INCIDENCE OF PESTICIDE RESIDUES IN FOODS PRODUCED IN TOBACCO GROWING AREAS IN THE PARDINHO RIVER BASIN, RS, BRAZIL

Nadir Hermes[1], Marco Flôres Ferrão[2] & João Paulo Machado Torres[3]*

[1] Central Analítica – Universidade de Santa Cruz do Sul. Avenida Independência, 2293, Bloco 11. Santa Cruz do Sul – RS. CEP 96815-900. [2] Departamento de Química e Física – Universidade de Santa Cruz do Sul. Avenida Independência, 2293, Bloco 12. Santa Cruz do Sul – RS. CEP 96815-900. [3] Laboratório de Radioisótopos Eduardo Penna Franca – Instituto de Biofísica Carlos Chagas Filho. Centro de Ciências da Saúde/UFRJ. Ilha do Fundão. Rio de Janeiro–RJ. CEP 21941-590.

*E-mail: nadir.hermes@gmail.com

ABSTRACT

The Vale do Rio Pardo region, where the Pardinho River Basin is located, is the main tobacco growing area of Brazil. Tobacco growing involves intense and regular use of pesticides. Among the risks to which producers are exposed is the use of the soil employed in tobacco seedling production for food cultivation. This soil can contain residues from successive pesticide applications. The objective of this work was to verify the possible contamination of these foods, seeking to elucidate the environmental problem related to tobacco growing. Therefore, 147 rural properties with less than 25ha in area, and located within the limits of the Pardinho River Basin, in the municipal districts of Gramado Xavier, Sinimbu and Santa Cruz do Sul, were selected. Of these rural properties, 23 used seedling soil for food cultivation. Residues of acephate, methamidophos, disulfoton, chlorpyrifos, and mancozeb were analyzed in 25 samples, such as sweet potato, cabbage, beans, watermelon, cucumber and tomato. No residues were found in these samples, showing that the families of the considered rural properties, during the 2000/2001 crop season, were not subjected to contamination by consumption of the food crops produced in the tobacco seedling soil.

Key-words: Foods, pesticides, tobacco growing.

RESUMO

INCIDÊNCIA DE RESÍDUOS DE PESTICIDAS EM ALIMENTOS PRODUZIDOS EM ÁREAS DE PLANTAÇÃO DE TABACO NA BACIA DO RIO PARDINHO, RS, BRASIL. A região do Vale do Rio Pardo, na qual a bacia hidrográfica do Rio Pardinho se insere, caracteriza-se por ser a principal região fumicultora do Brasil. O cultivo do tabaco nesta região envolve o uso intenso e sistemático de pesticidas. Entre os riscos que os produtores estão expostos está a prática de cultivo de alimentos no solo usado para a produção de mudas, o qual pode conter resíduos das sucessivas aplicações de pesticidas. Verificar a possibilidade de contaminação destes alimentos foi o objetivo deste trabalho, visando contribuir para a elucidação da problemática sócio-ambiental relacionada ao cultivo do tabaco, ainda pouco estudada. Para isso, selecionou-se 147 propriedades rurais de até 25ha, localizadas dentro dos limites da bacia hidrográfica do Rio Pardinho, nos municípios de Gramado Xavier, Sinimbu e Santa Cruz do Sul. Destas propriedades, foi verificado que em 23 foram utilizadas as áreas de canteiro de mudas para o cultivo de alimentos. Foram analisados os resíduos dos pesticidas acefato, metamidofós, disulfoton, clorpirifós e mancozeb, em 25 amostras de alimentos provenientes destas propriedades, entre os quais aipim, batata-doce, couve, feijão, melancia, pepino e tomate. As metodologias de análise empregadas foram a de evolução de dissulfeto de carbono para a determinação dos resíduos de mancozeb, e da cromatografia gasosa para os demais princípios ativos. Não foram encontrados resíduos nas amostras analisadas, mostrando que as famílias das propriedades consideradas, durante a safra 2000/2001, não estiveram sujeitas a contaminação pelo consumo dos alimentos produzidos no solo dos canteiros de mudas de tabaco.

Palavras-chave: Alimento, pesticidas, plantação de tabaco.

INTRODUCTION

This work was part of the study "Impacts of tobacco culture on the ecosystems and on human health in the area of Santa Cruz do Sul, RS", which was funded by the *International Development Research Centre* (IDRC) from Canada, and was coordinated by the Universidade de Santa Cruz do Sul (UNISC), in partnership with the Universidade de Campinas (UNICAMP) and with the Universidade Federal do Rio de Janeiro (UFRJ).

For some time tobacco has received special attention, mainly due to the damage smoking habit causes to human health. Only recently, other specific problems not related to tobacco consumption itself have been looked into by society and government entities linked to health and environment. Such problems are related to impacts to the environment and to human health caused by tobacco farming. However, up to now, only a few studies addressing these problems have been carried out.

In the area of Santa Cruz do Sul, southern Brazil, tobacco farming is the main agricultural activity. It began with the German immigrants in the 19th century, and became established with the introduction of tobacco companies at the beginning of the 20th century. Gradually, industries began to intervene in production and introduced the intensive and regular pesticide application.

The culture practice adopted by industries comprised periods of seedling production (seedbeds), land preparation, seedling transplantation, harvesting, curing and drying. The first steps are the ones involving the greatest quantity of agrochemicals - fertilizers and pesticides.

The present study was concerned mainly with seedling production, which is done in relatively small areas - around 45m^2 for each enclosure – and these are, sometimes, used subsequently for food growing. Depending on the extension of the area to be planted, more than one enclosure can be found on the same farm.

There are two types of seedling production: seedbeds or hydroponic systems, also called 'floats', where the seedlings develop in polystyrene trays, on a fertilized water layer (Figure 1). In both forms, significant quantities of insecticides and fungicides are applied.

The use of the float system aims to gradually replace the culture of seedlings in seedbeds. Thus, the use of the methyl bromide gas is abolished from the technological package. It was used to sterilize the soil before seeding. This is due to international agreements forbidding the use of this gas from 2005 onwards, since, not only is it extremely toxic but it also contributes to the depletion of the ozone layer.

In the Santa Cruz do Sul region, only a few properties have completely abolished the use of seedbeds during the year 2000 crop season, according to the first results of the study "Impacts of tobacco culture on the ecosystems and on human health on the area of Santa Cruz do Sul, RS" (unpublished observations). A questionnaire applied to 147 farmers in June and July 2000 showed that the majority of farmers used both systems, thus dividing seedling production. According to the study, 31% used only the conventional system, 22% used only the float system and 47% used both systems.

After harvesting the seedlings, during their transplantation, it was not difficult to find properties utilizing fertilized soil in the seedbeds for the

Figure 1. Types of seedbeds: conventional plant bed (left) and float system (right).

production of human foods, including cucumber, tomatoes, cabbage, cassava, sweet potato, beans and watermelon. However, along with chemical fertilization, the seedbeds regularly receive loads of insecticides and fungicides for about two months.

In view of these facts, the possibility of the foods produced there being contaminated with pesticide residues was investigated. It was hypothesized that the residues remain in the soil long enough to be absorbed by the crops grown in this area.

Concerning environmental pollution, the soil matrix is important for the context, as a final destination to pollutants, even if the pollutants are originally released into other matrices (Rieder *et al.* 2000).

Several studies, carried out in developed and developing countries, have pointed out food contamination due to the presence of pesticide residues in the environment (Andersen & Poulsen 2001, Leone *et al.* 1992, Liégois *et al.* 1992, Ramalho *et al.* 2000, Rigitano & Souza 1994, Sanghi & Tewari 2001, Sanghi & Sasi 2001, Zavati & Abakerli 1999).

In the present study, the sampling included 147 tobacco farms with up to 25ha located in the Pardinho River Basin, in the countryside areas of Santa Cruz do Sul, Sinimbu and Gramado Xavier. These areas are characteristically rural, and their economy is based on tobacco farming. Of the 147 farms, 23 (16%) used seedbeds for human food production. The foods sampled were analyzed by gas chromatography (organophosphorus pesticides) and by the carbon disulphide evolution method (dithiocarbamate pesticides).

The high cost of analysis was a limiting factor in this research, since all samples collected could not be analyzed. In spite of this, important observations were made regarding the cultivation of food crops in seedbeds, amongst other social and environmental implications of tobacco farming.

EXPERIMENTAL

SAMPLING

The study area included the Pardinho River Basin. The Pardinho River is a tributary of the Pardo River, and the area includes the towns of Gramado Xavier, Sinimbu and the city of Santa Cruz do Sul, in the central region of the state of Rio Grande do Sul (Figure 2).

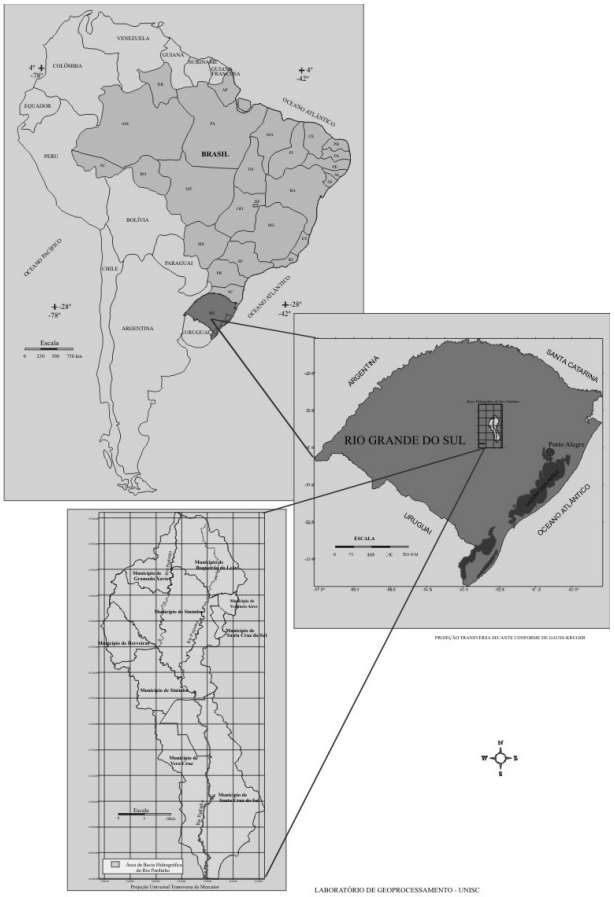

Figure 2. Location of the Pardinho River Basin.

In order to identify the farmers who grew foods for human consumption, a question was included in the sociodemographic questionnaire applied in June and July 2000 by the researchers of the project "Impacts of tobacco culture on the ecosystems and on human health in the area of Santa Cruz do Sul, RS". As a result, 22 farmers showed the intention to grow food crops in seedbeds (unpublished observations).

From November 2000 to May 2001, these farms were visited and the foods were sampled. Only one farm did not grow food crops, as had been indicated. On the other hand, food crops were sampled in two farms that had not declared the intention to grow them, totalizing 23 farms considered in the research. Table I lists the farms where samples were taken, as well as the food crops grown and the sampling dates. Table II shows the quantities, according to food type.

According to the availability of resources for the research, 25 out of 36 samples taken were selected. Food crops with only one sample collected were not considered, and to keep proportionality, samples were randomly chosen within each group. Table III lists the samples analyzed, collected from 20 tobacco farms.

Table I. List of tobacco farms and type of food sampled.

Owner Identification	District	Type of food	Sampling date
01	Sinimbu	cabbage, cucumber, tomato beans	12/07/2000 01/17/2001
02	Sta. Cruz do Sul	watermelon	01/31/2001
14	Sinimbu	cucumber	12/26/2000
19	Sinimbu	beans	03/03/2001
29	Sinimbu	beans	03/03/2001
44	Sta. Cruz do Sul	cucumber, tomato cabbage beet	11/25/2000 11/30/2000 01/03/2001
50	Sinimbu	sweet potato	05/07/2001
60	Sta. Cruz do Sul	sweet potato	05/03/2001
67	Sinimbu	lettuce, cucumber tomato	12/12/2000 01/16/2001
69	Sinimbu	cabbage	12/12/2000
81	Sinimbu	pumpkin	02/20/2001
84	Sta. Cruz do Sul	cassava watermelon	04/17/2001 01/20/2001
87	Sta. Cruz do Sul	beans, cucumber	12/07/2000
95	Sinimbu	cucumber	12/28/2000
100	Sta. Cruz do Sul	cucumber	01/13/2001
123	Gramado Xavier	cabbage	01/06/2001
125	Sinimbu	cassava melon	04/17/2001 01/18/2001
132	Sta. Cruz do Sul	watermelon	01/16/2001
134	Gramado Xavier	beans watermelon	01/22/2001 02/03/2001
135	Sta. Cruz do Sul	cucumber peanuts	12/08/2000 02/24/2001
137	Sta. Cruz do Sul	watermelon	01/20/2001
140	Sinimbu	sweet potato	05/08/2001
146	Sta. Cruz do Sul	watermelon	01/03/2001

Table II. Type of foods and number of samples taken.

Type of food	No. of Samples	Owner identification
Cassava	02	84, 125
Lettuce	01	67
Peanuts	01	135
Sweet potato	03	50, 60, 140
Beet	01	44
Cabbage	04	01, 44, 69, 123
Beans	05	01, 19, 29, 87, 134
Watermelon	06	02, 84, 132, 134, 137, 146
Melon	01	125
Pumpkin	01	81
Cucumber	08	01, 14, 44, 67, 87, 95, 100, 135
Tomato	03	01, 44, 67
TOTAL	36	

Table III. Type of food and number of samples analyzed for pesticide residues.

Type of food	No. of Samples	Owner identification
Cassava	02	84, 125
Sweet potato	03	50, 60, 140
Cabbage	03	01, 69, 123
Beans	04	01, 19, 29, 134
Watermelon	04	84, 132, 137, 146
Cucumber	06	01, 14, 44, 87, 95, 135
Tomato	03	01, 44, 67
TOTAL	25	

All samples were collected in zipped plastic bags, designed for freezing foods. At the laboratory, samples were washed under running water. They were then frozen whole, without any cutting, until

the analysis of residues was carried out, according to the method approved by the Food and Drug Administration (FDA 1994).

ANALYSIS OF ORGANOPHOSPHORUS PESTICIDE RESIDUES

Residues of acephate (Orthene, Acefato Fersol), disulfoton (Solvirex) and chlorpyrifos (Lorsban) were analyzed. Methamidophos, an acephate metabolite, was also considered for analysis. Although chlorpyrifos is not used in seedbeds, the possibility of food contamination was not discarded, since its half-life can be of up to one year, and tobacco fields usually are located near the areas where foods are grown.

Analyses were performed according to the methods recommended by the FDA (1994). Three methods were applied, according to the type of food:

Method 1: Used with samples of tomato, watermelon, cucumber and cabbage. 100g of sample was extracted with acetone, partitioned with acetone and dichloromethane, and filtered using charcoal and celite.

Method 2: Used with cabbage samples. 100g of sample was extracted with acetone, partitioned with petroleum ether and dichloromethane and filtered using charcoal, celite and magnesium oxide.

Method 3: Used with cassava, sweet potato and beans samples. 15g of sample was extracted with acetone and water, partitioned with petroleum ether and dichloromethane and filtered using charcoal and celite.

GAS CHROMATOGRAPHY

For identification and quantification of pesticides in the extracts obtained, a gas chromatograph, Varian 3300, on-column injection, column DB-1 (0.53mm x 30m) and a thermionic specific detector (TSD) were utilized.

The quantification technique used was that of the external standard, considering the average of area units for three injections.

Figure 3 shows the chromatogram of the standards and the chromatographic conditions used. The concentrations of the standards were: 0.8mg L^{-1} methamidophos, 0.78mg L^{-1} acephate, 0.60mg L^{-1} disulfoton and 0.74mg L^{-1} chlorpyrifos.

Glassware washing was tested through extraction with a blank (no sample) immediately after a recovery assay (with contaminated glassware). No interference was detected. The following glassware washing steps were taken: previous washing with acetone (distilled), running water and sponge (without detergent), soaking in alkaline Extran Merck 2.5% (v/v) for 24 hours, sulfochromic solution, running water, distilled water, oven drying, acetone, oven drying.

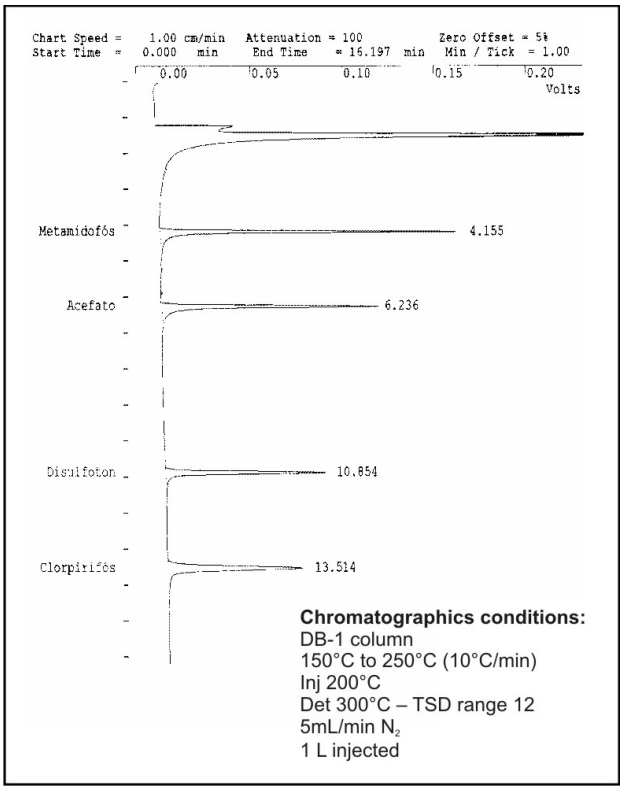

Figure 3. Chromatogram of organophosphorus pesticides.

DETECTION AND QUANTIFICATION LIMITS

For calculation of detection and quantification limits the following procedures were applied:

- Detection limit: successive dilutions of the working standard were injected until there was no peak integration in the analysis conditions. The concentration of the last dilution where signal integration was present was considered the detection limit.

- Quantification limit: 10 times the detection limit was used, and the equivalent concentration in the foods was calculated, considering the extracted quantity.

Table IV shows the detection limits (DL) found, as well as the quantification limits (QL), according to the extraction and purification methods applied for the organophosphorus pesticides considered.

Table IV. Detection limits (DL) and quantification limits (QL) of the employed methods.

Pesticide	Method 1		Method 2		Method 3	
	DL ($ng\ \mu L^{-1}$)	QL ($mg\ kg^{-1}$)	DL ($ng\ \mu L^{-1}$)	QL ($mg\ kg^{-1}$)	DL ($ng\ \mu L^{-1}$)	QL ($mg\ kg^{-1}$)
Methamidophos	0.0043	0.008	0.0043	NC*	0.0043	0.092
Acephate	0.0038	0.010	0.0038	NC*	0.0038	0.118
Disulfoton	0.0030	0.004	0.0030	0.004	0.0030	0.046
Chlorpyrifos	0.0037	0.006	0.0037	0.006	0.0037	0.069

SOURCE: elaborated by the authors. *NC: not calculated.

Method 2, applied to cabbage samples did not recover the acephate and methamidophos residues. This method had to be used after verifying that Method 1 produced an extract with many co-extractives for the cabbage samples, which could damage the chromatographic column.

For validation of the methods used for the analysis of organophosphorus pesticide residues, fortification of food samples was performed. These samples were obtained at the agro-ecological market of Santa Cruz do Sul, RS, Brazil, from farms located in the Pardinho River Basin. Fortification varied from 0.06mg kg^{-1} to 0.15mg kg^{-1}.

ANALYSIS OF DITHIOCARBAMATE FUNGICIDE RESIDUES

Residues of dithiocarbamate fungicide mancozeb (Dithane, Manzate) were analyzed according to the carbon disulfide evolution method proposed by Keppel (1969).

With this method, dithiocarbamate fungicide is heated with a solution of stannous chloride and hydrochloric acid to produce carbon disulfide, which is distilled, purified and collected in an ethanolic solution of cupric acetate and diethanolamine. Two yellow dithiocarbamate complexes are formed, which are measured by spectrophotometry. According to Keppel (1969), the quantification limit of this method is of 0.1mg kg^{-1}, for 500g of sample used in the test. In conformity with the available quantities of sample, different amounts were used for the analysis, and this caused the quantification limit to vary between 0.3, 0.4, and 0.5mg kg^{-1}.

For validation of the method, fortification of 1mg kg^{-1} of mancozeb was performed in watermelon samples from the agro-ecological market of Santa Cruz do Sul.

RESULTS AND DISCUSSION

In order to consider the sensitivity of the methods used, Table V shows the maximum residual limits (MRL) allowed for the analyzed types of foods, according to the Brazilian National Health Surveillance Agency (ANVISA 2001). For the

Table V. Maximum Residual Limits of pesticides in foods.

Food	Maximum Residual Limit ($mg\ kg^{-1}$)				
	Acephate	Methamidophos	Disulfoton	Chlorpyrifos	Mancozeb*
Cassava	-	-	-	-	-
Sweet potato	0.2	0.05	0.5	0.01	0.1
Beans	0.5	-	0.5	0.1	0.5
Cucumber	-	1	-	-	0.5
Cabbage	0.5	0.5	-	1	2
Tomato	0.5	-	0.5	0.5	3
Watermelon	-	0.5	0.5	-	0.5

SOURCE: ANVISA (2001), FAO/*Codex Alimentarius*(2001).
* Maximum residual limits refer to carbon disulfide.

methamidophos, which are not included in the Brazilian legislation, the MRLs are provided by the Codex Alimentarius, by the Food and Agriculture Organization (FAO 2001).

According to the data shown in Table IV, quantification limits for organophosphorus pesticides (acephate, methamidophos, disulfoton and chlorpyrifos) are well under the MRLs, showing the good sensitivity of the methods used. Except for sweet potatoes, the quantification limit for mancozeb equals the smaller MRL in the list, being also adequate for the study.

The results obtained in the recovery tests for method validation of the organophosphorus pesticides are given in Table VI. According to the FDA (1994), recoveries above 80% are considered complete. Recoveries of up to 110% are considered normal. Between 50% and 80%, the recoveries are considered partial and under 50% they are considered low.

For cabbage samples, low recoveries were only obtained with the application of method 2 (acephate and methamidophos) This method was used to eliminate co-extractives in cabbage, in order to prevent damage to the chromatographic column. Method 1 was applied to cabbage samples from farm number 69. The remaining cabbage samples were extracted with method 2.

The recovery obtained in the fortification test with the watermelon sample for method validation for mancozeb (dithiocarbamate) was of 82.9%.

Tables VII and VIII present the results obtained from the food sample analyses (see Table 3). None of the samples showed a positive result.

The first samples were collected in late November and in early December 2000 (Table I). That was about 160 days after the seedlings were removed in the low areas (Santa Cruz do Sul and Sinimbu countryside areas) and around 120 days in the high areas (Gramado Xavier countryside area). Considering the half-life of pesticides shown in Table IX, there probably was a higher chance for ETU (mancozeb metabolite), chlorpyrifos, thiabendazole and disulfoton residues to be still present in the soil.

Table VI. Results obtained from recovery tests for the analysis of organophosphorus pesticide residues, according to the type of food and method applied (n.r. = not recorded).

Food	Method	Recovery (%)			
		Acephate	Disulfoton	Chlorpyrifos	Methamidophos
Cassava	3	85.2	101.7	85.7	65.3
Sweet potato	3	91.6	110.9	90.5	76.5
Cabbage	1	99.4	71.4	70.8	111.8
Cabbage	2	n.r.	77.3	64.6	12.4
Beans	3	71.6	105.9	80.9	54.7
Watermelon	1	108.6	74.8	67.4	-
Cucumber	1	94.2	47.9	70.1	90.6
Tomato	1	97.4	73.3	73.0	96.5

Table VII. Results of the analysis of organophosphorus pesticide residues.

Food	Organophosphorus pesticide residues					
	Method 1		Method 2		Method 3	
	No. of samples analyzed	Positive residues	No. of samples analyzed	Positive residues	No. of samples analyzed	Positive residues
Cassava	-	-	-	-	2	0
Sweet potato	-	-	-	-	3	0
Cabbage	1	0	2	0	-	-
Beans	-	-	-	-	4	0
Watermelon	4	0	-	-	-	-
Cucumber	6	0	-	-	-	-
Tomato	3	0	-	-	-	-

Table VIII. Results of analyses of dithiocarbamate pesticide residues.

Food	Samples analyzed	Dithiocarbamate pesticide residues Positive residues >0.3 mg kg^{-1}	Positive residues >0.4 mg kg^{-1}	Positive residues >0.5 mg kg^{-1}
Cassava	2	-	-	0
Sweet potato	3	-	-	0
Cabbage	2	-	-	0
Beans	4	-	-	0
Watermelon	4	0	-	-
Cucumber	6	0	-	-
Tomato	3	-	0	-

Table IX. Half-life and water solubility of pesticides used on tobacco.

Pesticide	Half-life (days)	Water Solubility
acephate (methamidophos)	<3-6	790 g L^{-1}
	(2-12)	(90 g L^{-1})
disulfoton	7	25 mg L^{-1}
mancozeb (ETU)	1-7	6 mg L^{-1}
	(49-70)	-
imidacloprid	48-190	0.5 g L^{-1} (200°C)
iprodione	7-60	13 mg L^{-1}
thiabendazole	403	50 mg L^{-1}
chlorpyrifos	60-120	2 mg L^{-1}

Source: elaborated by the author, based on Oregon State University (2001).

However, the limitations of the assays for half-life determination have to be taken into account, and this parameter was used to construct the hypothesis in the present study. Due to the high cost and operational difficulties of field assays, half-life results usually have to be determined in the laboratory. Despite the cost, Rüdel *et al.* (1993) compared field results with laboratory assays and showed that the prediction is not sufficient. There are so many variables in the field that their reproduction in the laboratory is impossible. Therefore, it is possible that the half-life of the pesticides considered in this research for the soil of the Pardinho River Basin region is considerably different from that shown in the literature.

In fact, the analyses showed that there are no acephate, methamidophos, disulfoton, chlorpyrifos and mancozeb residues in the food samples of the listed farms. It is believed that, because food crops were not planted immediately after the seedlings were removed, the risk of contamination was greatly reduced.

On the other hand, the fact that residues were not detected could be attributed to the low water solubility of the tested pesticides, since plants absorb soluble compounds more easily. Among the pesticides used in seedbeds, only acephate has a high water solubility, but its half-life is of six days, at most.

FINAL CONSIDERATIONS

The food crops grown in tobacco seedbeds in the 20 farms selected in the countryside areas of Santa Cruz do Sul, Sinimbu and Gramado Xavier during the 2000/2001 harvesting period did not show contamination by acephate, methamidophos, disulfoton, chlorpyrifos and mancozeb residues.

Even though the analysis of pesticide half-life

led to the contamination hypothesis, this study has not confirmed it.

Although the number of analyzed samples was relatively low, not allowing inferences, the instances assessed showed that food contamination by pesticide residues in seedbeds cannot be inferred only from an analysis of their half-life. Factors such as time of planting after the seedlings are removed, soil type, and weather can play a determinant role.

For a safer evaluation of the risks to which tobacco farmers have been exposed in the last few years, it is recommendable that a study be carried out to investigate pesticide degradation, considering regional weather and soil type. However, a study of this nature would be time-consuming and highly expensive. At the present moment, it is probably more important to make an effort to avoid the use of enclosure areas.

The present study revealed that in 23 out of 147 farms considered (16%), seedbeds were used for the production of foods for human consumption. In previous years, this number was possibly higher, especially when the float system had not yet been introduced. Therefore, every farmer had a "well fertilized" soil available after the seedlings had been removed. In 2005, when the float system is adopted by all farmers, attention will have to be given to the final destination of the water in the system's pools, since the quantity of pesticides is the same as that applied in conventional seedbeds.

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