QUANTITATIVE ASSESSMENT OF ALACHLOR WITH ELISA TEST IN WATER USED IN MEAT TECHNOLOGY

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Background

Alachlor is a pesticide i.e,a herbicide, of a chlorine-acetanilide molecular $C_{14}H_{20}ClNO_2$ (Mr = 269,77) and the following structural formula:

$$CH_3\text{-}C = CH - CH = CH - C(CH_2CH_3) = CN(COCH_2Cl) - CH_2OCH_2CH_3$$

It is a herbicide that has effects on the narrow-leafed roots of genus *Graninea* (at the stage of germ) and serves for protection of crops: corn, soybean, sunflower and vegetables. It can be used individually, but it is more often used in combination with other herbicides-the triazines in particular. The time of currence is limited to the time of change (from the sowing to the pisking time). Maximum allowed concentration of alachlor in water is 0,1 ng/l, and 0,020 mg/kg in food products.

Objectives

Water is an important factor in the meat production technology and it influences the quality of meat products. The meat production lines, based on water needs, are supplied from various sources: city water plants, local water plants, as well as from some alternative sources such as superficial and deep wells. Since there is a frequent case of contact between agricultural soil (treated by herbicides-alachlor) - and natural sources of water-the alachlor residues may appear in the water. The authors have defined the objective of this paper to that end. The assessment of the content of alachlor residue in the water used for meat processing (in five production lines), was obtained from alternative sources. Sampling of the water has been done over the period from April until November 2001.

Methods

Quantitative assessment of the alachlor residue content in the water used in meat processing technology has been done with ELISA test at water samles from localities of Sarajevo Canton. A total of 20 water samples taken from five alternative sources have been analyzed.

1. *The test principles:*

The Alachlor Plate Kit uses polyclonal antibodies, which bind both alachlor compounds and the alachlor enzyme conjugate. Alachlor in the sample competes with the alachlor enzyme conjugate for a limited number of antibodies' binding sites. Antibodies that bind alachlor compounds are immobilized to the inside of the test wells. Since the same number of antibody binding sites are available in every well, each well receives the same number of alachlor enzyme conjugate molecules, a sample containing a low concentration of alachlor allows the antibody to bind many alachlor enzyme conjugate molecules. The result is a dark blue solution. Color is inversely proportional to alachlor concentration.

2. *The performance Characteristics:*

The Alachlor Plate Kit test does not differentiate between the related acetanilide residues, but detects their presence to differing degrees. The following table shows the value for 50 % Bo* and the approximate value for 90 % Bo which is a Lower Limit of Detection (LLD). All concentrations are in parts per billion (ppb). Table 1.

3. *The assay procedure:*

a) Alachlor was extracted from water samples with 5 ml of the dissolving solution petroletar/cyclohexane (1:1), per one liter of sample.
b) The extract was concentrated in the apparatus according to Kundern-Danisch.
c) In three parallel Plate Kit Test wells there were dosed per 80µl of the negative probe (0,00 ppb alachlor), 80µl of each calibrator (and 0,10ppb, 0,20ppb, 0,30ppb, 0,50ppb, 1,0ppb, 1,5ppb and 2,5ppb alachlor) and 80µl of each of the samples of water being tested.
d) By applying the same sequence of adding, each well was added two drops (~ 80µl) of Alachlor-Enzyme Conjugate.
e) After thorough mixing of the content of the well over one minute period, the plate was incubated at room temperature for a period of one hour.
f) The content of the well was then rinsed with cold running water 5 (five) times and the remaining water was thoroughly shaken off, but without any mechanical contact with the content of the wells.
g) According to the usual procedure 80µl of substrate and 40µl of chromogen were added to the wells.
h) The content of the wells was thoroughly mixed and incubated for additional 30 minutes at room temperature.
i) Each well was added 40µl of the stop-solution (5,0mol/l H_2SO_4).

4. *Spectrophotometric Measurement:*

Optical density of the generated blue coloring was simultaneously measured by cpectrophotometer "Idexx", USA product, at wavelength $\lambda = 450nm$ (with referential $\lambda = 650nm$). Nullification of the apparatus was done according to the air.

5. *Calculation:*

a) After measuring of the absorbency values for each well and calculating of the mean values-three for each sample-the negative control, the calibrators, and the analyzed water samples, we started calculating the values of % Bo, according to the following formula:

$$\%\ Bo = \frac{\text{average OD of calibrator or water sample}}{\text{average OD of negative control}} \times 100$$

OD - optical density
b) The calibration curve for values of % Bo of each calibrator was drawn, depending on the given alachlor concentrations (% Bo = f(conc).
c) The values of alachlor concentrations in the examined water samples were read.

Results and discussion

Through the analysis of 20 samples of water which was used in meat technology-taken from five production lines at the locality of Sarajevo Canton-in two samples there was determined an elevated concentration of alachlor, table 2. Alachlor concentrations varied between < 0,05ng/l and 0,14ng/l. The linear dependence of parameter % Bo of alachlor concentration was determined in the area of concentration ranging between 0,1ng/l, with linearity herein getting lost. Graph 1. The results were presented as value of < 0,05ng/l since the lowest measured concentration of calibrator was 0,1ng/l.

Conclusions

1. During the experiment the ELISA-test proved highly sensitive analytical method for detection of alachlor residua in water.
2. Insufficient differentiation of methods between similar acetanilide compounds overcomes with a varying sensitivity values, table 1.
3. Elevated content of alachlor residua in two samples of water was determined during the time of active agricultural activity (June 2001) and we therefore suggested the users of these water sources to abandon them.

Pertinent literature

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Table 1. Detection limits of acetanilide residues

Compound	90% Bo (LLD) = (ppb)	50% Bo
Alachlor	0.046	0.60
Alachlor ESA	0.13	1.1
Metalaxyl	10	> 1000
Acetochlor	2.5	36
Metalochlor	0.6	40
2-[(2,6-diethyl-phenyl) (methoxymethyl)amino]-2-oxoethane sulfonic acid	0,08	1.06

Graph 1.

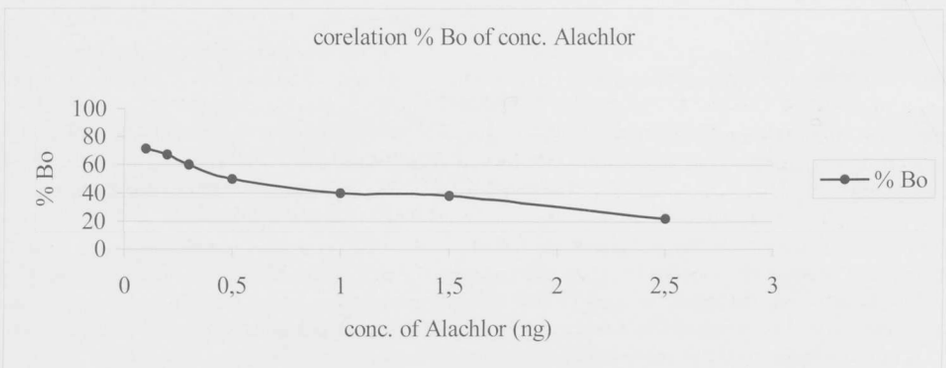

Table 2. Amount of alachlor in controled water samples

Department	Sample	Amount of alachlor ng/l	Department	Sample	Amount of alachlor ng/l
A	1	< 0,05	C	1	< 0,05
	2	< 0,05		2	< 0,05
	3	< 0,05		3	< 0,05
	4	< 0,05		4	< 0,05
	5	< 0,05		5	< 0,05
B	1	< 0,05	D	1	< 0,05
	2	0,14		2	< 0,05
	3	0,12		3	< 0,05
	4	< 0,05		4	< 0,05
	5	< 0,05		5	< 0,05