ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 5/6 2009

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LINURON, DDT AND ORGANOCHLORINE PESTICIDE RESIDUES IN PLANTS FROM NORTH-EASTERN POLAND

LINURON, DDT I POZOSTAŁOŚCI PESTYCYDÓW CHLOROORGANICZNYCH W MATERIALE ROŚLINNYM PÓŁNOCNO-WSCHODNIEJ POLSKI

Abstract: The aim of this study was to investigate the DDT, organochlorine and linuron pesticide residues in agricultural products and herbs from North-Eastern Poland in 2000–2007. Total analyzed 3399 samples: field vegetables, greenhouse vegetables, fruit, field crops and herbs.

The determination of 20 active substances: DDT (*p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD), aldrin, dicofol, dieldrin, endosulfan(α, β, sulfate), endrin, HCH (α-HCH, β-HCH, γ-HCH and δ-HCH), heptachlor, heptachlor epoxide(endo, exo), metoxychlor and linuron was carried by gas chromatography with selective detectors: EC and NP. Pesticide residues of linuron were detected only in carrot samples; DDT and metabolites of degradation in carrots, parsley, wheat, and flower of mallow, endosulfan in black curry and mushrooms; dicofol only in flower of mallow; lindan in parsley and wheat. Aldrin, heptachlor and metoxychlor were not found in any sample. Evaluation of detected contamination was carried out on the basis of appropriate regulation of Minister of Health on maximum residue limits (MRLs) in foodstuffs, on the basis of the register of biological substances in Poland and on the basis of EU directives.

Violations of MRLs were found in the case of endosulfan, degradation products of DDT, dieldrin, lindan, dicofol and linuron in small percent. After estimating long and short term risk of exposure to these compounds, it has been stated that food from North-Eastern Poland is safe considering the presence of organochlorine pesticides, and detection of pesticide residues was incidental.

Keywords: food contamination, organochlorine pesticides, DDT, linuron, monitoring, gas chromatography

Contamination of the environment and food by pesticide residues is an important topical issue in many areas of the world. Food has never been free of harmful components, but nowadays, the problem is serious. Pesticides were extensively used as insecticides, fungicides, herbicides in agriculture, for pest control in forestry and vector control in hygiene against diseases like malaria and typhus. Today residues of *p,p'*-DDT

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and other chlorinated pesticides are ubiquitously distributed and despite the ban in most industrialized countries in the early 1970s, significant concentrations are determined worldwide in water, sediment, agricultural commodities and soil samples of formerly affected environments [1–5].

Organochlorine (OCPs) pesticides were banned in Poland in 1975. OCPs pesticides act by binding to the acetylcholinesterase enzyme, disrupting nerve function, resulting in paralysis and death, yet their persistence in the environment is not well understood. The general population is mainly exposed to OCPs pesticides through the ingestion of contaminated foods (such as cereals, vegetables and fruits) which are directly treated with OCPs pesticides or are grown in contaminated fields. The long-term persistence of these pesticides, therefore, still poses problems for the environment, for farming in general and for organic farming in particular. The OCPs in fish, meat, rice, vegetables and mothers' milk [4] were found at high concentrations. The residues of pesticide could affect the final consumers especially when these commodities are freshly consumed. The total dietary intake of pesticide residues that remain on agricultural commodities are known as carcinogens and/or toxins, and therefore it is desirable to reduce these residues. The levels of pesticide residues are controlled by the maximum residue limits (MRLs) which are established by each country and sometimes cause conflicts because residue levels acceptable in one country could be unacceptable in another. Many countries have given high priority to monitor and control pesticide residues on treated crops [6–8]. Methods were developed and implemented as part of regulatory programs to detect products that exceed MRLs. In Poland, the MRLs of pesticide residues for specific commodities are established in Decree of Polish Minister of Health.

The aim of this study was to determine levels of organochlorine and linuron contaminants in vegetable, fruits, agricultural crops and herbs from north-eastern region of Poland called "Green Lungs of Poland" owing to its relatively clean environment. The analysis of organochlorine pesticide, DDT and its metabolites and linuron was performed by gas chromatography with specific EC and NP detectors.

Materials and methods

Fruit samples used in this study (Table 1) included: apples, currant, cherry, pear, plum, raspberry, strawberry, blueberry, berry and chokeberry, while vegetable samples included: road beet, cabbage, cauliflower, carrot, cucumber, onion, parsley, green been, broccoli, celery, tomato, leek; indoor cultivation: mushroom, paprika, tomato, lettuce, cucumber, cabbage. Among field crops were: potatoes, rape, wheat, barley, oat, rye, sugar-beets. The analysis was also conducted for herbs: folia: lemon balms, salvia, nettle, peppermint, apple and black currant, the flower: elder, chamomile, mallow; herb of St-John's-wort and horsetail. The main part of samples (2289 samples) were from government monitoring program in North-Eastern Poland conducted in 2000–2007 including Warmia-Mazury, Podlasie and Lublin region; remaining samples were from individual farm and gardeners from North-Eastern Poland (1110 samples) (Table 1). The sample size was at least 1 kg for small and medium size of fresh product. The

minimum weight for large sample size was 2 kg (for example broccoli and cabbage), where the unit was generally more than 250 g (Codex Alimentarius, 2000). The portion of raw agricultural commodity prepared as the analytical sample for determination of pesticide residues was carried out by matrix solid phase dispersion. A representative

Table 1

Number and kind of analyzed crops, samples with residues, above or under maximum residues level

Product range		No. of samples		No. of samples with residues	No. of samples < MRLs	No. of samples > MRLs
Commodity		M	O			
Agricultural crops 257 (M) 44 (O)	potato	117	31	nd	0	0
	rape	10	0	nd	0	0
	oat	12	0	nd	0	0
	winter-spring wheat	64	13	**2**	0	2
	barley	12	0	nd	0	0
	rye	7	0	nd	0	0
	sugar-beet	35	0	nd	0	0
Fruit 955 (M) 520 (O)	apple	338	134	nd	0	0
	currant	118	10	**17**	5	12
	cherry	176	121	nd	40	0
	pear	13	0	nd	0	0
	plum	21	0	nd	0	0
	rasberry	15	38	nd	0	0
	strawberry	260	151	nd	0	0
	blueberry	0	12	nd	0	0
	berry	0	12	**1**	0	1
	chokeberry	14	42	nd	0	0
Field vegetable 623 (M) 420 (O)	red beet	33	0	nd	0	0
	cabbage	129	0	nd	0	0
	carrot	110	51	**14**	11	3
	cauliflower	51	98	0	0	0
	cucumber	135	0	0	0	0
	onion	45	45	0	0	0
	parsley	7	0	**2**	0	2
	green been	35	0	0	0	0
	broccoli	25	115	0	0	0
	celery	3	0	0	0	0
	tomato	45	100	0	0	0
	leek	5	11	0	0	0

Table 1 contd.

Product range		No. of samples		No. of samples with residues	No. of samples < MRLs	No. of samples > MRLs
Commodity		M	O			
Greenhouse vegetable 454 (M) 56 (O)	mushroom	70	0	**1**	1	0
	paprika	17	4	0	1	0
	tomato	199	35	0	2	0
	lettuce	46	2	0	0	0
	cucumber	120	15	0	0	0
	cabbage	2	0	0	0	0
Herbs 70 (O)	folia of peppermint	0	18	0	0	0
	folia of apple	0	4	0	0	0
	folia of black currant	0	8	**2**	0	2
	flower of elder	0	8	0	0	0
	flower of chamomile	0	4	0	0	0
	flower of mallow	0	8	**2**	1	1
	herb of st-john's-wort	0	4	0	0	0
	herb of horsetail	0	4	0	0	0
	folia of salvia	0	4	0	0	0
	folia of nettle	0	4	0	0	0
	folia of lemon balm	0	4	0	0	0

M – samples from national monitoring in North-Eastern Poland, O – samples from individual farm and gardeners in North-Eastern Poland, nd – not detected, MRLs – maximum residues level.

portion of the analytical sample 2 was blended using a food processor and mixed thoroughly with the solid-phase materials (Florisil PR (Floridon Co., USA), Silicagel 60 (70–230 mesh, Merck) and neutral alumina (70–230 mesh, activity IB supplied by Merck). All adsorbents were activated by heating overnight at 150 ^{o}C before use, allowed to cool and stored in well-closed flask. Freshly activated adsorbents were used for this study. Anhydrous sodium sulfate p.a. used for drying was heated for 4 hrs. at 500 ^{o}C. The clean-up step was performed by column chromatography. The homogenous mixture was transferred into a glass chromatography column containing 5 g of anhydrous sodium sulfate, 2 g of deactivated alumina or activated silica gel. The analytes were eluted with solvents of increasing polarity in 15 cm^3 fractions: *n*-hexane-ethyl ether (9:1), *n*-hexane-ethyl ether (8:2), *n*-hexane-ethyl acetate (7:3). Elution was performed by gravity flow. The three fractions were combined and concentrated to about 1 cm^3 using vacuum evaporator with temperature programmed bath (40 ^{o}C). The final volume of the eluates was adjusted to 2 cm^3 by addition of *n*-hexane-acetone mixture (9:1).

Pesticide-grade ethyl acetate, acetone, *n*-hexane and anhydrous sodium sulfate were obtained from Merck (Darmstadt, Germany). Pesticide standards of DDT (*p,p'*-DDT;

p,p'-DDT; *p,p'*-DDE; *p,p'*-DDD), aldrin, dicofol, dieldrin, endosulfan(α, β, sulfate), endrin, HCH (α-HCH, β-HCH, γ-HCH and δ-HCH), heptachlor, heptachlor epoxide(endo, exo), metoxychlor, linuron were purchased from Dr. Ehrenstorfer (Ausberg, Germany). Individual stock standard solution of pesticide was prepared by dissolving 50–60 mg of each compound in 50 cm^3 of acetone and stored in amber bottles at 4 ^oC. A mixed standard solution was prepared from the stock solutions with a concentration of 10 mg · dm^{-3}. A series of calibration standards were prepared by diluting 10 mg · dm^{-3} of the mixed standard solution to produce a final concentration of 0.01; 0.1; 0.2 and 0.5 mg · dm^{-3} in acetone.

Gas chromatographic determination

The Agilent HP 6890 N gas chromatograph equipped with an ECD and with HP-5 fused capillary column (30-m length, 0.32-mm internal diameter, and 0.25-mm film thickness) was used. The injection port temperature was 250 ^oC and the detector temperature was 300 ^oC. The column temperature was programmed as follows: the initial temperature of 100 ^oC was increased at a rate of 10 $^oC \cdot min^{-1}$ up to 250 ^oC and held for 25 min, from 250 to 300 ^oC at a rate of 50 $^oC \cdot min^{-1}$ was used and held for 5 min at the final temperature. Helium carrier gas at a flow rate of 1.2 $cm^3 \cdot min^{-1}$ was used. Two microlitres of the extract were injected and the retention time of peak was compared with that retention time of the calibration standards (in matrices) to determine the residue quantitatively.

Results and discussion

GC-ECD provided good responses even at very low concentrations because of its selective and sensitive detectors.

Among 3399 analyzed samples (2000–20007 years) pesticide residues of organochlorine pesticides were detected in 20 samples of fruit (total 1475 samples of fruit), in 2 field crops (total 301 samples) and in 4 samples of herbs (total 70 samples). The pesticide residues of linuron and organochlorine compounds were detected in 18 samples of field vegetables and greenhouse vegetables (total 1553 samples). Among the determination of organochlorine pesticides (Table 2) in raw fruit were detected products of DDT decomposition and endosulfan, in vegetables – lindane (γ-HCH), products of DDT decomposition and herbicide – linuron. In samples of field crops were found products of DDT decomposition and lindane. In investigated herbs were detected dicofol, products of DDT decomposition and endosulfan.

Levels of determined pollutants in individual years are presented in Table 3.

Linuron is a selective herbicide which is most readily absorbed through the root system. It is labeled for use in soybean, cotton, potato, corn, bean, pea, winter wheat, asparagus, carrot, and fruit crops. It accumulates and metabolizes differently in different plants. Susceptible plants transport linuron through the foliage, while tolerant plants can metabolize linuron into inactive products. Linuron inhibits photosynthesis in susceptible plants causing them to lose color, wilt, and die. In crops, linuron has low residual action

Table 2

The mean levels of organochlorine and herbicide linuron in vegetables, fruit, agricultural crops and herbs in North-Eastern Poland 2000–2007

Active substance	LOD [mg · kg^{-1}]	Fruit (n = 1477)		Vegetable (n = 1560)		Agricultural crops (n = 275)		Herbs (n = 70)	
		Mean value [mg · kg^{-1}]	No. of positive samples	Mean value [mg · kg^{-1}]	No. of positive samples	Mean value [mg · kg^{-1}]	No. of positive samples	Mean value [mg · kg^{-1}]	No. of positive samples
Aldrin	0.005	nd	0	nd	0	nd	0	nd	0
p,p'-DDT	0.005	9.50	1	0.02	3	15.5	1	27.57	1
o,p'-DDT	0.005	1.05	1	nd	0	1.70	1	1.40	1
p,p'-DDE	0.005	0.44	1	0.025	1	0.37	1	0.27	1
p,p'-DDD	0.005	0.32	1	nd	0	0.75	1	0.81	1
Dicofol	0.005	nd	0	nd	0	nd	0	0.17	1
Dieldrin	0.005	nd	0	0.12	1	nd	0	nd	0
Endosulfan sum	0.005	0.14	17	0.3	1	nd	0	0.94	2
α-HCH	0.005	nd	0	nd	0	nd	0	nd	0
β-HCH	0.005	nd	0	nd	0	nd	0	nd	0
γ-HCH (lindane)	0.005	nd	0	0.06	1	0.45	1		
δ-HCH	0.005	nd	0	nd	0	nd	0	nd	0
Heptachlor sum	0.005	nd	0	nd	0	nd	0	nd	0
Metoxychlor	0.005	nd	0	nd	0	nd	0	nd	0
Linuron	0.005	nd	0	0.12	12	nd	0	nd	0

LOD – limit of detection.

Table 3

Detection of pesticide residues (concentration over maximum residue levels in agricultural crops, fruit, vegetables and herbs – marked in bold)

Year	Commodity	No. of samples with residues	Pesticide	Concentration [mg · kg^{-1}]	MRL [mg · kg^{-1}]
2000	carrot	1	*p,p'*-DDT	0.007	0.01
	carrot	3	linuron	0.18; **0.22**; 0.05	0.2
	black berry	2	endosulfan	0.08 ;0.01	0.05
2001	carrot	5	linuron	0.05; 0.05; 0.07; 0.07; 0.08	0.2
	parsley	1	γ-HCH	**0.06**	0.01
	wheat	1	γ-HCH	**0.45**	0.01
	black berry	7	endosulfan	**0.07**; **0.08**; **0.16**; **0.21**; **0.35**; **0.45**	0.05
2002	carrot	1	linuron	0.17	0.2
	black berry	3	endosulfan	**0.1**; **0.13**; **0.13**	0.05
	folia of black currant	2	endosulfan	**0.25**; **1.62**	0.05
	flower of mallow	1	dicofol	0.17	20
2003	carrot	1	linuron	0.04	0.2
	black-currant	3	endosulfan	**0.2**	0.05
	flower of mallow	1	*p,p'*-DDD *p,p'*-DDE *o,p'*-DDT *p,p'*-DDT	**0.81** **0.27** **1.40** **27.57**	0.01 0.01 0.01 0.01
2004	mushroom	1	endosulfan	0.03	0.05
	black-currant	3	endosulfan	0.02; 0.04; **0.3**	0.05
2005	parsley	1	*p,p'*-DDT	**0.07**	0.01
	wheat	1	*p,p'*-DDD *p,p'*-DDE *o,p'*-DDT *p,p'*-DDT	**0.75** **0.37** **1.70** **15.50**	0.01
2006	carrot	1	*p,p'*-DDT dieldrine *p,p'*-DDE	0.005 **0.12** **0.025**	0.01 0.01 0.01
	black berry	1	endosulfan	0.06	0.05
	blue berry	1	*p,p'*-DDD *p,p'*-DDE *o,p'*-DDT *p,p'*-DDT	**0.32** **0.44** **1.05** **9.50**	0.01 0.01 0.01 0.01
2007	carrot	2	linuron	0.02; **0.4**	0.2

and persistence. Linuron has been detected in 12 samples of carrots (0.02–0.4 mg · kg^{-1}). Two samples of carrots had linuron concentration below Maximum Residues Level (for carrots is 0.2 mg · kg^{-1}). The highest detected concentration was two times higher than Maximum Residues Level and average concentration in 12 samples of carrots were 0.12 mg · kg^{-1}. Endosulfan is a chlorinated hydrocarbon insecticide of the cyclodiene subgroup which acts as a contact poison in a wide variety of insects and mites. It is used primarily on food crops like tea, fruits, vegetables and on cereals. The breakdown product, endosulfan sulfate, has been observed in several field studies involving plants [9, 10]. The sulfate is more persistent than the parent compound, accounting for 90 % of the residue in 11 weeks. On most fruits and vegetables, 50 % of the parent residue is lost within three to seven days. Endosulfan residues have been found in 20 samples: blackcurrant berry, black berry, leaves of black currant berry and mushrooms at low concentrations. They have been detected in 0.01–0.45 mg · kg^{-1}. The average concentration in fruit was 0.14 mg · kg^{-1}, vegetables 0.03 mg · kg^{-1} and herbs 0.95 mg · kg^{-1}.

DDT has been banned in developed countries because of long-term persistence and accumulation but pesticides are still detected. The resignation from using DDT is an expression of the rule of the foresight, because it is not yet known how it is accumulated in fat tissues and how it influences health of people. DDT is classified as "moderately toxic" by the US National Toxicological Program and "moderately hazardous" by WHO. DDT is a persistent organic pollutant with a half life of 2–15 years, and is immobile in most soils. Breakdown products in the soil environment are DDE (1,1-dichloro-2,2-bis(*p*-dichlorodiphenyl)ethylene) and DDD (1,1-dichloro-2,2-bis(*p*--chlorophenyl)ethane) which are also highly persistent and have similar chemical and physical properties. These products together are known as total DDT. In this study DDT and its metabolite was detected in berry: *p,p'*-DDD – 0.32 mg · kg^{-1}, *p,p'*-DDE – 0.44 mg · kg^{-1}, *o,p'*-DDT – 1.05 mg · kg^{-1}, *p,p'*-DDT – 9.50 mg · kg^{-1}. Concentration of all metabolites were 11.31 mg · kg^{-1}. In one sample of wheat were found *p,p'*-DDD – 0.75 mg · kg^{-1}, *p,p'*-DDE – 0.37 mg · kg^{-1}, *o,p'*-DDT – 1.70 mg · kg^{-1}, *p,p'*-DDT – 15.50 mg · kg^{-1} isomers and in flower of mallow: *p,p'*-DDD – 0.81 mg · kg^{-1}, *p,p'*-DDE – 0.27 mg · kg^{-1}, *o,p'*-DDT – 1.40 mg · kg^{-1}, *p,p'*-DDT – 27.57 mg · kg^{-1}. The concentration of p,p'-DDT isomer in all analyzed cases was the highest. *p,p'*-DDT was detected in the parsley – 0.07 mg · kg^{-1} and carrot – 0.025 mg · kg^{-1}. Dieldrine was detected only in carrots and dicofol only in flower of mallow (0.17 mg · kg^{-1}). γ-HCH was detected in parsley 0.06 mg · kg^{-1} and wheat 0.45 mg · kg^{-1}. Aldrin, heptachlor and metoxychlor were not found in any sample.

Conclusions

The results show that linuron pesticide was only present in carrots. Detected organochlorine suggests that these pesticides were not in common use in fruit and vegetable samples found in this region. In order to minimize health risk as well as for enforcement activities, monitoring of pesticide residues is increasingly important and essential [6, 12].

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LINURON, DDT I POZOSTAŁOŚCI PESTYCYDÓW CHLOROORGANICZNYCH W MATERIALE ROŚLINNYM PÓŁNOCNO-WSCHODNIEJ POLSKI

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Abstrakt: Celem badań była ocena poziomu skażeń płodów rolnych oraz surowców zielarskich pochodzących z północno-wschodniej Polski pod kątem obecności linuronu, DDT i związków chloroorganicznych.

W latach 2000–2007 w Laboratorium BPŚOR w Białymstoku przebadano ponad 3000 próbek. Badane asortymenty to: warzywa spod osłon, płody rolne, owoce, surowce zielarskie. Oznaczenia 20 substancji aktywnych: czterech izomerów DDT (*p,p'*-DDT, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD), aldryny, dikofolu, dieldryny, endosulfanu(α, β, siarczanu), endryny, czterech izomerów HCH (α-HCH, β-HCH, γ-HCH i δ-HCH), heptachloru, heptachloru epoksydu(endo, exo), metoksychloru i linuronu wykonano metodą chromatografii gazowej z zastosowaniem specyficznego mikrodetektora EC. Pozostałości linuronu stwierdzono w uprawie marchwi, DDT i produkty rozkładu w marchwi, pietruszce, pszenicy, kwiecie ślazu, endosulfan w porzeczce czarnej i jagodzie, dikofol – w kwiecie ślazu, lindan w pietruszce i pszenicy, a dieldrynę w marchwi. Ocenę wykrywanych skażeń prowadzono zgodnie z rozporządzeniem Ministra Zdrowia i Opieki Społecznej w sprawie największych dopuszczalnych pozostałości w środkach spożywczych, rejestrem substancji biologicznych w Polsce oraz zgodnie z dyrektywą Unii Europejskiej.

Przekroczenia największych dopuszczalnych poziomów (NDP) stwierdzono w przypadku endosulfanu, produktów rozkładu DDT, dieldryny, dikofolu, lindanu i linuronu w niewielkim procencie. Po ocenie krótko- i długoterminowego ryzyka narażenia ludności na te związki stwierdzono, iż żywność pochodząca z Podlasia jest bezpieczna pod kątem obecności pestycydów chloroorganicznych, a stwierdzone obecności tych związków są incydentalne.

Słowa kluczowe: zanieczyszczenie żywności, DDT, linuron, pestycydy chloroorganiczne, monitoring, chromatografia gazowa