Polish J. of Environ. Stud. Vol. 17, No. 3 (2008), 321-326

Original Research

Study of Chlorothalonil Uptake in Selected Plant Materials

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Received: September 12, 2007
Accepted: November 30, 2007

Abstract

The objective of this work was to create a sorption model of different pesticides in plant material. The above-quoted model includes graphic curves describing the pesticide's behaviour in time (concentration level) depending on the place of sorption. Apart from curves the model also includes mathematical equations that allow us to predict the concentration of a pesticide in time function. The model has been developed based on research data obtained in a special experimental device.

This article accounts for the transportation model of chosen xenobiotics in plants. Chlorothalonil were used as a model pesticide. Chlorothalonil is a nonsystemic fungicide that has been used to control disease of many fruits, vegetables, and other agricultural crops. As a method of sample preparation supercritical fluid extraction was used. Gas chromatography with mass spectrometry was used for qualitative and quantitative analysis. The detection limit (LOD) of chlorothalonil was on level 0.01 μg/g. and the limit of quantification (LOQ) was level on 0.03 μg/g

Keywords: model, sorption, chlorothalonil, fungicide, supercritical fluid extraction, gas chromatography

Introduction

Agricultural development entails more intensive use of pesticides. Volatile xenobiotics derived from pesticides are one of the contaminants in vegetable foodstuffs [1, 2]. Detection and determination of these compounds are important tasks for an analyst. Chlorothalonil (2,4,5,6-tetrachloro-1.3 benzenedicarbonitrile) is an important, broad spectrum contact fungicide that has been widely used to control diseases of over 50 fruits, vegetables, and agricultural crops as well as on turf, lawn, and ornamental plants, for about 25 years [3]. The structure of chlorothalonil is shown in Fig. 1.

Its application to fruits and vegetables constitutes a menace to human health [4]. Contamination of plants or waters by xenobiotic compounds such as pesticides constitutes a serious environmental problem because of their potential toxicity and widespread use. Thus, their removal from the matrix has become an important task in which various methods are used (carbon adsorption, microbial degradation). Hydrolysis and photolysis can also contribute to decomposition [5]. Broad application of chlorothalonil stimulated research concerning its influence on the human organism. The series demonstrated indicates the influence of chlorothalonil and its derivatives on proteins [6].

Examining the process of vegetable foodstuff contaminants' uptake allows us to establish a model of such a process [7-10]. Creating the model allows us to predict the contaminants' accumulation places and the directions of their migration within a plant [11]. To create such a model, a laboratory investigation in a special system must be carried out. The system, which is used for the uptake of xenobiotics in plants, allows us to control temperature, insulation and time of saturation by pesticides. The control of these parameters presents the possibility to simulate natural processes in a laboratory environment.

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In the uptake process chlotothalonil is used. Lettuce and radish were used in the system as model plants. Authors proposed different systems to establish conditions of experiments [12-14]. These vegetables were used because they are popular as foodstuffs and for tillage they use pesticides. This investigation allows us to control concentrations of xenobiotics in plants and to state when a spray shower was used.

The objective of the present study was to draught curves which describe behaviour of chlorothalonil in model plants – lettuce and radish. Curves show the level of concentration of chlorothalonil in a chosen part of a plant dependent on the time after saturation. On the basis of curves we can estimate for how long a given pesticide will fulfill its protective function.

Experimental

Material and Methods

Lettuce *(Lactuca sativa)* and radish *(Raphanus sativus)* were grown on a horticultural farm in a greenhouse. After growth the plants were transported to the laboratory and placed in a system. Throughout the study carbon dioxide and helium from Praxair (Gliwice, Poland), anhydrous magnesium sulfate from POCH (Gliwice, Poland), and chlorothalonil (2,4,5,6-tetrachloro – 1,3 benzenedicarbonitrile) from Annopol (Warsaw, Poland) was used.

Apparatus and Analytical Conditions

For sample preparation we used a supercritical fluid extractor (SEKO-K Ltd., Czech Republic). Extraction parameters were: pressure 20 MPa, temperature 75 °C and 30 min. of extraction.

For qualitative and quantitative analysis GC-MS (Perkin Elmer, Norwalk, USA) was used. Separation was achieved on an RTX 35 column (Restek, Bellefonte, PA, USA) 30m x 0.25mm x 0.25µm. GC analysis was conducted according to the following temperature program GC: 50°C by 1 min., 15°C/min. to 120°C by 1 min., 5°C/min to 290°C by 5 min.

The MS operating parameters were as follows: electron impact ionization (EI), ionization energy: 70 eV, scan range: 35–350 u, scan rate: 0.4 scan per second, ion source and interface temperature: 200°C.

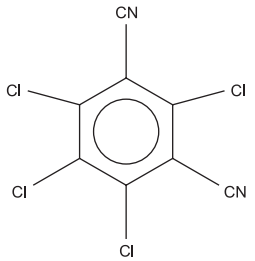

Fig 1. Structure of chlorothalonil.

Procedure of Research

Plants were grown in a greenhouse at 16-17°C, with no access to any external factor, like other pesticides. They were then transported to a laboratory in special containers, in isothermic conditions. In the laboratory they were placed in a system for determination of uptake of xenobiotics in plants. A phase of environment saturation with the chlorothalonil solution was carried out there. After saturation the plants were left in the system for the process of chlorothalonil uptake in the plant material. Saturation was done by sparkling upper parts of plants. Investigation was done before the achievement of the equilibrium state because we wanted to reproduce the agricultural conditions in a chemical laboratory. After each full 60 minutes of uptake process samples of both lettuce and radish were taken for analysis. Plant material was divided into three parts: upper (upper part of leaves), middle (middle part of leaves) and underground (roots) (Fig. 2).

All processes which have been placed in differents parts of the plant on the border of phases can be described by the set of equations. Changes are shown in Fig. 2. Mass change of pesticide can be described by the following equations (1-3):

Roots

mass change = ± diffusion from / to soil
+ mass flow within the transpiration stream
– metabolism

$$V_r \times \delta C_r/\delta t = (K_{aw} \times D_{a,eff} + D_{w,eff}) \times (C_w - C_r/K_{rw}) \times 2 \times L \times \pi / /[\ln(R_2/R_1)] + Q_w \times (1 - \mathrm{TSCF}) \times C_w - \lambda_r \times V_r \times C_r \quad (1)$$

Stem

mass change = + mass flow with the transpiration stream from soil
– mass flow within the transpiration stream to leaves
+ mass flow within the phloem from leaves
– mass flow within the phloem to seeds
– metabolism

$$V_{st} \times \delta C_{st}/\delta t = Q_w \times (C_w \times \mathrm{TSCF} - C_{st}/K_{stxy}) + Q_p \times \times (C_l/K_{lw} - C_{st}/K_{stxy}) - \lambda_{st} \times V_{st} \times C_{st} \quad (2)$$

Leaves

mass change = + mass flow within the transpiration water from stem
± diffusive flux from / to air
– mass flux within the phloem into the stem
– metabolism

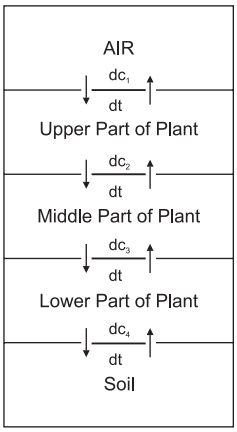

Fig. 2. The structure of plant and surroundings. Arrows symbolize possible paths of xenobiotics in plants.

$$V_l \times \delta C_l/\delta t = Q_w \times C_{st}/K_{stxy} + A \times g_{total} \times (C_a - C_l/K_{la}) - Q_p \times \times C_l/K_{lw} - \lambda_l \times V_l \times C_l \quad (3)$$

Each part of the plant was homogenized, mixed with anhydrous magnesium sulphate and was extracted by a supercritical fluid extractor (SFE) [15]. After extraction chromatographic analysis was carried out in a gas chromatographer equipped with a mass spectrometer (GC-MS). The experimental part ended after 8 hours from the sample collection. In that experiment the quantitative results of chlorothalonil level in plant material were acquired, respectively: $^1/_2$, 1, 2, 3, 4, 5, 6, 7, and 8 hours from the sample taking.

Results and Discussion

As a result of the saturation process of plants a series of quota and data were gained, showing the manner of chlorothalonil for acting in an investigated environment (soil/plant and plant/air). The results were gathered in the graphs of interdependence between the concentration of the adsorbed chlorothalonil and time that had passed since the saturation until the moment of taking a sample of plant material and the sample preparation in a chromatographic analysis phase. As it was explained above, each plant was divided into three parts: an upper part of leaves (Area 1), a middle part of leaves (Area 2) and a lower part (Area 3, underground – root). The partitioning of lettuce is shown below (Fig. 3). The equations of curves below, describing concentration levels of chlorothalonil in the chosen part of the plant, facilitate the understanding of the process (Fig. 4).

Table 1 shows concentrations of chlotothalonil in lettuce in differents parts. Also presented is the decrease in pesticides between consecutive measurements. Changes in Area 1 are bigger than in Area 2. This is a result of differences in building of those plant parts. In both cases, lettuce as well as radish, chlorothalonil was not detected in the third part (3 Area), that is the underground part (roots) of plants. A diagram of radish partitioning and the results of the uptake process in individual plant parts is shown in Fig. 3.

Changes of concentration level in time was shown in Fig. 5. In Table 2 concentrations of chlorothalonil in radish in differents parts was shown. Decreases in pesticide between consecutive measuremets are also presented. Changes in Area 1 are bigger than in Area 2. This is a result of differences in building those parts of the plant. Area 1 has more complicated building that Area 2. The differences were described below and concentrate on the concentration level. Equations of curves describing concentration levels of chlorothalonil in chosen parts of lettuce and radish are showed in Table 3. In this table also coefficient of determination – R^2 is shown.

The curves (Figs. 4, 5) above show certain regularity in the behaviour of chlorothalonil in analyzed plant material. The longer the time period between the saturation process and the analysis phase, the lower the chlorothalonil concentration in a plant part analyzed. This regularity proves right for both lettuce and radish. When analyzing chlorothalonil in radish and lettuce, it is possible to conclude that the highest concentration occurred after a half an hour of the saturation process (3.3 μg/g for upper part for lettuce and 2.2 μg/g for upper part for radish). The main part of chlorothalonil was adsorbed in leaves and in the air stay only low concentration in this small part. From this time more important are the processes in the plant. In time, the concentration decreased to 1.5 μg/g for upper parts and to 0.2 μg/g for

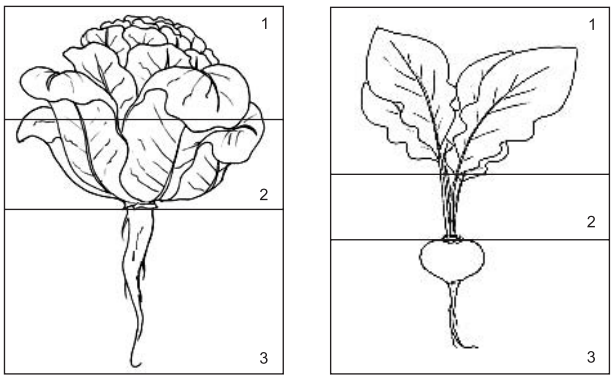

Fig. 3 The partition of lettuce and radish into 3 parts: 1 Area – upper, 2 Area – middle, 3 Area – lower.

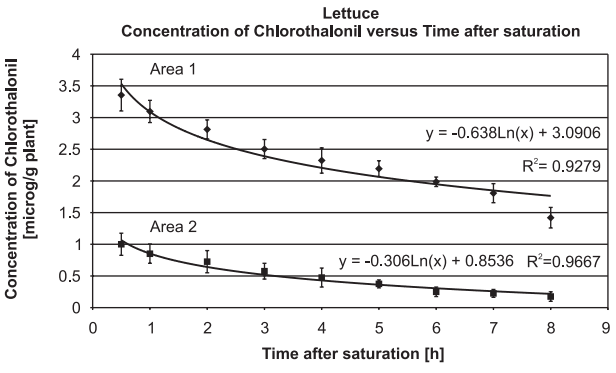

Fig. 4. Lettuce Areas 1 & 2.

Table 1. Composition concentrations of chlotothalonil in lettuce and its differences between next measurements.

Lettuce Area 1									
Time [h]	0.5	1	2	3	4	5	6	7	8
Concentration [μg/g]	3.354	3.096	2.812	2.503	2.322	2.193	1.987	1.806	1.419
Δ Concentration [μg/g] = C1 – C2		0.258	0.284	0.31	0.181	0.129	0.206	0.181	0.387
Lettuce Area 2									
Time [h]	0.5	1	2	3	4	5	6	7	8
Concentration [μg/g]	1	0.85	0.725	0.575	0.475	0.375	0.25	0.225	0.175
Δ Concentration [μg/g] = C1 – C2		0.15	0.125	0.15	0.1	0.1	0.125	0.025	0.05

middle parts for lettuce. The higher concentration in the first period in lettuce than in radish is the result of a greater leaf surface. It means that higher surfaces of leaves have a higher possibility to adsorb pesticides. The final concentration for radish was: 1.3 μg/g for upper part of plant and 0.6 μg/g for middle part. The pesticides were not detected in lower (underground) parts for both plants: lettuce and radish. The decreasing concentration of chlorothalonil in a plant might be an effect of a degradation process [4]. A biodegradation process proceeds faster in aqueous systems. Linear velocity of pesticide in phloem transport is an average of 20-200 cm/h. From the curves above we can draw no evidence of the influence on transport of chlorothalonil in plant. Poor transport in a plant is caused by low solubility of chlorothalonil in water, the structure of the plant and by the chemical and physical properties of a compound. Figure 2 shows us the structure of plants and surroundings.

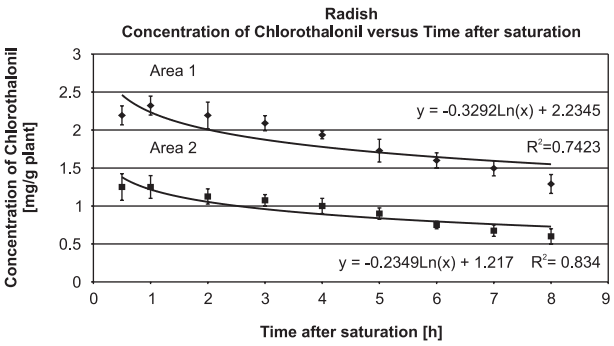

Fig. 5. Radish Area 1.

Chlorothalonil is not a volatile compound and that fact proves that its concentration in the air is zero. But this pesticide creates the aerosol, which can be dewiding as a spray. These facts reduce our model to 3 elements. Determination of compounds in a lower part (underground – roots) of plant shows us that level of concentration of chlorothalonil is zero. But that fact only demonstrates that there is no connection between the lower part of a plant and the middle part that transports any xenobiotics like chlorothalonil. Results show that only two parts of plant are very important for pesticide transport in the plants. Curves describe behaviour of chlorothalonil in different parts of a plant. The application of anhydrous magnesium sulphate instigates better contact between SF's and samples and it can reduce the dead volume effects.

Conclusions

The use of a miniature greenhouse (system for the sparkling plants by the pesticides) for the experiment

Table 2. Composition concentrations of chlorothalonil in lettuce and its differences between next measurements.

Radish Area 1									
Time [h]	0.5	1	2	3	4	5	6	7	8
Concentration [μg/g]	2.193	2.322	2.193	2.09	1.935	1.729	1.6	1.496	1.29
Δ Concentration [μg/g] = C1 – C2		-0.13	0.129	0.103	0.155	0.206	0.129	0.103	0.206
Radish Area 2									
Time [h]	0.5	1	2	3	4	5	6	7	8
Concentration [μg/g]	1.25	1.25	1.125	1.075	1	0.9	0.75	0.675	0.6
Δ Concentration [μg/g] = C1 – C2		0	0.125	0.05	0.075	0.1	0.15	0.075	0.075

Table 3. Equations of curves describing concentration level of chlorothalonil in chosen part of lettuce and radish.

Plant	Area 1	R^2	Area 2	R^2
lettuce	y = – 0.638 ln (x) + 3.0906	0.9279	y = – 0.306 ln (x) + 0.8536	0.9667
radish	y = – 0.3292 ln (x) + 2.2345	0.7424	y = – 0.2349 ln (x) + 1.217	0.834

enabled the lessening of the influence of such outer factors as wind or rainfall. A chromatograph with mass spectrometer was used but also the application of supercritical fluid extraction and gas chromatograph equipped with an electron capture detector constitutes a very useful tool for the analysis of chloroorganic pesticides in plant material. Our model is composed of 4 elements: air, upper part of plant, middle part of plant and lower part of plant This model is used to describe pesticide's behaviour in plant.

Values of R^2 (Table 3) show us better correlations between time and concentration of pesticide for lettuce than radish. The diagram curves show the behaviour of chlorothalonil in laboratory conditions, and constitute an important information source for the evaluation of chlorothalonil behaviour in natural environmental conditions.

Abbreviations

N_{dr} - sum of the diffusive flux of chemical to the roots in air- and water-filled pores (kg/s);
K_{aw} - partition coefficient of air to water (the dimensionless Henry's law constant);
K_{rw} - partitioning coefficient between roots and water;
$D_{a,eff}$ - effective diffusion coefficient in air-filled soil pores (m^2/s);
$D_{w,eff}$ - effective diffusion coefficient in water-filled pores (m^2/s);
C_w - concentration in the external (soil) solution (kg/m^3);
C_r - concentration in the root (kg/m^3);
L - total length of the roots (m);
N_{pst} - flux of the xenobiotic within the phloem from the leaves to the stem (kg/s);
Q_p - flow of the assimilation stream (m^3/s);
C_l - concentration in the leaves (kg/m^3);
A - leaf area (m^2);
C_a - concentration of xenobiotic in air (kg/m^3);
K_{lw} - partition coefficient between leaves and water in the assimilation stream
K_{la} - partition coefficient between leaves and air, and is calculated from the ratio K_{lw} / K_{aw}.
R_1 - radius of the roots;
$R_2 - R_1$ - diffusion length;
R_2 - radius of a deficiency zone surrounding the roots.
N_{tr} - mass flow of xenobiotic (kg/s) with the transpiration water remaining in the roots

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