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Metazachlor residues in soil and rape seed

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Abstract: The purpose of the work was to analyse metazachlor contamination of the soil and metazachlor contamination of rape seeds. Monitoring tests were carried out during the 2010–2012 time period, on winter and spring oilseed rape fields located in south-western Poland. Soil and seed samples were collected at harvest time. The determination of metazachlor residues was conducted using gas chromatography with electron capture detection (GC/ECD). Based on the analysis of a total of 59 soil samples and 59 rape seed samples, metazachlor residue was detected in 45% of the soil samples of winter oilseed rape and in 71% of the soil samples of spring oilseed rape. Metazachlor contamination of rape seed was detected in 29% of winter rape samples and in 53% of spring rape samples. The concentration of assayed residue did not exceed 0.0005–0.0102 mg/kg. There were significantly higher amounts of metazachlor residue determined for the soil and seed samples of spring oilseed rape. None of the analyzed samples of oilseed rape seed showed a residue content exceeding the Maximum Residue Level (MRL).

Key words: metazachlor, MRL, residue monitoring, spring and winter oilseed rape

Introduction

Oilseed rape (*Brassica napus* L.) belongs to the most important crops in Poland. This plant provides a considerable source of raw material for the oil and fat industry as well as the energy industry. The cultivation involves mainly winter oilseed rape. Its spring cultivars have always been only marginally significant, since they are low yielding (Muśnicki *et al*. 1997). Spring oilseed rape was often cultivated as a pre-sowing plant after frost damage in winter oilseed rape. These days, due to plant breeding development and improvement in the cultivation regime, interest in the spring form has been growing (Murawa and Warmiński 2005). One of the basic factors influencing high yielding of oilseed rape is appropriate cultivation technology, including protection against pests. Insects cause considerable losses in oilseed rape (Lemańczyk *et al*. 1997; Murawa and Warmiński 2004). Protection of rape against weeds is equally important (Franek 2000). Metazachlor belongs to those major substances recommended for weed control, both in winter and spring oilseed rape cultivation. Metazachlor constitutes an active ingredient (independent or as a component of a mixture) of numerous herbicides.

Metazachlor – 2-chloro-N-(pyrazol-1-ylmethyl)acet-2′,6′-xylidide belongs to the class of chloroacetanilide herbicides. This herbicide acts as an inhibitor in lipid biosynthesis. Metazachlor affects cell division and tissue differentiation. It is applied as a herbicide pre-emergency and early post-emergency to control winter and annual grasses and broad-leaved weeds (Rouchaud *et al*. 1992). It is absorbed through the hypocotyls and roots. Metazachlor half-life (DT_{50}) in different soil types ranged from 1 to 2 months under laboratory conditions (Walker and Brown 1985; Allen and Walker 1987).

New EU regulations (Directive 2009/128/EC) in plant protection are aimed at a national reduction in pesticide application. New strategies include developing methods suitable to local conditions. The use of chemicals is to be reduced while safeguarding desired effectiveness.

The research on pesticide residues assesses the effect of applied chemicals on the environment and human health (Łozowicka *et al*. 2012a; Nowacka *et al*. 2012). Moreover, long-term monitoring allows for the analysis of the consequences of the introduced changes, and for choosing the best methods minimizing the risk resulting from the use of chemical plant protection.

The purpose of this work was to investigate metazachlor contamination of soil and rape seeds based on the monitoring tests done on winter and spring oilseed rape fields located in south-western Poland.

Materials and Methods

Monitoring research was conducted in 2010–2012, on fields of oilseed rape. In three growing periods, shortly before harvest, the samples of soil and rape seed were collected from 59 cultivated fields (42 – winter oilseed rape and 17 – spring oilseed rape). Interviews were conducted among the field owners, who stated that in most cases, the previous crops were cereals (mainly wheat and winter

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barley). The fields were established on different soils (pH = = 5.7–6.4, C_{org} = 1.1–1.9%). The size of the controlled rape fields was diverse and ranged from 4 to 12 ha. On selected fields, the farmers applied herbicides containing metazachlor. Herbicide treatments and introduced amounts were done according to instructions regarding the terms and the doses set up by herbicide producers. Herbicides were applied before oilseed rape emergence and shortly after its emergence. Winter oilseed rape was sown at the turn of August to September, while spring oilseed rape – at the turn of March to April. The harvest took place from the middle of July to the first days of the third decade of August.

All collected soil and rape seeds samples were examined to detect any metazachlor residue. Each soil and seed sample was analyzed three times. Repeatability of the analytical results was satisfactory, with relative standard deviations (RSDs) not exceeding 5% of the mean values.

Samples taken from each field were well mixed and stored in polyethylene bags at –20°C until sample extraction was to be done. The analytical procedure consisted of three elementary processes:

1) Extraction of the analyzed substance from the matrix. Soil samples were homogenized, and then passed through a 2-mm sieve. The rape seeds were ground in a blender. Soil or seeds portions (3 × 10 g) were mixed with 3 × 2.5 g of Diatomaceous Earth (Dionex® ASE® Prep DE) and transferred in stainless steel cells. Extraction was done by Accelerated Solvent Extractor Dionex ASE 350 (Dionex®, CA, USA) [extraction solvent – acetone (33 ml per cell for two cycles of extraction), temperature 40°C, extraction time – 20 min, and pressure – 0.2 MPa]. The combined extracts (from three cells) were then slowly evaporated under a nitrogen stream until dry.
2) Cleaning of the extract.
Dry residue from the extraction was dissolved with 30 ml of water and subjected to SPE (Solid Phase Extraction) column (capacity – 3 ml, sorbent bed – 0.5 g of octadecyl, 40 μm) (Bakerbond®, J.T.Baker®, Philipsburg, NJ, USA). A cartridge of SPE was preconditioned with water (3 ml) and then methanol (3 ml). A thirty ml extract solution was loaded (small portions) onto the cartridge (wet sorbent bed) and the eluate was discarded. Analytes were eluted with 2 ml acetone (injection solvent).
3) Final determination.
A gas chromatography Varian CP 3800 equipped with software Varian GCMS 2000 and an electron capture detector (Varian®, CA, USA) were used to carry out the final determination. Throughout the entire experiment, a VF-5ms capillary column (30 m × 0.25 mm × × 0.25 μm film thickness) (Varian®, CA, USA) was used. Nitrogen was used as the carrier gas at a flow rate of 1.5 ml/min. Chromatographic separation was performed at the column oven where the initial temperature was held at 120°C for 7 min. The temperature was then ramped at 10°C/min to 200°C and held 17 min, and finally programmed at 5°C/min to 230°C which was held for 20 min. Injector and detector temperatures were set at 230 and 300°C, respectively. Aliquots of 1 μm of the samples were injected.

The recovery of metazachlor from soil and rape seed was determined by analysing fortified samples. Analysation was carried out at four concentration levels in three replicates. The obtained results (overall and per fortification level) are detailed in table 1. The quantification limit of the method was 0.0002 and 0.0005 mg/kg for 30 g of soil and seed rape sample, respectively.

Table 1. Recoveries of metazachlor residues

Sample	Fortification level [mg/kg]	Average recoveries [%]
Soil	0.0005	100.2
	0.001	90.1
	0.01	92.3
	0.1	87.5
Rape seed	0.0005	79.6
	0.001	90.3
	0.01	87.2
	0.1	85.7

Results and Discussion

Within the three-year research period, 59 soil samples and 59 samples of rape seed were subjected to analysis. Metazachlor residue was detected in 19 and 12 soil samples (winter and spring oilseed rape, respectively), which provided for 45.2 and 70.6%, respectively, of all the examined samples. As far as the seed was concerned, these values equaled 12 and 9 (28.6 and 52.9%, respectively) (Table 2).

Table 2. Number of samples and range of detected residues

Residues	Winter oilseed rape	Spring oilseed rape
soil		
Number of samples	42.0	17.0
Samples with residue	19.0	12.0
% of samples with residue	45.2	70.6
Range of detected residue [mg/kg]	0.0010–0.0098	0.0018–0.0232
seed		
Number of samples	42.0	17.0
Samples with residue	12.0	9.0
% of samples with residue	28.6	52.9
Range of detected residue [mg/kg]	0.0005–0.0064	0.0008–0.0102

When comparing the obtained results with the previous data attained for seed of winter oilseed rape, it is possible to state that the percentage share of the samples contaminated with herbicide residue did decrease considerably. In the research conducted in 2000–2008, (Kucharski and Domaradzki 2009) in the area of south-western Poland, over 160 samples of winter oilseed rape were examined. On average, metazachlor was found in 43% of the samples, which, compared to the obtained results, proved that the percentage share of samples with

residue diminished approximately by 40%. Similar results were noticed when determining the concentration of the examined residue. In this research, the concentration range was from 0.0005 to 0.0064 mg/kg, while in a previous investigation the residue value reached a maximum of 0.0094 mg/kg. Because of the wider range of the previously conducted research (9 years and fourfold number of samples) it is difficult to compare obtained results, yet a downward trend can definitely be noted. Such a noticeable tendency seems to show that farmers do follow the instructions by herbicide producers and the latest programs of oilseed rape protection. Yet the previous results regarding metazachlor residue in the soil, do not seem to confirm these findings (Kucharski and Sadowski 2011). On winter oilseed rape fields, in soil samples collected from 0–15 cm at the time of harvest, there was 0.0072–0.0108 mg/kg of detected metazachlor residue. In both experiments, the maximum quantity of herbicide residue reached similar levels.

On the basis of the research, it is possible to state that the frequency of detected residue and its value are significantly higher in the case of spring oilseed rape (soil and seed). If the treatments take place in the spring, the period from herbicide application to the harvest is about by 6 months shorter. This means the active herbicide ingredient did not get a chance to decompose to the degree it reached in the case of the treatment done in the autumn. The period of autumn application is long enough to expect that chemical analysis will not show any herbicide residue in the soil and in seed, or that their concentration will remain at the limit of detection for the analytical method.

None of the examined samples showed a herbicide residue concentration of a similar value or one exceeding the permissible level (MRL – Maximum Residue Level). For metazachlor, MRL was 0.1 mg/kg (Regulation 396/2005), while maximum values obtained in this research did not exceed 0.0064 mg/kg for winter rape seed, and 0.0102 for spring rape seed.

The research results involving metazachlor residue in oilseed rape seed, prove that the use of herbicides will not result in a threat to humans or the agricultural environment (in relation to accepted standards). Nevertheless, it should be stressed that all the samples originated from the fields where each cultivation treatment and herbicide application was controlled, and the instructions by the producer had been followed. It is not possible to exclude sporadic incidents exceeding permissible values. Such cases are usually caused by a lack of knowledge on the farmers part, spraying devices which are in bad condition, exceeding the recommended dosage, the use of fake herbicides or a situation where the farmer purposely damages his/her field because they are counting on being compensated (Sadowski and Kucharski 2005). On the basis of claims connected with the assessment of the results regarding the flaws mentioned above, it is possible to state that exceeded standards of concentration occur in 10–20% of cases, *i.e.* 8–15 samples per year.

Investigations which monitor plant protection chemical residues in crops are carried out on a considerably larger scale by appropriate auditing units (Łozowicka *et al*. 2012b; Nowacka *et al*. 2012). Such investigation, though, differ methodologically and mainly deal with fungicides and insecticides. The samples of most fruit and vegetables sent for control do not possess any records specifying which pesticides were applied. The assay of residue is conducted according to multi-residue methods, which allow for the determination of several tens of substances in one analysis. Compared, though, with the single-residue methods used in our work, the above mentioned multi-residue methods are characterized by a lower limit of quantitation, typically at the level of 0.01 mg/kg (Grzegorzak *et al*. 2012). The differences in both the range and research methodology make the direct comparison of the results impractical.

Moreover, based on the surface and groundwater results of the long-term monitoring research from the south-western part of Poland, it could be stated that metazachlor residues were only found twice in surface water (0.0001 and 0.00008 mg/kg) (Sadowski and Kucharski 2006).

Conclusions

Metazachlor residue was detected in soil and seed samples (winter and spring oilseed rape). Significantly higher amounts of metazachlor residues were determined for the soil and seed samples of spring oilseed rape. None of the analyzed samples of oilseed rape seed showed residue contents exceeding the MRL permitted values. Based on water monitoring research and observations of fields, it was not proved that metazachlor had any negative influence on the successive crops or on surface and groundwater contamination of the south-western Poland.

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