H_{18}N_6O_6S$. Molecular weight 410.4. Colourless crystals. Melting point 169–172 °C; (tech., 140–161 °C). Vapor pressure <8 x 10^{-7} mPa (25 °C). Kow logP = –1.8 (pH 7). Solubility in water 7.4 g/l (pH 7), acetone 18, ethanol 4.5, chloroform, DMF 64, acetonitrile 23, toluene 0.370, hexane <0.02, dichloromethane 160 (all in g/kg, 25 °C).

A large quantity of nicosulfuron formulation (22) during 2004-2012 year had been studied in Ukrainian Institute of Ecohygiene and Toxicology named Medved L.I.

Wide use of nicosulfuron containing herbicides in Ukraine and presence of harmful toxicological effect make actual the risk assessment of nicosulfuron residues for Ukrainian consumer.

MATERIALS AND METHODS

The objective of the study were to conduct toxicology- hygienic assessment for nicosulfuron-containing herbicide and to determine the residue levels of nicosulfuron in green plant maize and grain.

All tested Formulations and technical grades nicosulfuron were obtained from manufacturer.

Toxicity testing was conducted in Institute of Ecohygiene and Toxicology named Medved L.I. in the range of tests according to requirement for pesticide state registration in Ukraine [2]. All needed test (acute oral, dermal, inhalation toxicity, dermal and eye irritation, skin sensitization) were performed according to OECD test guidelines.

Field trials were conducted in Ukraine during 2004-2012 in two main agroclimatic zone in Ukraine (Polessye and Forest Steppe). Nicosulfuron residue decline studies in maize carried out in accordance to the basic principles outlined in the guidelines [2,14]. Experimental plots received the normal agronomic practices through experimental period. Treatment was carried out by knapsack sprayer equipped with one nozzle. The different commercial formulation nicosulfuron (WG, CS) was used. The plots were treated at maximum dose rate recommended by manufacturer up to 60 g a.i./ha. Maize growth stages were from 12 to 20 BBCH. Untreated plot was left to serve as control.

Sampling was performed from various places of the experimental plots according to the FAO/WHO recommendations and Ukrainian guidelines [2,14]. Samples of maize plants were taken 1 h after pesticide application. Subsequent samples were taken 3,6,9,12,30 and 90 days after treatment. During experiment, a control sample was taken in each sampling time. After collecting the samples were placed into polyethylene containers and frozen at — 18°C.

Residues of nicosulfuron were analyzed by HPLC-UV method evaluated for the determination of parent nicosulfuron in different plant matrices an LOQ of 0.01 mg/kg. Also we used the results of research presented by manufacturers and literature data [6-13].

RESULTS AND DISCUSSION

Results of our own research and literature data [3-5] showed that nicosulfuron in accordance to the Hygienic classification of pesticides by hazard [17] belongs to the pesticides of hazard class 4 (acute oral and dermal toxicity, primary skin irritation, sensitization effect,): to pesticides hazard class 3 (acute inhalation toxicity), to pesticides hazard class 2 (primary eye irritation). Toxicity class limit for nicosulfuron — 3 (harmful if contact to the mucous membranes of the eyes). Nicosulfuron was found to be of low acute toxicity by the oral, dermal, and inhalation routes.

Nicosulfuron is absorbed rapidly but only to a limited extent (about 40%) in the rat following oral administration. It is widely and uniformly distributed in the body and is excreted mainly via faeces (63 — 73%) and urine (23 — 28%). The half life — 12-24 hours. It is largely excreted unchanged (70 – 86 %) [3-5]. In chronic experiments no observed effect level (NOEL) for nicosulfuron were set for dogs males 147 mg/kg and females 587 mg/kg; for mice male — 199,3 mg/kg and females — 1259 mg/kg; for male rats — 199,3 mg/kg and females 254,4 mg/kg [3-5,10].

No mutagenic activity was observed when tested in four strains (TA97A, TA98, TA100, and TA1535) of Salmonella typhimurium. In vitro chromosomal aberration tests in cultured human lymphocytes indicated negative responses at the concentrations from 40 to 470 ug/ml. Nicosulfuron assayed with or without metabolic activation in vitro in Chinese Hamster Ovary (CHO) cells was nonmutagenic at the concentrations from 4 to 465 ug/ml and a micronucleus assay in mouse bone marrow cells was negative at dose levels from 500 to 5000 mg/kg. An unscheduled DNA synthesis study in rat hepatocytes did not cause any DNA damage in rat hepatocytes at the concentrations from 0.04 to 470 ug/ml. Carcinogenic and teratogenic activity of nicosulfuron also has not been revealed [3-5,10].

Approved in Ukraine nicosulfuron acceptable daily intake (ADI) — 2.0 mg/kg.

In the soil (depending on the content of organic matter, the amount of absorbed bases, pH, moisture, density and mechanical composition of soils) nicosulfuron half-life was 4-20 days. According to accepted in Ukraine approaches and in according to [17] nicosulfuron belongs to pesticides of 3rd class of hazard according to the stability criteria in the soil. The main degradation product of nicosulfuron in the soil is amino-2-sulfamoyl-N,N-dimethylnicotinamide (ASDM) [6-13].

According to criteria of soil migration nicosulfuron belongs to pesticides of 3rd class of hazard. The main metabolite — ASDM belongs to the 2nd class of hazard according to criteria of soil migration.

Field evidence indicates that nicosulfuron remains within the top 50 cm of the soil after application, and its use will not result in significant groundwater contamination.

In water nicosulfuron hydrolytically stable at pH 7; 9 and the temperature of 25°C. The half-life at pH 5 is 18 days. The main degradation product in the aquatic environment is 2-amino-4,6-dimethoxypyrimidin (ADMP). Under photolysis conditions T50 of nicosulfuron at pH 9.1; 7,3 and

5.0, was respectively 69, 46 and 9 days. The main product of the degradation is also ASDM. In the dark under the same conditions T50 — 136, 69 and 53 days respectively. [6-13].

Our research devoted to stability of nicosulfuron in water at pH 7-7,2 and temperature 16-18 °C revealed half-life 18,88 days. According to it nicosulfuron belongs to 1 class of hazard.

Metabolism of nicosulfuron was investigated for foliar application on maize, using 5-14C-pyrimidinyl and 2-14C-pyridyl labelled nicosulfuron. Metabolism of nicosulfuron in plants proceeds in two ways: hydrolysis of sulfonylurea bonds, resulting in the formation of pyridynsulfonamid and pyrimidinamine and hydroxylation of 5 pyrimidin ring followed by conjugation.

In maize, 102 days after application, the highest TRR was identified in straw (0.082–0.13 mg eq./kg), whereas in grain the TRR was low (0.001-0.003 mg eq/kg) for pyridyl and pyrimidinyl labels respectively. In both studies, nicosulfuron was the predominant residue for all harvest times, accounting for 29 % (0.03 mg eq/kg) and 54 % TRR (0.06 mg eq/kg) 102 days after treatment for pyridyl and pyrimidinyl labels respectively [6-13]. None of the identified metabolites was considered to be toxicologically significant. Therefore it was concluded that the residue definition in maize could be limited to nicosulfuron for risk assessment and monitoring purposes.

Field trials on maize in the USA have shown that if the pesticide used with a maximum rate — 70 g of active substance/ha on 45-128 day residues of nicosulfuron in grain and green plants of maize were not detected. The limit of determination was 0.05 mg/kg. Nicosulfuron residue also not found in the different maize factions after processing [6-13].

In Ukraine in accordance to [18] for nicosulfuron were recommended and approved following hygienic standards:

MRL, mg/kg of maize — 0,2 (LOQ by HPLC — 0,01), maize (oil) — not required. SRLI in the air of the working zone, mg/m^3 1,0 (LOQ by HPLC — 0,01). SRLI in ambient air, mg/m^3 0.01 (LOQ by HPLC — 0,008). MAC in water, mg/dm$^{3:}$ 0,001, (LOQ by HPLC to 0.0005). APC in the soil, mg/kg: 0,2 (LOQ by HPLC — 0,01).

Results of our own experiments showed that nicosulfuron containing herbicides in accordance to the Hygienic classification of pesticides by hazard [17] belongs to the pesticides of hazard class 4 (acute oral and dermal toxicity, primary skin irritation, sensitization effect,): to pesticides hazard class 3 (acute inhalation toxicity, primary eye irritation). Toxicity class limit for nicosulfuron containing herbicides – 3.

For assessing real quantity of residues in agricultural commodities we studied dissipation of nicosulfuron in agro-climatic conditions of Ukraine. Field trials on maize were conducted with different nicosulfuron formulation. In each study one application have been made with different formulation at rates up to 60 g a.i./ha. Field trials was conducted in two agro-climatic zones of Ukraine (Polesye and forest-Steppe) at maize growth stages from 12 to 20 BBCH. In 2004-2011 the plan of studies included sampling of plant, starting from the day of application until the day of harvest. Field design of studies for each herbicide included from 4 to 6 sampling intervals. Along with this, we study the samples collected near the harvest of treated maize. In 2012, taking into account the results of the previous studies, the number of sampling interval had been reduced to two.

Our experiments showed that after application of herbicides the nicosulfuron residues in green plant at day 0 ranged from 0.06 mg/kg to 1.6 mg/kg. In three cases, nicosulfuron residues founded in green plants on the 3rd day after application were 0.012 mg/kg, 0.09 mg/kg and 0.43 mg/kg correspondingly. In one case nicosulfuron had been found in green maize plants after 7th day of application. On the 9's and 10-days after application nicosulfuron did not found in green plants and maize cobs.

Herbicides residues in grain and straw were not detectable at harvest time in all studied samples. In the soil of the treated areas near the harvest time nicosulfuron residue also were not found.

As quantifiable residues of nicosulfuron were not revealed in the treated crops , therefore investigation the effect of industrial and/or household processing is no needed.

Taking into account toxicology and hygienic assessment of nicosulfurone herbicides and obtained results of nicosulfuron residues in maize plant we recommend to reduce the existing MRL of nicosulfuron in maize from 0,2 mg/kg to 0.01 mg/kg (LOQ by HPLC — 0.01 mg/kg). Also we recommend to decrease the number of sampling interval from 4-6 to two (cobs and grains of the harvest).

Regulation and control of nicosulfuron residues in maize oil is not needed due to application of herbicides at early stages of maize, fast disappearance of residues, negligible residues at harvest time and low lipophylity of nicosulfuron. Pre-harvest interval (PHI) also is not needed. The possible daily intake of nicosulfuron for Ukrainian regional diet were significantly lower than allowable level (0.01 %). Use of nicosulfuron herbicides for maize protection will not result in a consumer exposure exceeding the toxicological reference value.

CONCLUSIONS.

1. Toxicity class limit for nicosulfuron and for nicosulfuron containing herbicides – 3.

2. According to stability and migration criteria in soil, nicosulfuron belongs to the pesticides hazard class 3.

3. On the basis of conducted research had been recommended to reduce the existing MRL for nicosulfuron in maize from 0,2 mg/kg to 0.01 mg/kg (LOQ by HPLC — 0.01 mg/kg).

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

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