ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 11 2009

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ASSESSMENT OF SELECTED PESTICIDES CONTENT IN FOOD PRODUCTS IN ACCORDANCE WITH POLISH LAW IN FORCE

OCENA ZAWARTOŚCI WYBRANYCH PESTYCYDÓW W PRODUKTACH SPOŻYWCZYCH ZGODNIE Z OBOWIĄZUJĄCYM W POLSCE PRAWODAWSTWEM

Abstract: The tests covered the determination of the residue content of pesticides belonging to the groups of pyrethroids and dithiocarbamates in tomatoes and strawberries. Vegetables and fruits coming from the area of the Lower Silesian Province were tested. On the basis of the test results it has been established that: in none of the samples subjected to the chemical analysis for the dithiocarbamate residue content any exceedance of MAR defined in the Health Ministry Order of 16th May 2007 concerning the maximum allowable pesticide residue levels in foodstuffs or on their surface was found; in none of the samples subjected to the chemical analysis for the pyrethroid content any residues of: bifenthrine, cypermethrin, deltamethrin were found.

Keywords: insecticides, fungicides, toxicity, maximum allowable levels

Plant protectants contribute to better and higher-quality crops. However, their use in agriculture entails the risk of soil and surface water contamination. They also disturb the ages-old natural balance and can be toxic if used in larger quantities [1–3].

The increasing pollution of the natural environment with harmful compounds calls for periodic investigations and continuous monitoring. This is so because of the unintended (industry, transport, urbanization) and intended (agriculture) effects of human activity on the natural environment whose integral part are plant and animal food resources [4].

Besides the food nutritional value, food safety is a principal factor having a bearing on human health. Therefore food monitoring is essential in order to evaluate food quality with regard to the health of a population. Food quality monitoring is critical for assessing the exposure of the population to pesticide residues in foodstuffs. Pesticide

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tests indicate the pesticides which occur in the largest amounts in food, and the food products which most often contain their residues. It is necessary to monitor the residues of chemical plant protectants in food also because of their toxic action and common use in agriculture [5, 6]. According to the current concepts behind the various ecological programmes (HACCP, Sustainable Development, Good Agricultural and Veterinary Practice), it is crucial to determine the life cycle of products (pesticides in this case). By monitoring the content of pesticides in plant products one can assess the health safety of prospective consumers [7–9].

Pesticides

Pesticides are a widely used and so far the most effective way of protecting crops and plant products against pests. Besides having a beneficial effect on plant production such compounds may have adverse side effects due to the fact that generally they have toxic properties. The aim of determining the permissible levels of pesticide residues in the total mass or on the surface of foodstuffs is to protect the health of consumers [10–15].

Chemical substances which are to be used as pesticides should be characterized by:

– selective toxicity (high toxicity towards pests and low toxicity towards the other organisms),

– persistence in the environment (a time of residence in the environment sufficiently long for pest destruction),

– high degradability (once the function of a pesticide is fulfilled, it should quickly decay),

– no tendency to bioaccumulation in animal or plant organisms.

Pesticides are classified according to different criteria, the most important of which are: the application direction and mode of action, the chemical composition and toxicity.

According to toxicity, pesticides are divided into 5 classes. Class affiliation depends on LD_{50}, ie a lethal dose expressed in milligrams of a toxic substance per kilogram of body mass, which after a single administration causes death of 50 % of the tested population of animals. This applies to tests on animals and to acute toxicity determination. The classes of pesticide toxicity to mammals, being in force in Poland, are shown in Table 1 [16].

Table 1

Classes of pesticide toxicity to mammals, being in force in Poland [16]

Toxicity class	LD_{50} [mg/kg body mass]	Degree of hazard
I	up to 50	poisons
II	51–150	poisons
III	151–500	harmful substances
IV	501–5000	harmful substances
V	above 5000	practically harmless

With regard to their action, pesticides are divided into:

1) zoocides – animal control products:
   – insecticides – for the control of insects,
   – rodenticides – for the control rodents,
   – molluscicides – for the control molluscs (slugs and snails),
   – nematicides – for the control nematodes,
   – larvicides – for the control larvae,
   – aphidicides – for the control aphids,
   – ovicides – for the control insect and mite eggs;
2) fungicides – for the control fungi and oomycetes;
3) herbicides – for the control weeds;
4) growth regulators – stimulating or inhibiting plant life processes:
   – defoliants – removing leaves,
   – desiccants – removing moisture,
   – deflorants – removing excessive flowers;
5) acaricides (miticides)– for the control mites;
6) algicides – for the control algae;
7) bactericides – for the control bacteria.

Environmental persistence is the most decisive factor as regards the range of application of pesticides. As concerns their environmental persistence pesticides have been divided into four groups, as shown in Table 2.

Table 2

Division of pesticides with regard to their environmental persistence [16]

Group	Environmental persistence
Highly persistent	above 18 months
Persistent	up to 18 months
Non-persistent	up to 6 months
Fast decaying	up to 3 months

The pesticide residue level in crops depends on the pesticide decomposition time. The amount of the remaining pesticide must be reduced to zero or to a level harmless to people [17]. Pesticides are characterized by high biological activity and may have side effects, especially when introduced in higher doses into soil. Surface application pesticides do not penetrate deep into plants, remaining only on their surface. Some may penetrate deeper into plant tissues. The action of other pesticides is systematic – via the root system they penetrate the whole of the plant.

Chemical plant protectants when administered in the doses recommended by the manufacturer have only slight side effects which quickly disappear. Only when the dose is increased several times, the disturbances are stronger and longer lasting [14].

Insecticides

Insecticides are the most popular plant protectants used to kill insects. This group includes derivatives of chlorinated hydrocarbons. Since the latter do not undergo quick decomposition their accumulation in the environment is particularly dangerous. The half-life of chlorinated hydrocarbons in soil and the time of their activity against some pests are counted in years. The compounds are well soluble in the body where they are retained for a long period of time. The toxic residues of chemical plant protectants, taken up in small doses over a longer period, contribute to chronic poisonings. Since the substances accumulate slowly in the body, the poisonings progress latently without showing any pathological symptoms for years.

Synthetic pyrethroids belong to the group of insecticides which are derivatives of chrysanthemum acid, characterized by a wide spectrum of action against insects. Their toxicity to mammals is relatively low in comparison with, for example, phosphoorganic insecticides or carbamates. They have been passed as fit for use in the protection of vegetables, potatoes, fruits, plants grown for industrial purposes and forests. This wide range of their use creates a danger that they will be retained in plant and animal foodstuffs. Pyrethroids are generally characterized by considerable environmental persistence, high insecticidal activity and low toxicity towards warm-blooded organisms. Their drawback is low stability and quick decomposition under the influence of external factors, particularly light, whereby they are used only for pest control in closed spaced. In the 1970s light-resistant pyrethroids, such as cympermethrin and fenvalerate (Fig. 1), were produced [19].

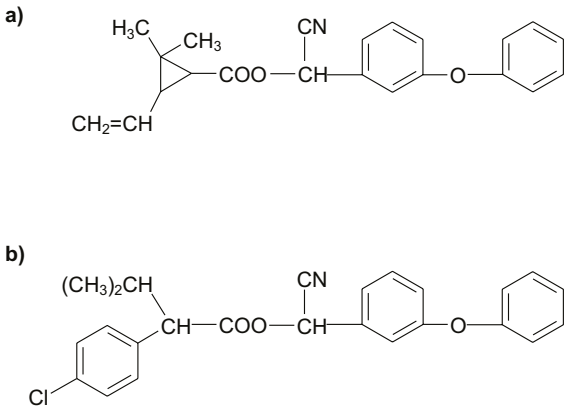

Fig. 1. Light-sensitive pyrethroids: a) cympermethrin (LD_{50} = 250–500 mg/kg – rat orally), b) fenvalerate (LD_{50} = 450 mg/kg – rat orally) [19]

Fungicides

Fungicides are substances which kill fungi. Fungicides include, among others, derivatives of dithiocarbamic acid whose chemical formula is as follows (Fig. 2) [19, 20]:

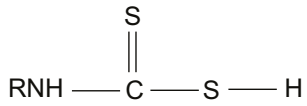

Fig. 2. Formula of derivative of dithiocarbamic acid [19, 20]

The principal fungicides are:

– inorganic substances – sulphur, barium and calcium polysulphides, copper(II) salts;

– organic substances – dithiocarbamates, benzimidazole (benomyl) derivatives and mercury- and tin-organic compounds.

Dithiocarbamates are one of the main groups among agricultural fungicides. Their toxicity is low. The probable lethal doses for humans range from 50 mg/kg (thiuram) to 5–15 g/kg (zineb). They get into the body via the alimentary canal and the respiratory system and accumulate either in the thyroid or the sexual glands. Most of them remain in the body for as long as a week (except for thiuram – retained up to a month), undergoing transformations to more toxic metabolites. They are solids characterized by different water solubility (lithium group salts – well soluble, heavy metals salts and disulphides – slightly soluble) [19].

Dithiocarbamates are analyzed in terms of CS_2.

Legal requirements

The maximum allowable residues (MAR) of pesticides in Poland are specified by the Health Ministry Order of 16th May 2007 concerning the maximum allowable pesticide residue levels in foodstuffs or on their surface.

The Order specifies [10]:

– the maximum allowable pesticide residue levels in plant foodstuffs or on their surface, except for grain, treated in Annex 1 to the Order;

– the maximum allowable pesticide residue levels in grain or on its surface, as wells as used for grain fumigation, treated in Annex 2 to the Order;

– the maximum allowable pesticide residue levels in animal foodstuffs or on their surface, treated in Annex 3 to the Order;

– the maximum allowable pesticide residue levels in baby (up to the 12th month of life inclusive) and small child (up to the age of 3 years) foodstuffs, specified in regulations issued on the basis of art. 26 of the act of 25 August 2006 concerning food and nutrition safety, treated in Annex 4 to the Order.

Materials and methods

Sample taking principles

The principles of taking pesticide residue samples are specified in the National Hygiene Institution procedure: ‘Methods of Taking Samples of Plant and Animal Products for Testing Compliance with MAR’ [21].

The sample size depends on the size of fruits or vegetables and the kind of plant product. Table 3 shows the minimum laboratory sample size to be used in tests for the presence of pesticides [21].

Table 3

Minimum laboratory sample size in pesticide content tests [21]

Product classification	Product	Minimum size of each laboratory sample
Small size fresh products, units < 25 g	Berries, peas, olives	1 kg
Medium size fresh products, units generally 25 g to 250 g	Apples, oranges	1 kg (at least 10 units)
Large size fresh products, units generally > 250 g	Cabbage, cucumbers, grapes (in bunches)	2 kg (at least 5 units)

Pesticide residue determination

Dithiocarbamate residue determination

The spectrophotometric determination of dithiocarbamates and thiuram disulphide consists in heating up a sample with hydrochloric acid and tin(II) chloride in order to release carbon disulphide from the residues of dithiocarbamates present in it. Carbon disulphide is removed from the system and purified through distillation and subsequently collected in an ethanol solution of copper(II) acetate and diethanolamine. The absorbance of the reaction products was measured at a wavelength of $\lambda = 435$ nm. The determination was made in accordance with Polish Standard PN-EN 12396-1:2002 [22].

The analytical sample was comminuted only to a degree allowing it to pass through the reaction flask neck since the rate of decomposition of dithiocarbamate residues increases with the degree of sample size reduction.

10 cm^3 of sodium hydroxide solution was added to the first washer connected to a Liebig condenser while 15 cm^3 of a colour reagent was added to each of the next two washers equipped with Widmer spirals. The weighed out 200 g analytical sample was placed in a three-necked flask. After the apparatus was properly closed, 240 cm^3 of tin(II) chloride solution in hydrochloric acid was added by means of a dropping funnel and the flask was heated until boiling. The system was kept boiling for 1 hour. The content of the first washer was transferred to a measuring flask with a capacity of 25 cm^3 and made up to the mark with ethanol. The absorbance of the reaction products was measured at a wavelength of $\lambda = 435$ nm against a reference prepared from 15 cm^3 of the colour reagent and 10 cm^3 of ethanol.

Pyrethroid residue determination

The pyrethroid residue testing consists in extracting pyrethroids from plant material into a methanol-water solution, back-extracting to *n*-hexane and determining the compounds in the hexane extract by gas chromatography [18].

The comminuted plant material sample was weighed out in the amount: strawberries – 50 g and tomatoes – 50 g. Then 100 cm^3 of methanol were added. The mixture was shaken in a mechanical shaker for 1 h. The obtained suspension was filtered under diminished pressure through a Büchner funnel, using hard filter paper previously wetted with methanol.

20 cm^3 of the filtrate, 20 cm^3 of *n*-hexane and 30 cm^3 of 5 % were taken into a separator of 250 cm^3 capacity and shaken for 5 minutes. The bottom methanol-water layer was drained into another separator and the hexane solution remaining in separator I was filtered through fluted filter paper (with 2 g of anhydrous sodium sulphate) into a round-bottomed flask with a capacity of 250 cm^3.

Solvent (*n*-hexane) in the amount of 10 cm^3 was added to the methanol-water solution in separator II and then extracted for 5 minutes. The methanol solution was transferred to separator III and the hexane solution was filtered into the round-bottomed flask. Extraction was repeated one more time using 10 cm^3 of *n*-hexane and the obtained filtrate was combined with the other ones collected in the flask. The sodium sulphate left on the funnel was rinsed out with 10 cm^3 of *n*-hexane.

The hexane solution was evaporated on a vacuum pan to a volume of about 1 cm^3 at a temperature of 40 ^oC and spread on a column (filled in the following way: a wad of glass wool in the column outlet, about 2 g of anhydrous sodium sulphate, 17 g of Florisil and then about 2 g of anhydrous sodium sulphate; the column was washed out with 50 cm^3 *n*-hexane in order to clean it from substances making chromatographic analysis difficult).

The sample was eluted with 150 cm^3 of a mixture of acetone and *n*-hexane at a ratio of 2:98. The eluate was thickened on the vacuum pan to a volume of about 5 cm^3 and quantitatively transferred to a measuring flask with a capacity of 10 cm^3 and made up to the mark with *n*-hexane.

Results and discussion

Results of chemical analyses of dithiocarbamate residues in fruits and vegetables

Eight samples of strawberries and eight samples of tomatoes, taken in the Lower Silesian province, were tested for the presence of dithiocarbamates.

The dithiocarbamate residue content was calculated as a mass fraction of residue w, in milligrams per kilogram of the product, expressed in terms of CS_2 [22]:

$$w = \frac{m_c}{m_t}$$

where: m_c – the mass of released carbon disulphide (read off the analytical curve) [μg];
m_t – the mass of the tested material (prior to the removal of any parts, eg stones) [g].

The arithmetic mean of two simultaneously performed determinations was adopted as the result. Table 4 shows the dithiocarbamate residue content in strawberry and tomatoes samples, in terms of carbon disulphide.

Table 4

Dithiocarbamate residue content in strawberry and tomatoes samples in terms of CS_2 in mg/kg of strawberries/tomatoes

Sample no.	Dithiocarbamate residue content in sample in terms of carbon disulphide [mg/kg]	
	strawberries	tomatoes
1	0.100	0.090
2	0.125	0.090
3	0.095	0.005
4	0.090	0.100
5	0.095	0.110
6	0.085	0.155
7	0.085	0.095
8	0.190	0.090

The above results showed no exceedance of the allowable dithiocarbamate content, which for strawberries is 2.0 mg/kg of the product (according to the Health Ministry Order of 16th May 2007). The tested samples contain slight amounts of dithiocarbamate residues and so they do not pose hazard to living organisms. No exceedance of the allowable dithiocarbamate residue content, which is 3.0 mg/kg of the product for tomatoes, was determined in any of the samples. The slight amounts of the pollutants in the tested plant material are within the limits of MAR, defined in Annex 1 to the Health Ministry Order of 16th May 2007.

Results of chemical analyses of pyrethroid residue content

The pyrethroid content in the plant material [mg/kg] of the product was calculated from this formula [18]:

$$C_x = \frac{C \cdot V_1 \cdot V_3}{m \cdot V_2}$$

where: C_x – the concentration of the tested substance, mg/kg of the product;
C – the concentration in the extract obtained from the analyzed sample [$\mu g/cm^3$];
m – the sample mass taken for analysis [g];
V_1 – the volume to which the thickened eluate was made up [cm^3];
V_2 – the amount of filtrate taken for analysis [cm^3];
V_3 – the amount of solvent taken for extraction [cm^3].

Sixteen samples (eight samples of tomatoes and eight samples of strawberries) were tested for pyrethroid residue content. The pyrethroid residue content in the strawberries and tomatoes samples is shown in Table 5.

Table 5

Pyrethroid residue content in strawberries and tomatoes samples [mg/kg] of strawberries/tomatoes

Sample No.	Pyrethroid residue content [mg/kg]					
	bifenthrine		cypermethrin		deltamethrin	
	strawberries	tomatoes	strawberries	tomatoes	strawberries	tomatoes
1	< 0.006*	< 0.01*	< 0.02*	< 0.04*	< 0.03*	< 0.05*
2	< 0.006*	< 0.01*	< 0.02*	< 0.04*	< 0.03*	< 0.05*
3	< 0.006*	< 0.01*	< 0.02*	< 0.04*	< 0.03*	< 0.05*
4	< 0.006*	< 0.01*	< 0.02*	< 0.04*	< 0.03*	< 0.05*
5	< 0.006*	< 0.01*	< 0.02*	< 0.04*	< 0.03*	< 0.05*
6	< 0.006*	< 0.01*	< 0.02*	< 0.04*	< 0.03*	< 0.05*
7	< 0.006*	< 0.01*	< 0.02*	< 0.04*	< 0.03*	< 0.05*
8	< 0.006*	< 0.01*	< 0.02*	< 0.04*	< 0.03*	< 0.05*

* Below the determinability limit.

No pyrethroids were found in the tested strawberries and tomatoes samples. According to the Health Ministry Order of 16 May 2007 concerning the maximum allowable pesticide residue levels in foodstuffs or on their surface, the Maximum Allowable Pyrethroid Residue Levels in the strawberries are [7]:

– bifenthrine – 0.5 mg/kg of the product,
– cypermethrin – 0.05 mg/kg of the product,
– deltamethrin – 0.2 mg/kg of the product;

and the Maximum Allowable Pyrethroid Residue Levels in the tomatoes are [10]:

– bifenthrine – 0.2 mg/kg of the product,
– cypermethrin – 0.5 mg/kg of the product,
– deltamethrin – 0.3 mg/kg of the product.

Conclusions

The tests covered the determination of the residue content of pesticides belonging to the groups of pyrethroids and dithiocarbamates in tomatoes and strawberries. Fruits and vegetables coming from the area of the Lower Silesian Province were tested. On the basis of the test results it has been established that:

– In none of the samples subjected to the chemical analysis for the dithiocarbamate residue content any exceedance of MAR defined in the Health Ministry Order of 16th May 2007 concerning the maximum allowable pesticide residue levels in foodstuffs or on their surface was found.

– In none of the samples subjected to the chemical analysis for the pyrethroid content any residues of: bifenthrine, cypermethrin, deltamethrin were found, which is in compliance with the Health Ministry Order of 16th May 2007 concerning MAR of plant protectants in foodstuffs or on their surface.

The use of chemical plant protectants in the manufacturer recommended doses guarantees that the side effects are slight and quickly recede. Only when the dose is increased many times, the disturbances are stronger and last longer.

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OCENA ZAWARTOŚCI WYBRANYCH PESTYCYDÓW W PRODUKTACH SPOŻYWCZYCH ZGODNIE Z OBOWIĄZUJĄCYM W POLSCE PRAWODAWSTWEM

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Abstrakt: Przeprowadzone badania obejmowały oznaczenia zawartości pozostałości pestycydów z grupy pyretroidów i ditiokarbaminianów w pomidorach i truskawkach. Badaniu zostały poddane owoce i warzywa

pochodzenia krajowego z terenu województwa dolnośląskiego. Na podstawie uzyskanych wyników można stwierdzić, że w żadnej z próbek poddanych analizie chemicznej, w kierunku zawartości ditiokarbaminianów nie stwierdzono przekroczeń NDP określonych w Rozporządzeniu Ministra Zdrowia z dnia 16 maja 2007 r. w sprawie najwyższych dopuszczalnych poziomów pozostałości pestycydów, które mogą znajdować się w środkach spożywczych lub na ich powierzchni; w żadnej z próbek poddanych analizie chemicznej, w kierunku zawartości pyretroidów nie stwierdzono pozostałości: bifentryny, cypermetryny, deltametryny.

Słowa kluczowe: insektycydy, fungicydy, toksyczność, najwyższy dopuszczalny poziom