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FACULTY OF ENVIRONMENTAL SCIENCES AND ENGINEERING

DOCTORAL SCHOOL IN ENVIRONMENTAL SCIENCES

PARTICULARITIES REGARDING PESTICIDE USE AND EXPOSURE IN RURAL AREAS

- SUMMARY OF DOCTORAL DISSERTATION -

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CONTENTS

The summary of the doctoral dissertation presents the personal experimental research results, general conclusions and a selective bibliography. The summary was edited using the same notations for chapters, tables, pictures or figures used in the text of the thesis.

Keywords: pesticide, spray drift, exposure dose, pesticides degradation.

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1. INTRODUCTION

Ensuring a proper environmental quality and protecting it - as a necessity of survival and progress - is an issue of major concern and certain actuality for the social evolution, the main objectives being the adoption of solutions to mitigate pollution and increase the overall environmental quality.

With the population growth - which more than doubled during the last 50 years, reaching seven billion in 2013 - and the need for food, agricultural production increased similarly, even if the productive arable lands expanded only by 10% (Köhler & Triebskorn, 2013). The population growth forecast of almost 10 billion in 2050, determines the need of further changes in agriculture, livestock and manufacturing industries in terms of large-scale production, by doubling the present agricultural production (Ray et al., 2013).

Increasing agricultural production is the preferred option in comparison with the expansion of cultivated areas (deforestation, drainage, etc.). In these circumstances, crops protection by wide and intense use of pesticides became common, with an impact of global importance. Side effects are usually outsourced, being more severe for the society as a whole than for the agricultural sector in which they operate, and therefore stimulation of corrective measures is poor. Nowadays, organic farming is expensive and not capable of a production to ensure food on a large scale.

Generally, the adverse effects of pesticides upon the environment depend on the properties of incriminated substances, soil characteristics, and also plant species and environmental conditions. The ability to persist in the environment and/or to bio-accumulate is also relevant for the effects on the environment and living organisms. Pesticides are mainly adsorbed to target organisms and may spread in environmental factors. It is important to note that post-application drift and displacement of pesticide residues may cause unintended adjacent contamination as well as possible human exposures.

Agriculture is one of the most dangerous activity sectors globally, being associated as well as livestock with an increased risk of diseases among workers. Along with conditions through injury that occur in farmers using machinery, handling natural and artificial fertilizers and various chemicals including pesticides, continue to be associated with injuries, diseases and even deaths in modern farms.

The worldwide use of different groups of pesticides leads to global cross-contamination and intentional/unintentional exposure nearly all people are inevitably exposed. Organophosphates constitute a large class of pesticides that are used mainly in agricultural practice. As a result, organophosphates are involved in more occupational poisoning cases than any other single class of insecticides. Organophosphorous pesticide residues have been detected at levels above the limit of quantification, and sometimes even exceeding maximum residue levels (MRLs) in many agricultural products; therefore, low-level dietary exposures to organophosphorus pesticides is very likely. Occupational exposures to organophosphorus pesticides dwarf environmental exposures; however, special populations, such as farm workers and children, may receive higher exposures. Therefore, with the widespread use of pesticides, concerns regarding their impact on human health are more pronounced.

Long-term contact with pesticides may disrupt the function of different mechanisms and systems of human organism: the nervous, endocrine, immune, reproductive, renal, cardiovascular systems and respiratory tract. There is much evidence on the association between pesticide exposure and incidence of human chronic diseases, including cancer, Parkinson's disease, Alzheimer's disease, multiple sclerosis, diabetes, chronic diseases, cardiovascular and renal diseases and aging in general. Studies performed in recent years have focused on the mechanism of phenomena occurrence due to toxicity of compounds and targeted effects on fetal growth process both in human and animal experimental models. Although epidemiological studies have shown inconsistent results especially in children, however some observations have been reported that require further study.

Human health risk assessment is the process by which it is estimated the nature and likelihood of adverse health effects in people who may be exposed to chemicals in contaminated media in the environment, at present or in the future, assuming four successive steps: hazard identification, assessment of the dose-response relationship, exposure assessment and risk characterization (EPA, 2006). Any risk assessment should take into account that the effects vary from one person to another and in order to justify this variability, uncertainty factors become part of the risk assessment.

Biological measurements of chemicals and their metabolites in tissues and body fluids may be used to estimate the past and present exposure to chemicals, in cases where analytical methods are available.

In case of organophosphatic pesticides, availability of suitable biomarkers for absorbed doses from occupational and non-occupational exposure is still a critical issue, especially since due to organophosphatic pesticides metabolism and their rapid excretion the utility of a single biomarker measurement is uncertain. The biomarkers of exposure for organophosphatic pesticides are commonly those measured in blood and urine, but research in the field proposes new matrices such as saliva, sweat, hair, amniotic fluid, meconium, etc.

Data on environmental contamination and human exposure to pesticides are relatively limited in Romania, studies being generally focused on organochlorine pesticides class. In Romania, a number of authors have pointed the contamination of soil, water, sediments, food and dust in residences in investigated areas showing in some cases high concentrations exceeding the legislated standards and identifying as contamination mechanism the remote transport process and volatile isomers deposition from secondary sources (Tarcau et al., 2013; Ene et al., 2012; Ferencz & Balog, 2010; Neamțu et al., 2009; Covaci et al., 2006).

Also regarding organochlorine pesticides, there are studies in Romania that targeted exposure biomarkers in colostrum (Cioroiu et al., 2010), serum (Dirtu et al., 2006) and hair (Covaci et al., 2008) showing different degrees of exposure. The daily intake estimates in case of breast-fed children (Cioroiu et al., 2010) showed that some health-protection standards have been exceeded. On the other hand, the daily intake calculated either via dietary intake only or via dietary intake and/or in-home dust led to similar results from other European studies and highlighted the importance of exposure to indoor dust for the pesticide body burden. In relation to organophosphate pesticides, studies in Romania are restricted in number and addressed in addition to the development of their analysis methods from environmental factors (Culea et al., 1996) also their measurement from indoor dust (Dirtu et al., 2012), as well as in vitro evaluation of decontamination absorption and desorption of organophosphate compounds from the skin and synthetic membranes (Mircioiu et al., 2013).

Only one study focused on habits of pesticide use on small farms in rural areas, showing the lack of knowledge regarding environmental protection measures and one's own health during pest control activities (Gurzău et al., 2008).

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The initial assumption in this thesis is that at present, in small farms (of family type) in rural areas there is a lack of knowledge/errors regarding the methods of crop protection and pest control.

This thesis addresses issues aimed at achieving an appropriate level of knowledge regarding pesticide use in a rural area, quantification of area extension affected by pesticide application by an experimental model for spray drift, knowledge of pesticides transfer processes in soil and plants under area-specific conditions, as well as estimation in experimental model of potential effects induced upon human health of an organophosphatic pesticide based on calculated exposure doses. Not lastly, the thesis aimed at raising public awareness on the correct use of pesticides based on the principle of respect for the environment and human health and promoting alternative methods for plant protection.

6. STUDY DESIGN

In order to achieve the proposed objectives, a study design was developed consisting of consecutive steps and Sâncraiu locality in Cluj County as the target rural area.

The study started with a questionnaire-based investigation of a representative populational sample, which aimed to obtain information regarding the pesticide use habits in family farms, demographic data, data on lifestyle and personal history pathological data.

The second step of the study, based on information regarding the protection measures to be taken by the operator during pesticide application, was to assess the respiratory health status of questionnaire-based investigated subjects.

As an assessment baseline of the potential health effects associated with pesticide use, a study was conducted on morbidity structure and dynamics for the population of Sâncraiu locality over a representative period of time (2003-2013).

Starting from the premise that health damage can also occur outside the spraying area, an experimental model was developed on spray drift of a less dangerous pesticide of moderate toxicity (organophosphatic) in field conditions.

Since exposure to pesticides can also occur during re-entering the spraying area (contact with contaminated soil), the next step was to develop an experimental model for the study of an organophosphatic pesticide degradation/transfer in soil and vegetation in field conditions.

Biomonitoring of exposure to chlorpyrifos-methyl by analyzing 3,5,6-trichloro-2-pyridinol metabolite (TCP) in urine after the spraying process in two different situations in relation to wearing or not the personal protective equipment.

Exposure dose calculation on the basis of data obtained in experimental models regarding chlorpyrifos-methyl concentrations in air and soil and effects assessment on the basis of the reference dose.

Development of measures on proper pesticides use and alternative methods for plant protection in order to reduce the impact on the quality of life in investigated rural area.

7. STUDY AREA

The study area for this thesis was Sâncraiu locality (geographic coordinates: 46°34′49″N 22°43′20″E46°34′49″N 22°43′20″E) (Figure 5).

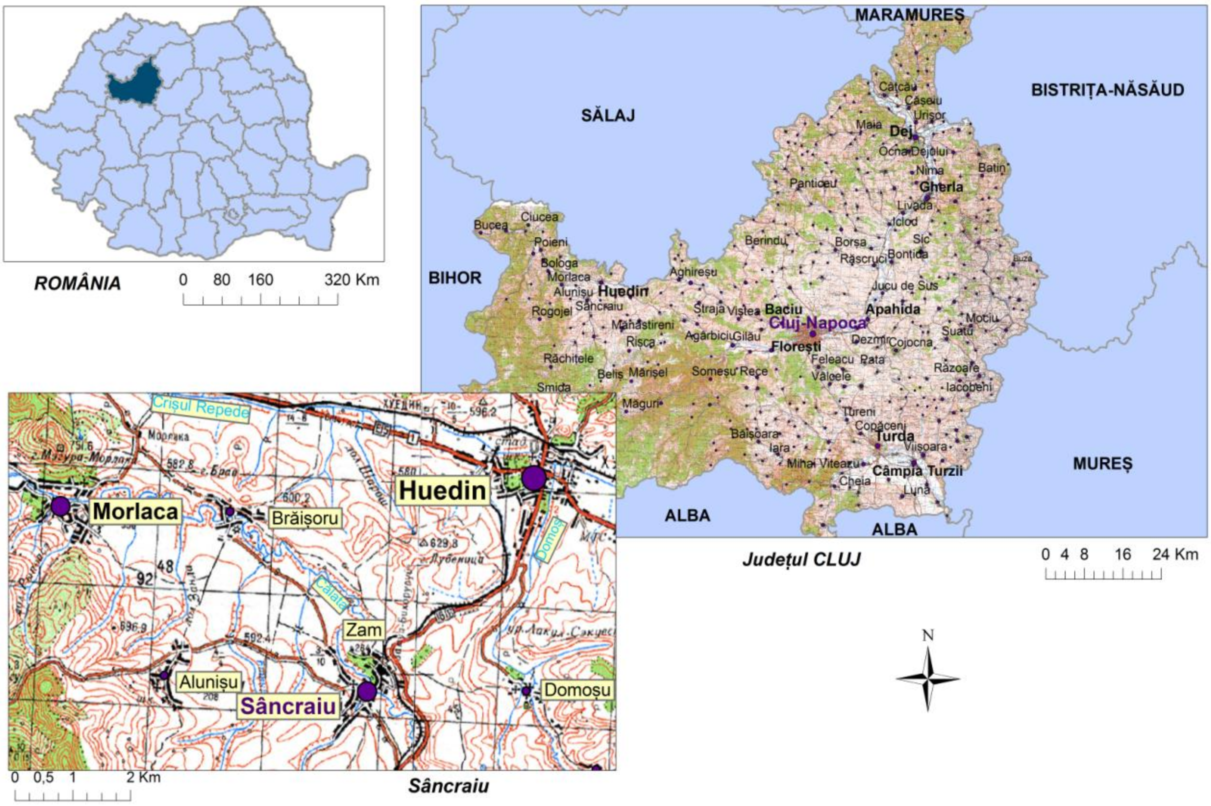


Figure 5: Framing of Sâncraiu locality in the area

Sâncraiu is a locality in Cluj County, Romania that consists of five villages (Sâncraiu village - village residence, Alunişu, Domoşu, Brăişoru, Horlacea). Sâncraiu locality has a total area of 56.83 km^2 and is located at an average altitude of 600 m, at a distance of 56 km from Cluj-Napoca municipality and at 5 km from the first city, Huedin.

According to the 2002 census the total population of Sâncraiu locality was of 1856 inhabitants, in which Sâncraiu village had 1172 inhabitants (Varga, 2007).

8. CASE STUDY ON HABITS OF PESTICIDES USE IN SÂNCRAIU LOCALITY

Despite the progress achieved in the environmental domain, research concerning effects of environmental pollutants upon health are insufficient at present, not only in Romania but around the world. In order to achieve these goals, a very well organized system is needed. Focus should be set on the relationship between population exposure to environmental pollutants and human health. Symptoms and diseases should be evaluated according to past and present exposure to pollutants of concern while sources of exposure should be investigated in terms of their effects upon human health (Gurzău et al., 2008).

In order to determine the magnitude of the problems related to the use of pesticides, a populational study was conducted during 2011 in the rural area of Sâncraiu locality, Romania.

Materials and methods

On the basis of a written consent, 100 persons were included in the questionnaire-based survey. The questionnaire consisted of open and closed questions. Demographic data, data on lifestyle, personal pathological antecedents, general information on the activities within the farm were collected. Databases and processing were performed using Excel software.

Results

The respondents sample included 100 persons residing in Sâncraiu locality, among which 50% were women, aged between 22 and 89 years old, the average age for the entire sample being 50.71 years old. One third of the respondents (33%) declared that they completed 8 primary classes, for 30% of cases the answer was "high school", 6% graduated a faculty, 26% graduated a vocational school and other 5% completed less than 4 years of high school.

Because pesticides can enter the body via many routes, namely by dermal, oral, ocular and/or respiratory pathways, the questions in this section focused mainly on food, tobacco and alcohol consumption (both the amount consumed and frequency and characteristics of consumption). 75% of respondents declared that they eat vegetables at least once a day both from their own production and from other sources. Vegetable consumption varied generally from once a week to 3 times a day. 44% of respondents declared that they eat fruits at least once a day (consumption most commonly reported by respondents).

Regarding the personal history of alcohol consumption as well as the alcohol consumption at the time of filling in the questionnaire, 16% of respondents declared that they drink alcohol 2-4 times a week, the history of alcohol consumption most frequently reported being 1 or 2 alcoholic drinks during the previous year before the questionnaire application.

Regarding smoking, 79% declared that they do not smoke and 21% stated that they smoke. Cigarette consumption for smokers was in average of 20 cigarettes a day. 14% of the smokers stated that they have smoked for less than 5 years, 14% between 5 and 10 years, 29% for 10-20 years and 43% have smoked for more than 20 years. Consumers of alcohol and tobacco have a higher vulnerability towards exposure to pesticides through absorption of larger quantities of toxic substances. Workers who smoke cigarettes while applying pesticides are at risk of exposure via the digestive pathway also.

61% of respondents declared that they own the farm where they live and work. It must be noted that, given the specific area and age of respondents, most of them (46%) stated that they have worked in the farm in question for a period longer than 30 years.

Regarding the land cultivation activity within the farm, 26% of respondents declared that they have performed this activity for a month (30 days) during the last agricultural season, 27% between 31 and 100 days and 20% for more than 100 days.

Regarding the use of harvesters or other agricultural machinery, 84% of respondents stated that they have never used them. Only 5 respondents declared an intensive use of agricultural machinery within the farm. Planting is another important activity in the economy of a farm and also an exposure route. 25% of respondents stated that that they have never performed a planting operation, 63% declared that they have planted for a period of 1-5 days and 11% between 6 and 25 days during the last agricultural season.

According to the questionnaire results, the activity in farms is mainly non-mechanized in Sâncraiu area. Manual harvest of crops has been reported as high as 49%, leading to an increased risk of pesticide contamination via dermal contact.

It has been found that animal breeding is an important activity within a farm. In most cases, several species are reared, among which the most important are: cattle (both for meat and milk) and swine. Potatoes and wheat were the most important crops for the population in study, as observed following the questionnaire application and interpretation.

Respondents answered YES (75%) when asked whether they have personally prepared or applied pesticides during their lifetime, among which 27% stated that they have applied those pesticides for more than 30 years.

Pesticides formulation and application are part of the regular activities performed by respondents besides farming and animal breeding. According to respondents' statements, the process of pesticides application is performed over a period of 5-9 days each year (90%). One respondent declared that he uses pesticides for a period longer than 40 days/year.

With regard to pesticides application, 95% of respondents (who use pesticides) use a spraying device with a tank carried on the back by the operator (backpack type sprayer). However, some farmers (9%) used a tractor-mounted sprayer and 11% used a manual sprayer.

29% of pesticides operators repair themselves the spraying or preparation equipment, without resorting to specialists.

The types of pesticides most commonly used in Sâncraiu locality during 2010 were insecticides for crops (87%), pesticides for weed control (37%) and insecticides for animals (29%), the insecticides for pets ranking last (3%).

All 75 respondents stated that they purchase the pesticides in liquid form, but 31 of these persons purchased pesticides also in solid form of powders and granules. Respondents store pesticides in various places on their property: 1 person out of 75 stores them in the house, 4 in the garage, 28 in the cellar, 39 in external auxiliary buildings and only 2 respondents do not store them at all in the household.

Calypso (77.3%) is the most widely used pesticide in Sâncraiu locality, followed by Decis (49.3%).

In Sâncraiu locality, the most widely used pesticides are in Class II (WHO, 2009a, Universitatea din Hertfordshire, 2013) - moderately hazardous - belonging to the classes of neonicotinoid, respectively pyrethroid insecticides with thiacloprid and deltamethrin as active substances.

Regarding the personal hygiene habits, most respondents (76%) wash their hands and arms immediately after pesticides application; only some of them (49.33%) bathe or shower completely immediately after application. 90% of the 75 respondents who use pesticides change their working clothes immediately after preparation and application of pesticides, even when a small amount of pesticide gets on the clothing. 8 people out of 75 change their clothes only at the end of the workday. 49.33% of pesticides applicators wash their working clothes separately from other clothes, but still a large number of them (20%) wash their working clothes together with the family clothes.

76% of all people who use pesticides in agriculture have never used protective equipment, this being due to limited education regarding the pesticides handling. Gloves and protective face masks that are very important for the prevention of pesticides poisoning are used to a small extent (Figure 17).

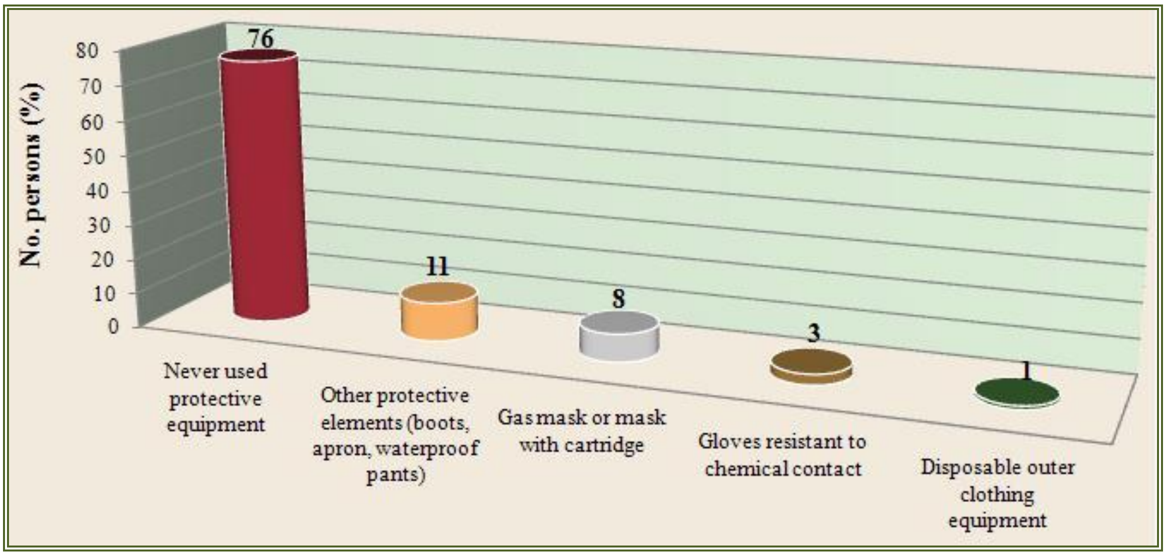


Figure 17: Protective equipment use by investigated population

Working in agriculture can become dangerous in many ways, thus the use of protective equipment is required. The personal protective equipment does not prevent accidents, but may prevent or reduce injuries or even death. The protective equipment should be carefully chosen in order to fit the users.

The extensive pesticides use in agriculture represents the most important route of exposure for the rural population. Public concern regarding the impact of pesticides on human health has significantly increased lately, a series of studies highlighting the potential effects that cause mainly chronic diseases. Application of pesticides in agricultural practices pose a high risk as polluting substances may be carried by the wind at long distances from the target areas and determine adverse effects upon populations located outside the areas of chemicals application. Children are more susceptible to the effects of pesticides due to increased exposure through food and breast milk, and a less developed immune system and higher life expectancy, having time to develop diseases with long latency period. (Gurzău et al., 2008).

CONCLUSIONS

The investigated population has past and present exposure to pesticides due mainly to non-mechanized agricultural activity.

A characteristic of the investigated populational group was that 27% of them applied pesticides for more than 30 years.

On the whole, investigated persons declared an average duration of pesticide application between 30 and 100 days per year, noting that 20% of respondents apply pesticides for more than 100 days per year.

The investigated population prepares/applies its own pesticides and repairs/maintains the spraying equipment by itself.

Pesticides most widely used by the community in study are Calypso and Decis, belonging to the classes of neonicotinoid and pyrethroid insecticides, being moderately hazardous.

Some of the respondents have used during their lifetime pesticides that at present are off the market (DDT, Regent).

Alcohol and tobbaco consumption existent in the investigated community increases vulnerability to toxic substances (pesticides), all the more as the declared respiratory and cardiovascular chronic pathology is frequent.

Cereals, vegetables and/or fruits produced in their own farms are intended mainly for family consumption.

The most widely used applicators are those in the form of spraying devices, especially for insecticides used on crops.

Pesticides are stored in different places on people's properties.

During pesticides application, respondents do not use full protective equipment.

Regarding the personal hygiene habits, most respondents (76%) wash their hands and arms immediately after pesticides application; only some of them (49.33%) bathe or shower completely.

90% of the 75 respondents who use pesticides change their working clothes immediately after preparation and application of pesticides, even when a small amount of pesticide gets on the clothing. 8 people out of 75 change their clothes only at the end of the workday.

Public education regarding pesticides handling is completely missing.

9. ASPECTS OF MORBIDITY IN THE POPULATION OF SÂNCRAIU LOCALITY

A particularly high risk of exposure to hazardous chemicals is associated with pesticides use in agriculture, especially organophosphate pesticides that are the most commonly used insecticides on a large scale worldwide. Exposure to pesticides has been associated with an increased incidence of non-Hodgkin's lymphoma, multiple myeloma, sarcoma of soft tissue, lung sarcoma and pancreatic, stomach, liver, gall bladder and bladder cancers, Parkinson's disease, Alzheimer's disease and impairment of reproductive function. Other chronic diseases generally associated with exposure to pesticides are asthma, cardiovascular disease, chronic obstructive pulmonary disease. Given these findings, identification of populations at risk is a very high priority.

9.1. Pathological antecedents in the investigated group

Materials and methods

A sample of 100 inhabitants was selected of the general population of Sâncraiu locality and invited to give their consent to participate in a questionnaire-based study. These questionnaires collected data on a number of diseases known to be related to exposure to pesticides. Databases and processing were performed using Excel software.

Results and discussions

The pathological personal antecedents reported by persons enlisted in this phase of the study consisted of acute and chronic diseases prevailing cardiovascular diseases and pneumonia, respectively. Other types of chronic pathology encountered in order of frequency were: nervous system diseases, renal diseases (excluding renal lithiasis), asthma, COPD, diabetes, chronic bronchitis, lung emphysema, Hodgkin lymphoma, cancer with other locations and depression.

After questionnaire processing, several subjects were identified for whom various symptoms were positively related to the use of pesticides. 5.33% of these subjects sometimes report skin irritations, 4% report eye irritations and 1.33% headaches and sometimes dizziness. Other subjects associate the frequent pesticides use with chest discomfort (4%) followed by eye (2.67%) and skin (1.33%) irritations, nausea, vomiting, headaches, fatigue, nervousness, and depression (1.3 %).

In this study, only 3 persons reported chronic respiratory diseases and they were not those who reported association of cough with pesticide exposure. Moreover, subjects who reported association of cough with pesticide exposure were smokers and therefore association of this specific exposure with respiratory symptoms is difficult to be confirmed.

9.2. Characterization of the general population's health status in Sâncraiu locality

Materials and methods

In order to characterize the population health status in Sâncraiu locality, data on the number and types of diseases (ICD 10 coding) were collected from the records kept by the local family doctor. Data were extracted for the period 2003-2013, when healthcare was provided by the same local physician. We calculated the specific causes morbidity and age and prevalence of chronic diseases. Cause-specific morbidity and age groups shows the frequency of new cases of a specific disease in a given territory and over a period of time. In the case of prevalence, chronic disease occurs only once as a new case.

Age groups and cause-specific morbidity was calculated using the formula:

$$\frac{\text{Nr. cazuri noi de boală "X" la vârsta "Y"}}{\text{Nr. locuitori de vârsta "Y"}}\ X\ 100\,000$$

The prevalence of chronic diseases, accounting for all new and old disease cases, was calculated using the formula below:

$$P_r = \frac{b_n + b_v}{L}\ X\ 100$$

where:

Pr = the total prevalence

bn, bv = new diseases discovered and previously known diseases

L = the average number of residents

Results and discussions

Cause-specific morbidity and age groups registered with GP during 2004-2013 was calculated by dividing the number of cases to 100,000 inhabitants.

Chronic respiratory diseases in children reflect specific morbidity 4390.2 $^{0}/_{0000}$ (specified by asthma morbidity was 487.8 $^{0}/_{0000}$). Specific morbidity by anemia (2682.9 $^{0}/_{0000}$) mainly nutrition, is a surprising fact for a locality with a relatively high standard of living. The registered chronic diseases in children included also heart disease and chronic hepatitis. The incidence of acute diseases is dominated by diseases of the upper respiratory tract (257,987.8 ± 109,534.3 $^{0}/_{0000}$) and lower respiratory tract (92012.2 ± 63895.2 $^{0}/_{0000}$), respectively. Other acute diseases with much lower incidence, but recorded in children from Sâncraiu locality are allergic dermatitis, urticaria and erythema, conjunctivitis, gastritis and duodenitis.

Incidence ranking of chronic diseases in adults (average values 2004-2013) showed that the group of diseases that dominate the morbidity spectrum in Sâncraiu locality is that of cardiovascular diseases (5933.5 $^{0}/_{0000}$) and among them ischemic heart diseases and hypertension are of overwhelming majority (1451.0 and 573.0 $^{0}/_{0000}$). At big difference, chronic respiratory diseases occupy the second place (1099.8 $^{0}/_{0000}$), this group of diseases including diseases of the upper and lower tracts and chronic pulmonary heart. Other groups of diseases follow in descending order including malignancies that occupy the 5th place (360.4 $^{0}/_{0000}$).

Regarding incidence of acute conditions calculated for the same period, acute diseases of the upper and lower respiratory tracts (13123.8 ± 3910.7 $^{0}/_{0000}$ respectively 8539.7 ± 4183.7 $^{0}/_{0000}$) occupied the first two places, also for children. Other acute diseases presented much lower incidences and on the whole, incidence of acute diseases as ranking is the common one among adult population.

The following is the annual evolution of recorded diseases among adults and children between 2003-2013 in Sâncraiu locality.

The diagrams for children and adults were superposed to the reporting scale - the left side for children and the right side for adults. Due to the irregular curve of incidents, a 3rd order polynomial trendline was applied. Polynomial trend is used to describe nonlinear phenomena.

Nutritional deficiencies anemia observed mainly in children, but not excluded among adults, had variable incidences: in children ascending values between 2006-2009 with peaks in 2005, 2009, 2010 and 2012 and the lowest values recorded in 2013, in adults the highest values were recorded in 2008 and a downward curve between the years 2010-2013.

Specific morbidity by conjunctivitis has fared irregular both in children and in adults, ascertaining the maximum value in children and minimum in adults in the year 2010.

Acute infections of the upper and lower respiratory tract (specific morbidity), between the years 2004-2013, showed a decreasing trend in both cases.

Chronic diseases are generally characterized by their slow progression and their long term duration, and are considered to be the main cause of death throughout the world. There is evidence, even though limited, of exposure to pesticides and other types of chronic human diseases, including chronic fatigue syndrome, autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis (Mostafalou & Abdollahi, 2013).

With regard to chronic diseases of the upper respiratory tract (Figure 21) an irregular evolution is observed with a downward trend in children between 2004-2006 and 2010-2013. Unlike children, incidence variability was lower in adults for this type of diseases.

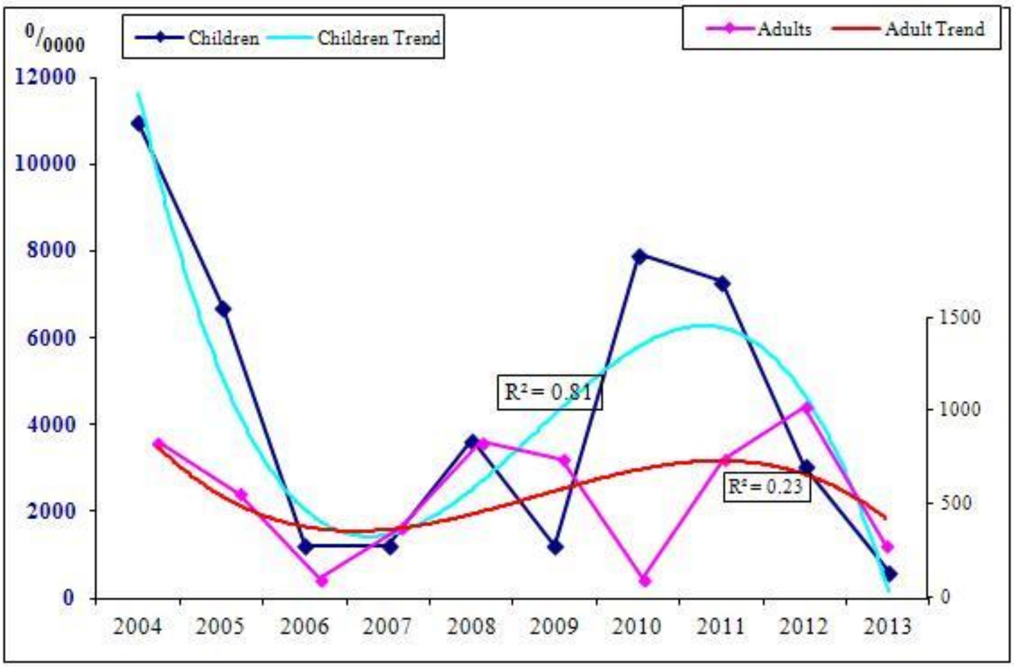


Figure 21: Specific morbidity due to chronic upper respiratory disease

Among chronic lung diseases, asthma remains a disease with increasing frequency and the most severe consequences worldwide. The incidence of asthma in children was investigated during years in study, among which two years (2006 and 2012) had maximum values and four years (2004, 2007, 2011 and 2013) in which no case of disease was recorded. The incidence evolution is more fluctuating in adults than in children.

Skin diseases (allergic contact dermatitis, urticaria and erythema) have recorded a variable but similar evolution in adults and children, with maximum values in 2004, 2009 and minimum values in 2007, 2012.

Malignant tumors were centralized in the family doctor’s records only since 2005, having a prevalence with a significant upward trend since 2007, with a peak recorded in 2013.

Among the group of metabolic diseases, a net upward trend was observed in the prevalence of diabetes, with a number of new recorded patients between 0.4 and 0.64% in 2006. The increasing observed prevalence since 2008 (similar to the national phenomenon) is mainly due to the national health programs conducted between the years 2008-2009 that allowed the diagnostic of an increased number of new cases.

Among cardiovascular diseases very common in the investigated community hypertension recorded high prevalence (with a number of new records above 2 %) in 2006, 2007, 2009 and 2010. The upward trend was also observed for ischemic heart disease with a contribution of newly recorded patients over 2 % in 2005 and 2007.

Prevalence of the existent cerebrovascular diseases is relatively constant ranging from 20 to 24 %, with records of new cases over 1 %, only in 2006 and 2009.

Regarding chronic lung diseases, the chronic obstructive pulmonary disease (COPD) had a clear upward trend, displaying a 1 % increase of newly recorded cases.

Chronic pulmonary heart which is a severe disease has registered a number of patients in family doctor's records with an upward trend in recent years (2010-2013); another characteristic is that there are years in which no new case was recorded (2003, 2009 and 2013). Regarding cirrhosis and chronic hepatitis, the same upward trend has been observed, with less than 0.5 % new recorded cases as in all years, except in 2008 when there was a prevalence of 0.56 % for new entries. Regarding the prevalence of chronic renal insufficiency, it had an upward trend only between the years 2003-2008, with many years in which no case was recorded.

9.3. Frequency by trimesters of diseases that could be associated with pesticide use (2004-2013)

In order to see to what extent exposure to pesticides could lead to immediate effects among children and adults, the trimestrial frequency of specific diseases was calculated. Cumulative (adults and children) allergic contact dermatitis, eczema, urticaria and erythema had highest values during trimesters II and III, possibly caused by natural allergens (pollen).

Conjunctivitis calculated also as trimestrial frequency for adults and children was around 30% in trimester III and with a lower and equal frequency in trimesters I and II.

Evolution of trimestrial distribution of conjunctivitis in adults and children considered separately shows that among children the frequency decreases from trimester I towards trimester IV while among adults the frequency was relatively evenly distributed with a peak in trimester III.

Unlike adults, the highest frequency of children with allergic and vasomotor rhinitis appears during the studied period (2004-2013) in trimester II followed by trimester III, fact that does not exclude the allergic component.

Trimestrial evolution of asthma frequency in adults reveals a clear seasonal aspect, the highest frequency being identified during spring and suggesting a predominant allergic nature/component of the disease. The presence of highest frequencies of asthma in trimesters III and IV (cold season) suggests the infectious nature/component in children as during this season circulation of virus strains with respiratory tropism and re-entry of children in collectivities are the major causative factors of the disease (Figure 29).

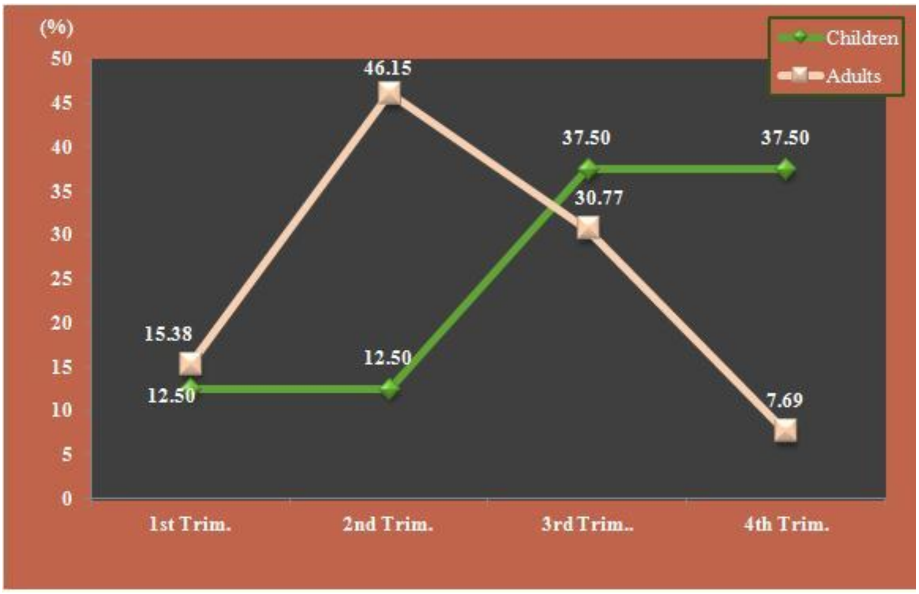


Figure 29: Quarterly Distribution (%) of asthma

Also in relation to the cold season, frequency of chronic bronchitis and COPD in adults is net superior compared to other times of the year.

Allergic contact dermatitis, urticaria and erythema develop in parallel, almost superposed as frequency (in adults and children) in trimesters II and III of the year.

Chronic diseases are generally characterized by their slow progression and long term duration, being considered at present the main cause of death all over the world. There are data, even if limited, regarding exposure to pesticides and various types of human chronic diseases, including chronic fatigue syndrome, autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis (Mostafalou & Abdollahi, 2013).

CONCLUSIONS

The personal pathological antecedents of subjects included in study reported as response to the questionnaire included diseases of the nervous, cardiovascular, respiratory and renal systems, and cancers with different locations. Among reported chronic diseases, cardiovascular diseases and pneumonia prevailed among acute diseases.

A small number of subjects reported symptoms positively associated with pesticide use namely skin irritations (sometimes 5.33%, frequently 1.33%), eye irritations (sometimes 1.33%, frequently 2.67%), headaches (1.33%), frequent chest discomfort (4%) and other symptoms such as nausea, vomiting, headaches and fatigue.

Morbidity in children (average between 2004-2013) is dominated by chronic respiratory diseases (first place - 4390.2 $^{0}/_{0000}$) and asthma (third place - 487.8 $^{0}/_{0000}$).

With regard to chronic diseases of the upper respiratory tract, an evolution with downward trend was observed in children between 2004-2006 and 2010-2013. In particular, asthma in children had oscillating incidence ranging from high values to no registration of cases.

Acute conditions were dominated as incidence by those of upper respiratory tract (257987.8 ± 109534.3 $^{0}/_{0000}$) and lower respiratory tract (92012.2 ± 63895.2 $^{0}/_{0000}$), respectively.

The group of diseases that dominate morbidity in adults in Sâncraiu locality is the one of cardiovascular diseases (5933.5 $^{0}/_{0000}$), its increased incidence being mainly due to ischemic heart diseases and hypertension.

Chronic respiratory diseases (1099.8 $^{0}/_{0000}$) represent the second cause of morbidity in adults, including diseases of the upper and lower respiratory tracts and chronic pulmonary heart; and unlike children, they had a lower variability in incidence (excepting chronic pulmonary heart). Asthma, with much lower incidences had a much more oscillant evolution than in children.

Malignant tumors had an average incidence of 360.4 $^{0}/_{0000}$ during 2005-2013, with an upward trend and a peak recorded in 2013.

Just as in case of children, acute conditions have been dominated by those of the upper and lower respiratory tracts with significant downward trends in both populational groups.

Other acute diseases with much lower incidence, but recorded in children and adults are allergic dermatitis, urticaria and erythema, conjunctivitis, gastritis and duodenitis with similar evolutions during the studied period.

Regarding the prevalence of chronic thyroid diseases, diabetes, cardiovascular diseases, COPD, chronic pulmonary heart, cirrhosis and hepatitis, it had an upward trend but with a very different contribution of new patients annually. Most new cases of diseases (average 2003-2013) were caused by hypertension (16.3 $^{0}/_{00}$) and ischemic heart disease (15.7 $^{0}/_{00}$). Cerebrovascular diseases had a relatively constant prevalence with an average contribution of 8.15 $^{0}/_{00}$ of new cases annually.

Allergic contact dermatitis, urticaria and erythema have evolved in parallel, almost superposed as frequency in adults and children, in trimesters II and III of the year.

The trimestrial distribution of conjunctivitis frequency in adults and children considered separately is different with the maximum value in children in trimester I and in adults in trimester III.

Unlike in adults, the highest frequency in children with allergic and vasomotor rhinitis appears for the studied period (2004-2013) in trimester II followed by trimester III, fact that does not exclude the allergic component.

The trimestrial evolution of asthma frequency in adults reveals a clear seasonal aspect, the highest frequency being identified in spring and in children during the cold season (trimesters III and IV). Also in relation to the cold season, frequency of chronic bronchitis and COPD in adults is net superior compared to other times of the year.

10. RESPIRATORY STATUS IN RELATION TO EXPOSURE TO PESTICIDES IN SÂNCRAIU LOCALITY

Respiratory diseases are currently a major clinical problem with respect to agricultural workers. An increased risk of respiratory problems such as asthma and chronic bronchitis among farm workers has been reported in studies (Mostafalou & Abdollahi, 2013; Hernandez et al., 2011; Kimbell-Dunn et al., 2001; Radon et al., 2001). Exposure to pesticides in relation to agricultural activities has been associated with an increased risk of respiratory symptoms (Slager et al., 2009; Sprincc et al., 2000). Farm workers are usually exposed to a wide range of different chemicals. Contact with these substances occurs during preparation, handling hoses, washing contaminated clothing and applying treatments to animals and it is not limited only to product application (Neice et al., 2005).

Materials and methods

In order to determine the magnitude of the problems related to pesticide use, a study was conducted concerning the population of Sâncraiu rural area, Romania, during 2011 (Lovász & Gurzău, 2011). From the group of 100 subjects investigated on a questionnaire-based study that began in 2011, 39 subjects agreed to participate in the respiratory status assessment based on performing several pulmonary function tests.

Spirometry is the most commonly used pulmonary function testing.

Quarterly Distribution (%) of asthma for this thesis a portable spirometer/peakflow-meter Microloop manufactured by CareFusion was used, a closed-circuit device (the entire breathing cycle, inhalation/exhalation, is achieved through the mouthpiece) that can measure 41 spirometric parameters and can store data and results for more than 2000 subjects.

Estimation of associations between pesticide exposure and pulmonary function was performed using Excel software. The statistical processing used the t-test (Student’s) for differences between averages, the "χ^2 test" (Pearson’s chi-squared test) for frequencies, P-value ≤ 0.05 was determined for the level of significance and Wilcoxon test for the determination of differences size between results.

Results and discussions

Out of a total of 39 subjects, 66.7% were women and 33.3% men, respectively, aged between 22 and 72 years old, the age average being 44.94 years with a standard deviation of 13.44 years.

The average for the forced vital capacity (FVC) determined in the investigated sample was 3.63 ± 0.97 L, representing 101.64% of the calculated theoretical values. For the examined subjects, the average for the forced expiratory volume in one second (FEV_1) was 2.94 ± 0.78 L, i.e. 95.90% of the theoretical value.

The forced expiratory flow during some given intervals (FEF 25-75%) shows significant differences compared to the theoretical values. For FEF 25% there are no significant differences between the measured values and theoretical ones, instead for FEF 50% and 75% the difference is highly significant ($p=0.002$). Other respiratory function parameters showed no significant differences between the measured values and the calculated theoretical values.

Respiratory function is influenced by many factors, e.g. smoking, home heating, occupational exposure and exposure to other pollutants. Application of pesticides is a risk factor affecting the respiratory function.

A next step of the study consisted in the assessment of differences between respiratory functions in the group that apply pesticides in relation to the group that does not apply pesticides.

Student's t-test of significance was applied between subjects that do not apply pesticides and those who apply pesticides from the percentage averages (determined from estimated values) and Wilcoxon test was applied between the determined values and expected ones for each group separately. Student's t-test does not show significant differences, while Wilcoxon test has a greater significance ($p<0.01$) for FEV_1 for subjects that apply pesticides without protective equipment ($T=10$, $p<0.01$).

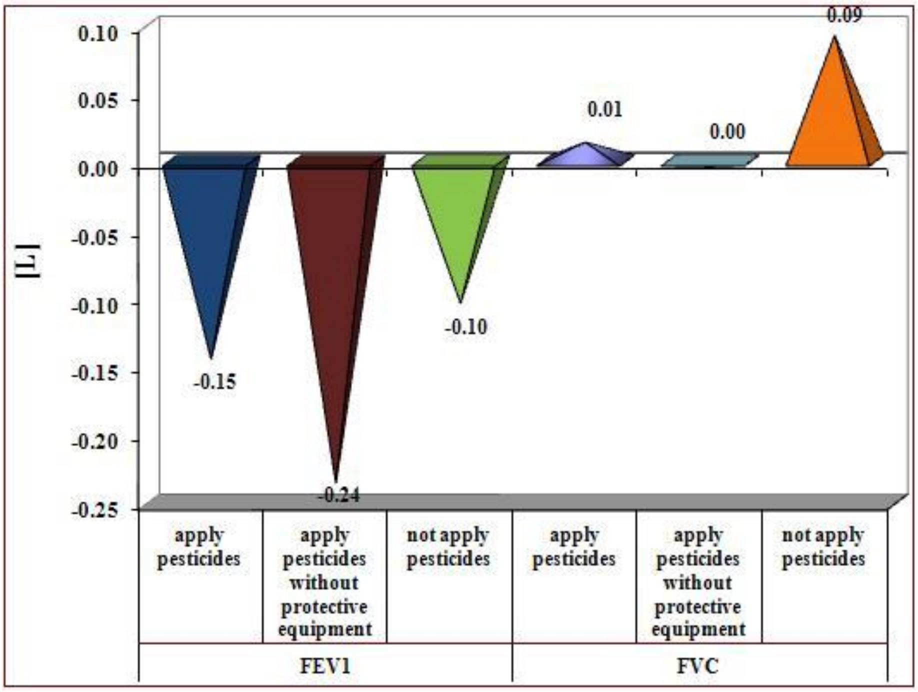


Figure 31: Differences in the performed respiratory function compared to the expected one

As shown in Figure 31, there are differences between the group exposed to pesticides and the unexposed one, but they do not reach the level of significance value for averages, also chi-squared tests (χ^2) do not show significant differences in terms of the percentage determined from the calculated theoretical one.

Other parameters also showed no significant differences neither as averages, nor as frequencies.

Wilcoxon test shows significant differences in all cases (PEF, FEF 25, FEF 50, FEF 75, FEF 25-75%) between determined values and estimated ones. In all cases $p<0.01$, except for FEF 75 for subjects that do not apply pesticides, where $p<0.02$, but note that T is lower for subjects who apply pesticides without protective equipment, which shows that differences between determined values and estimated ones are greater.

The peak expiratory flow (PEF) in subjects that apply pesticides (subjects using protective equipment and those who do not use protective equipment) was determined in a ratio of 93.96% from the estimated one, in subjects who apply pesticides without protective equipment PEF was 92.22% determined from the estimated one and subjects who do not use pesticides had the highest percentage of PEF from the estimated ones (99.75%).

Student's t-test of differences between the average values for subjects who do not apply pesticides and those who apply pesticides without protective equipment is significant ($p<0.05$) in cases of FEF 50, 75 and 25-75%.

The figure below (Figure 35) shows that subjects who apply pesticides without protective equipment had in all cases lower values of percentages determined from the estimated ones.

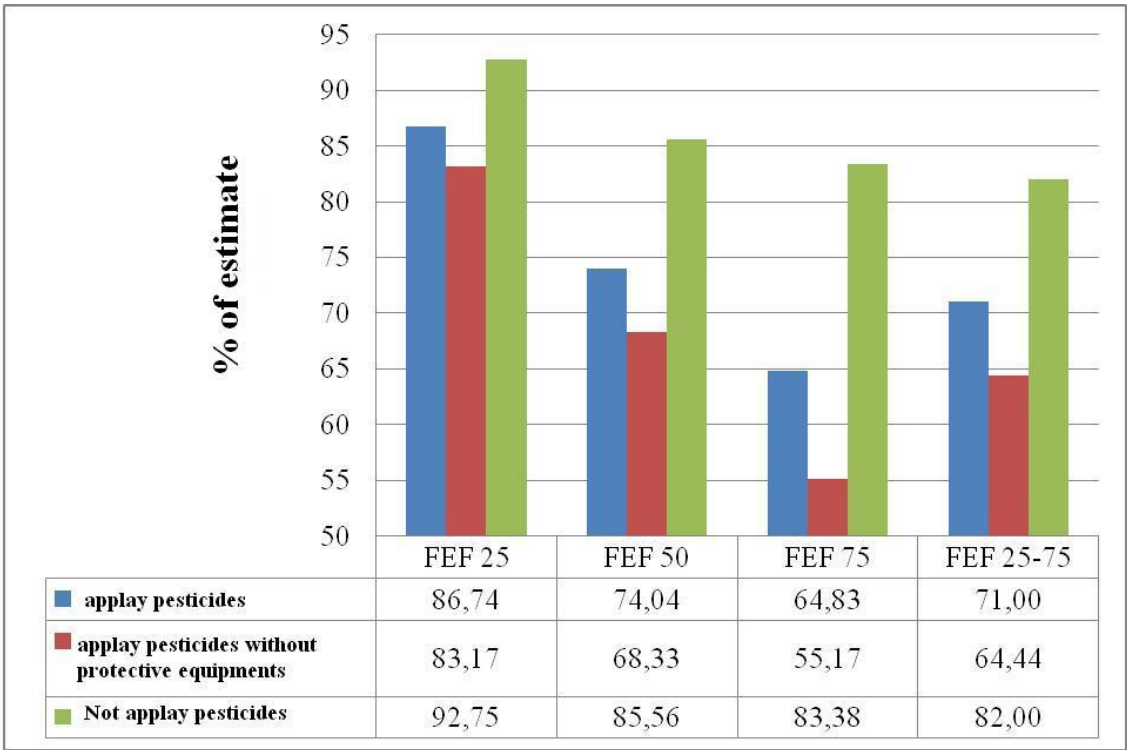


Figure 35: Average percentage values (determined from estimated ones) - forced expiratory flow during some given intervals (FEF 25, FEF 50, FEF 75 and FEF 25-75) for the group that

apply pesticides, the group that apply pesticides without protective equipment and the group that do not apply pesticides

Students's t-test was performed on the differences between averages determined by subjects who do not apply pesticides and those who apply pesticides with and without protective equipment. In the case of FEV_1/FVC the difference of averages is close to the limit of significance (p=0.06).

Wilcoxon test shows greater significant differences between the determined values and estimated ones for subjects who apply pesticides without protective equipment (p<0.01).

In the case of FEV_1/FVC ratio (which can show the existence of respiratory problems) χ^2-test shows significant differences between subjects who apply pesticides and those who do not apply pesticides when this ratio is lower than 80%.

As shown in the figure below (Figure 37) for the group that apply pesticides without protective equipment there is a big difference between the actual age and estimated one by the spirometry device, i.e. pulmonary age (-7.25 years), this difference being significant p=0.04. If we take the whole group that apply pesticides (with and without protective equipment) the difference is close to the limit of significance (p= 0.08). For the unexposed group there is very small difference between the actual age and pulmonary age (-0.35 years).

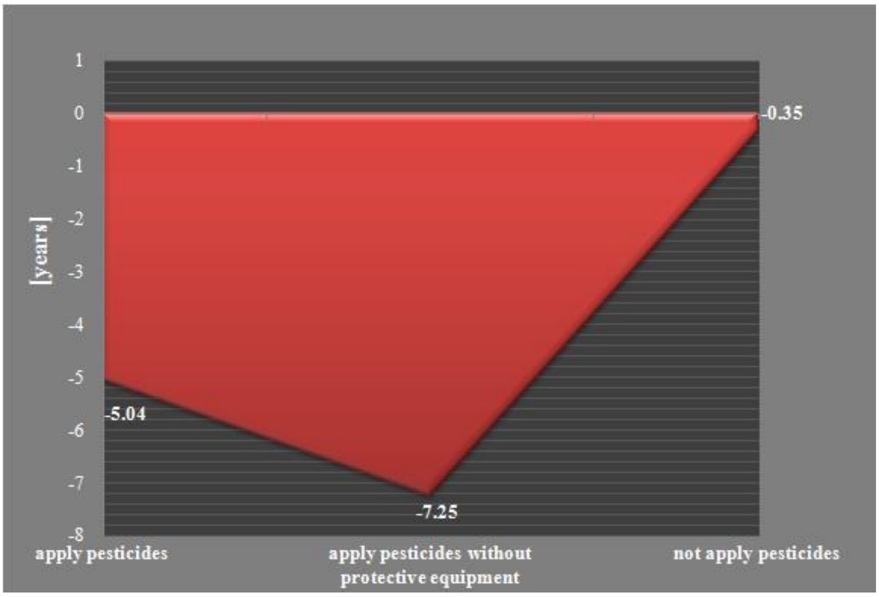


Figure 37: Difference between the actual age and pulmonary age estimated according to exposure to pesticides

Pesticides are potential risk factors for respiratory diseases among farmers, most research suggesting association with asthma and related symptoms. Several specific pesticides were associated with wheezing among farmers and artificial pesticide applicators in the health impact assessment in relation to agricultural activities (Hoppin et al., 2002; Hoppin, 2006). Pesticides have been associated with asthma among farmers (Hernandez et al., 2011; Slager et al. 2009;

Senthilselvan et al., 1992). Respiratory diseases, including chronic bronchitis are an important cause of morbidity among farmers and their families (Schenker, 2000).

CONCLUSIONS

On the whole, for the investigated group there are no significant differences between the tested pulmonary function values and those estimated by age, sex, height and weight, with the exception of parameters FEV 50% and 75%, for which it is possible to consider a degree of bronchial obstruction.

For all explored respiratory functional parameters, differences were observed between the group that apply pesticides and the group that do not apply pesticides, but they do not reach the limit of statistical significance.

There is a special situation in the case of the estimated pulmonary age which is 4.59 years higher for subjects exposed to pesticides and smoking in comparison to unexposed subjects, for whom there is no difference.

Considering that the most commonly used applicators are in form of spray devices in accordance with the results of pulmonary exploratory tests, the respiratory status of the subjects that use pesticides could be affected.

11. AIR DISPERSION OF THE PESTICIDE CHLORPYRIFOS-METHYL – EXPERIMENTAL MODEL

Since inhalation could be an important pathway of exposure to pesticides, this thesis evaluated the exposure of farmers to chlorpyrifos-methyl during an experimental spraying session. This study was conducted in May 2013 outside the built-up area of Sâncraiu locality on a waste farmland in order to highlight under field conditions the air dispersion of chlorpyrifos-methyl (O, O-dimethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate), a compound belonging to the group of organophosphate pesticides and the calculation of exposure dose by inhalation both in adults and children. The commercial product used was RELDAN 22EC, which has chlorpyrifos-methyl as the active substance and acts as an acaricide-insecticide belonging to toxicity class III (moderately hazardous according to WHO and slightly toxic according to U.S. EPA) (HSDB).

Materials and methods

In order to measure the concentration of pesticides in air during spraying, 3 spraying sessions were performed using the manual pump (Volpi) with the nozzle adjusted to the smallest holes (fine sprays) of 20 minutes each with insecticide RELDAN 22 EC, having chlorpyrifos-methyl 225 g/l as the active substance. Chlorpyrifos-methyl is a broad-spectrum pest-control organophosphate pesticide, acting through contact, ingestion and vapors in cultures of fruit trees, vegetables, vines and warehouse pests.

The spraying solution was prepared on the spot from 10 l of water and 22 ml Reldan 22 EC before each spraying session. Spraying with the manual pump was performed at a height between 0.5 m and 1.5 m from a fixed point downwind. Air samples were collected using sampling pumps with sorbent tubes (OVS-2 tube: 13 mm quartz filter, XAD-2 140/270 mg). The sampling pump was set and calibrated to a flow rate of 1 L/min by means of a rotameter prior to each sampling series. Sorbent tubes were fixed at heights of 0.9 (respiratory level of children) and 1.5 m (respiratory level of adults) and at distances of 0 m, 2 m and 5 m downwind at the spraying site. After each spraying session, the sorbent cartridges were changed.

Meteorological conditions (temperature, humidity, pressure, wind speed and direction) have been monitored during each experiment by means of Irox Pro X weather station installed at 1.5 m above the ground.

Sprayings were performed using full protective equipment (gas masks, goggles, chemical-resistant gloves, anti-chemical coveralls and rubber boots).

Samples were transported to the laboratory in cool boxes and then stored in a refrigerator until analysis.

The sampling method for measuring the pesticide concentration in air was in compliance with NIOSH manual of analytical methods, number 5600 (NIOSH 5600, 1994). The plastic cap and retainer ring made of polytetrafluoroethylene (PTPE) of the sorbent tube were removed during analysis. The quartz filter and XAD-2 frontal section were transferred to a 4-mL vial and the short socket of polyurethane foam together with XAD-2 spare section were transferred to a separate 4-mL vial.

The desorbtion solvent (2 mL acetone/toluene solution: 1/9) was added to each vial and let to rest for 30 minutes. The sample was then extracted in an ultrasonic bath for 30 minutes. By means of a Pasteur pipette the liquid phase was transferred in 1.5 ml sample vials and analyzed by a gas chromatograph coupled with mass spectrometer. (GSMS-QP2010 Plus).

Quality control was performed using specific methods for analysis accuracy assessment, in accordance with the standards and certifications set by the Romanian Accreditation Association.

For ANOVA statistical analysis, t-test (Student's) and r-test (Pearson-Bravais) were used.

Estimation of chlorpyrifos-methyl exposure doses

Exposure by inhalation

On the basis of the lowest, the highest and average concentrations of chlorpyrifos-methyl measured during the experiment, the daily inhaled dose in children (6-8 and 12-14 years old) and in adults was calculated using a public utility program, Exposure Dose Calculator belonging to ATSDR (Agency for Toxic Substances and Disease Registry) within CDC (Center for Disease Control and Prevention), which is used for evaluation in the United States.

The exposure factor represents how often and for how long a person is exposed to a contaminated environmental factor and takes into account the frequency, duration and time of exposure (ATSDR, 2005). In this case, the exposure factor is equal to 1, representing a daily exposure. Body weight is used in the equation for calculating the exposure dose because when exposed to the same amount of a substance, people with a lower body weight will receive a relatively higher dose from that substance in comparison to people with higher body weight (ATSDR, 2005).

Results and discussions

During the experiment 18 samples have been collected for the determination of chlorpyrifos-methyl concentrations in air.

Chlorpyrifos-methyl concentrations measured in the air during the three spraying series varied between 0.0003 mg/m^3 and 0.0219 mg/m^3. The maximum concentration during the three spraying series (0.0219 mg/m^3) was measured at a height of 1.5 m and at a distance of 2 m from the application site. The lowest concentration (0.0003 mg/m^3) was recorded at a wind speed of 1.6 m/s at the application site (at 0 m), at heights of 0.9 m and 1.5 m (Figure 40).

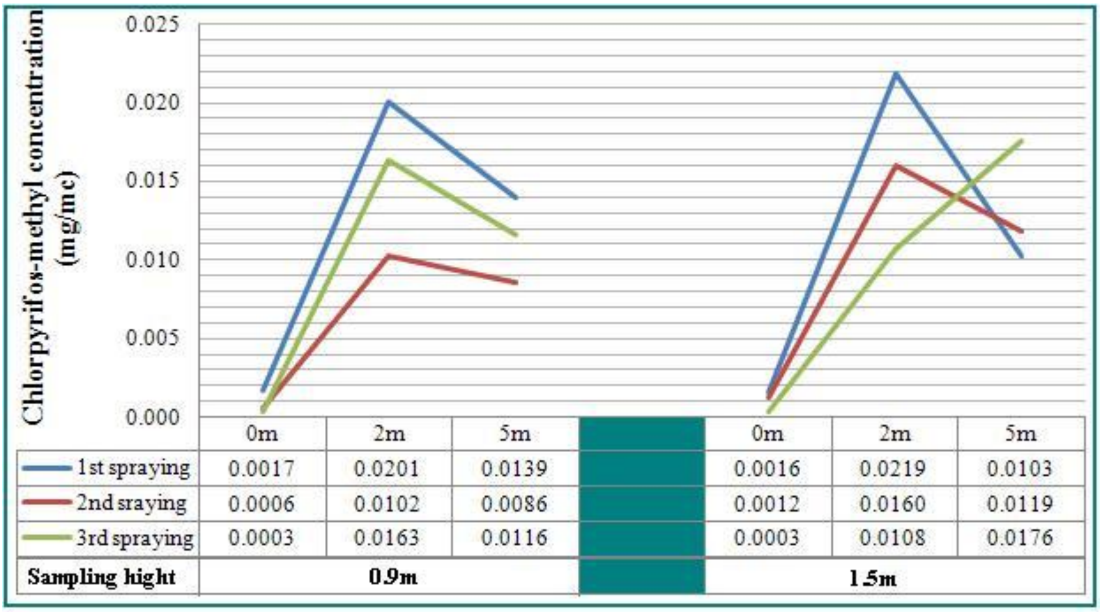


Figure 40: Chlorpyrifos-methyl concentrations in the air during the three spraying series.

With the increase in wind speed, chlorpyrifos-methyl concentrations measured in air decreased, both at 0.9 m and 1.5 m heights, at the application point (0 m). At distances of 2 m and 5 m, concentrations changed only at the height of 1.5 m, decreasing in the first case and increasing at 5 m distance. Air temperature, humidity and pressure were relatively constant in all cases.

ANOVA test for a single factor shows that there are no significant differences between sprayings ($p=0.73$) with respect to chlorpyrifos-methyl concentrations measured in air.

The correlation r-test (Pearson-Bravais) shows that there are no correlations between chlorpyrifos-methyl concentration and the sampling height ($r = 0.064$). The correlation r-test - Pearson-Bravais - unilateral test (one tail) (for a $p=0.05$ at 18 "r" determinations must be minimum 0.4).

Student's t-test for average concentrations measured at 0.9 m and 1.5 m shows no significant differences ($p=0.8$) vertically.

On the other hand, Student's t-test for differences between average concnetrations shows that there are very significant differences between samples collected horizontally at 0 m, 2 m, 5 m, respectively ($p<0.001$) (Table 19). The same thing is demonstrated by applying ANOVA test for a single factor ($f=33.6$ and $p<0.001$). Chlorpyrifos-methyl concentrations at 0 m and 2 m decrease dependent on the wind speed, while at 5 m distance concentrations increase at 1.3 m/s and 1.6 m/s, respectively.

Starting from the lowest, the highest and average concentrations of chlorpyrifos-methyl measured during the experiment, the daily inhaled dose in children (6-8 and 12-14 years old) and in adults was calculated. The daily doses determined in each case were compared with the acceptable daily intake (ADI) (WHO, 2009b).

The results showed that the daily dose in relation to different exposure levels (concentrations) decreases with age, this aspect being most obvious for the highest pesticides concentrations taken into consideration. At similar pesticides concentrations, the daily dose for children is higher in comparison to adults. In our experimental model, the calculated daily dose does not reach ADI, the calculated values representing a different percentage of ADI (80.4% for children aged 6-8 years old, 61.5% or 49.2% for children aged 12-14 years old and below 50% for adults).

Regarding gender, the same exposures are associated with higher daily doses for men versus women, both in children and adults, being due to the specific rate of inhalation.

CONCLUSIONS

Chlorpyrifos-methyl concentrations measured in air ranged from 0.0003 mg/m^3 to 0.0219 mg/m^3.

The measured average concentrations showed that there were no significant differences of dispersion vertically, while very significant differences were highlighted between concentrations measured in samples collected (dispersion) horizontally from 0 m, 2 m, 5 m, respectively.

The main factor that influenced chlorpyrifos-methyl concentrations in the deviation horizontally was the wind speed by observing an increase of concentrations at larger distances from the spraying point.

The obtained data showed that pesticide dispersion dependent on the wind speed leads to the loss of a significant quantity of the substance outside the spraying area. These losses can pose health risks for the population located outside the spraying area at least equal or greater in comparison to people exposed on the spraying site.

The daily dose calculated in relation to different levels of human exposure decreases with age; same exposures are associated with higher daily doses for men versus women, both in children and adults.

In this experimental model, the calculated daily dose represented between 50% and 80.4% of the acceptable daily intake, being thus lower than the protective values for human health.

12. DEGRADATION IN SOIL AND ABSORPTION BY PLANTS OF THE PESTICIDE CHLORPYRIFOS - METHYL - EXPERIMENTAL MODEL

The behavior of a pesticide determines its fate in the environment components, including plants. Pesticides can be absorbed, accumulated, metabolized in plants and/or released into the environment. These processes determine both the impact of pesticides on plants and the characteristics of pesticide residues.

Our research was aimed at monitoring degradation in soil and uptake by plants of the pesticide chlorpyrifos-methyl in field conditions and calculation of the exposure dose to contaminated soil (dermal contact and ingestion) in adults and children.

Materials and Methods

The experimental model of the pesticide chlorpyrifos-methyl degradation and absorption by plants was conducted between 05.10.2013 - 27.04.2014 and consisted of:

(05/10/2013) Selection of the study area (225 m2), uncultivated soil from a rural agricultural area, outside Sâncraiu city limits, which was not worked in the last 10 years.

Selection of 10 sampling sites, of which five areas (A-E) where the ground was covered with vegetation (marked in green) and 5 areas (FJ) where we removed vegetation and prepared the soil with the spade to about 20 cm depth (marked with brown) in each area on a 1 m^2 surface. To prevent accidental grazing an ad with information was put regarding the recent pesticide spraying of the land.

(06/10/2013) Extraction of the first set of soil samples from 10 sampling sites considered as time zero before spraying chlorpyrifos methyl; samples were collected from two depth 0–5 cm and 30 cm. Preparing the spray solution with RELDAN 22 EC, containing the active substance chlorpyrifos-methyl 225g/l (100 ml RELDAN 22 EC to 10l water).

For the spraying solution we used a manual pump with the spray nozzle set to the smallest holes (fine spray), the spraying being done at the height of approx. 50 cm above the ground (tip pointing down). Spraying was carried out for 15 minutes without respiratory protective equipment and without gloves, but wearing rubber boots, long pants and a long-sleeved gown. In this way we simulated the equipment described by the vast majority of people participating in the study based on a questionnaire (Lovász & Gurzău, 2013). Weather conditions (temperature, humidity, pressure, wind speed and direction) were monitored during spraying with IROX Pro X Weather Station.

30 minutes after spraying and soil samples were collected from the 10 areas of said sample at a depth of 0-5 cm and 30 cm.

(13/10/2013) Soil sampling was performed after 7 days since spraying: in total 20 samples of soil sample were harvested from the 10 areas at the established depths. (Note that in the time interval after the spraying event, a week followed in which it rained for two days and the temperature has not dropped below 0 ° C at night).

(20/10/2013) Soil sampling at 14 days from the spraying event: a total of 20 soil samples from 10 sampling sites at depths determined have been collected.

(27/10/2013) Soil sampling at 21 days from the spraying event: a total of 10 soil samples have been collected, only from the 5 sampling areas without vegetation, at both fixed depths (0 – 5 cm and 30 cm) (because the samples dated from 20.10.2013, chlorpyrifos-methyl pesticide has not been found). A sample of mixed vegetation (only green leaves without roots) was gathered from the 5 sampling areas.

(24/11/2013) Repeating the collection of samples from the soil sampling areas without vegetation - a total of 10 samples (4 weeks after the last sampling and 49 days after spraying), under conditions of temperature drop. A sample of mixed vegetation (only dry leaves without roots) was gathered from the 5 sampling areas.

(16/02/2014) Soil sampling at 133 days after spraying, from the 5 sampling sites and the two bare areas, where the vegetation has been removed, totaling 14 soil samples. We have taken again samples of the plants (leaves only dry, roots) from the five sampling areas.

(27/04/2014) Sampling of spring vegetation (203 days from the spraying event) from the 5 sampling sites where the vegetation has not been removed since the beginning of the experiment.

500 grams of soil were collected each time from each sampling site mentioned above, in plastic bags, labeled and then shipped to the laboratory in insulated boxes, where they were stored in the refrigerator until analysis (within 24 hours).

Approx. 100 grams of plant mix was sampled from the five vegetation zones, the samples were collected without roots in a plastic bag, labeled and transported to the laboratory in insulated boxes and stored in the refrigerator until analysis (within 24 hours). The following plants were identified from the samples: *Alopecurus pratensis, Festuca pratensis, Poa pratensis, Plantago lanceolata, Ranunculus acris, Taraxacum officinale, Achillea millefolium, Trifolium pratense*. The harvested plants are inedible for humans; however they are fit for animal consumption (important for the toxic intake and entering the food chain).

Sampling of the soil and plants in the laboratory

The vegetation taken from the five areas on the same day, was homogenized and sampled after the quartering method.

The soil was homogenized and sampled after quartering. The soil samples were not mixed together and were analyzed separately.

From the collected soil samples we determined the following: pH, moisture (solids), total nitrogen (N), organic material, density, grain size, adsorption capacity, humus and chlorpyrifos-methyl.

For the vegetation samples, we analyzed only for the chlorpyrifos-methyl pesticide content.

Methods for determining organophosphate pesticide - Chlorpyrifos - methyl in soil and vegetation

The analysis of plant and soil samples was performed by adapting the method used by Sánchez-Brunete et al (Sánchez-Brunete et al., 2004).

The plant material was analyzed for two situations: after being washed three times with distilled water and without being washed.

Soil samples were prepared from polypropylene extraction columns (syringes) by placing the two circles of filter paper in the base, while the lower orifice is obstructed and adding 5 grams of soil sample sieved through a 2 mm sieve.

Samples of vegetation of 5 grams each (washed and unwashed), were introduced into the extraction column made of polypropylene (bottles with a conical bottom).

Plant and soil samples were extracted with 4 ml ethyl acetate, for 15 minutes, in an ultrasonic water bath, at room temperature. The ethyl acetate extract was selected as the solvent because of the good results obtained by Sanchez-Brunete et al. (Sánchez -Brunete et al., 2004). The water in the bath was adjusted to match the extraction solvent from the columns, which were supported in a vertical position.

After extraction, the solvent was filtered and collected into tubes. Samples of soil and vegetation were back-extracted with 4 ml of ethyl acetate (15 min). The solvent used for the extraction of the soil samples was filtered and washed with 1 ml of additional solvent.

The total extracts were collected into tubes and then concentrated using a slow stream of nitrogen to a volume of 1 ml and stored at 4 °C until analysis by GC - MS.

GC - MS analysis was performed with a gas chromatograph equipped with a split/splitless automatic injector and a mass spectrometer as detector (Shimadzu GC - MS QP 2010 Plus NCI). The separation of compounds was performed in a fused silica capillary column (TG - 5MS), 5% phenyl polysiloxane as nonpolar stationary phase (30 m, 0.25 mm internal diameter and 0.25 μm thick film). Operating conditions were as follows: injection port temperature of 240 °C, helium carrier gas at a flow rate of 1.49 ml/min, high pressure injection (310 kPa for 1 min). Column temperature was set to 80 °C, then programmed 10 °C/min up to 260 °C and then held for 1 min. Total analysis time was 19 minutes and the balancing time was 2 minutes. The injection volume was 8 μl. MS detector was operated using the electron impact ionization mode. Ion source temperature was set at 250 °C and the GC - MS interface temperature at 280 °C. Figure 45 shows the mass spectrum for chlorpyrifos-methyl.

The analysis was performed with selected ion monitoring (SIM) using a target ion (m/z 125) and two qualification ions (m/z 286 and 109). Ions of interest were determined by injecting standard pesticide solution in the same chromatographic conditions, using full scan with mass/charge ratio from 60 m/z to 500 m/z. Quantification was based on the graphical representation of the concentration of standard solutions with respect to the peak area of the target ion. Standards were prepared in the extraction solvent in the 1.0-5.0 µg/ml range. MS response was a linear calibration curve with a correlation coefficient R=0.9999.

Quality control analysis was performed by analysis of blank samples and of control samples of known concentrations for each set of tests. Blank samples were below the limit of quantification of the method (0.005 mg/ml) and control samples were within the range of $X_0 \pm 2*s$, where X_0 is the average of the standard solution of 3.0 µg/ml made in days and s is their standard deviation.

<u>Calculation of exposure dose by ingestion and dermal contact</u> (hands) to chlorpyrifos-methyl from soil

Exposure by ingestion – soil

Oral daily intake and exposure dose to chlorpyrifos-methyl were calculated using the Dose Exposure Calculator software, developed by ATSDR from the CDC for a reference population: infants under one year of age with a body weight of 10 kg, children between 1 and 6 years of age and with a body weight of 16 kg, adults aged between 19 and 65 years and weighing 70 kg, and children aged 1 to 6 years with a body weight of 16 kg, which manifest *pica* behavior (eating soil).

Chlorpyrifos-methyl concentrations considered when estimating exposure doses were: the highest concentration, the smallest and the average, obtained from the experiment (30 minutes, 7 days, 14 days, 21 days to 49 days and 133 days after pesticide spraying) of the 5 areas without vegetation from depths between 0-5 cm and 30 cm.

The exposure factor shows how often and how long a population is exposed to contaminated soil and takes into consideration the frequency, duration and time of exposure (ATSDR, 2005). In this case, exposure factor is equal to 1, representing daily exposure.

Body weight is used in the equation for calculating the exposure dose, because when exposed to the same amount of a substance, people with a lower body weight will receive a relatively higher dose of the substance compared to people with higher body weight.

Exposure by skin contact – ground

In order to estimate the exposure doses and daily intake of chlorpyrifos-methyl through dermal contact, we used the Exposure Dose Calculator software belonging to ATSDR, from the CDC.

The total adhered soil depends on the surface of exposed skin, which is different for each age group. In the present study we considered hands as the only area of skin that may come in contact with chlorpyrifos-methyl.

The bioavailability factor is the amount of the substance that is absorbed into the body of a person, and is a percentage of the total amount of the substance that actually enters the blood and is available to affect a person. In this case the bioavailability factor is equal to 1, which represents 100% of the total.

Exposure factor depends on the frequency and duration of exposure, and in our case we estimated that the population is exposed to chlorpyrifos-methyl 20 days/year. Body weight influences the size of the dose of exposure, so people with lower body weight will receive a relatively higher dose of a substance compared to people with higher body weight. Computing scenarios used to estimate exposure doses and daily intake are: children 1-11 years with a body weight of 30 kg, adolescents 12-17 years with a body weight of 50 kg and adults 18-70 years with a body weight of 70 kg.

Results and Discussion

1. **Chlorpyrifos-methyl concentrations in the samples of soil and vegetation**

1a. Soil

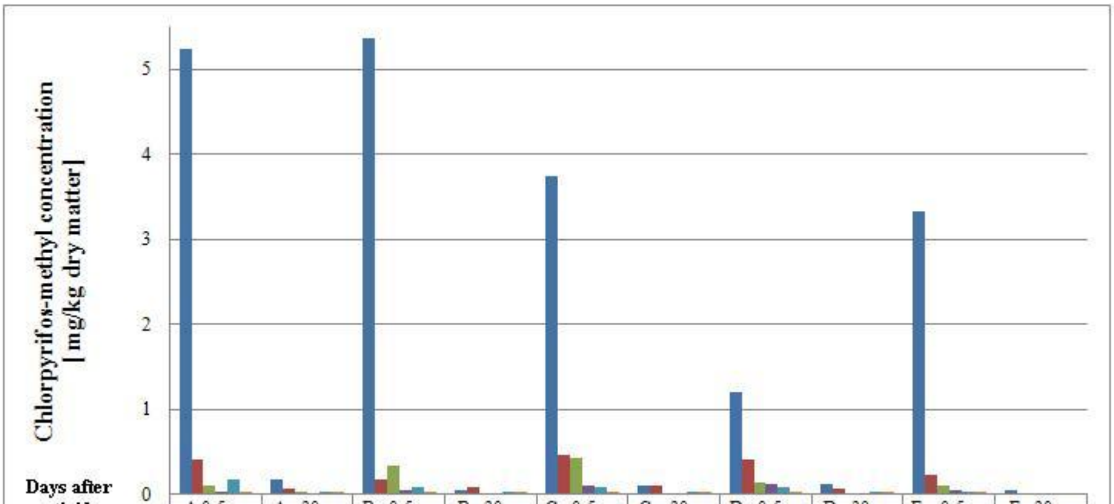
The initial state of the field from the point of view of presence of the pesticide chlorpyrifos-methyl in soil at the start of the experiment, was characterized by concentrations of the substance involved in the experiment below the detection limit of the analytical method (<0.02 mg/kg dry matter) both in bare soil and on soil prepared in advance.

The pesticide failed to disperse in soils covered with dense vegetation, except for very small quantities. At 30 minutes after spraying with the pesticide RELDAN 22EC (with chlorpyrifos-methyl as the active substance), the substance was identified only in area G, both in the surface (0.0525 mg/kg dry matter) and in depth (0.0395 mg kg dry matter) and also in two other areas (I and J) only at 30 cm depth. 7 days after application of the pesticide clorpyrifos-methyl, its concentration in the G area was below the detection limit of the method, while in the I and J areas, the concentration increased compared to samples taken 30 minutes after spraying; furthermore, during this sampling step, the pesticide has been identified in a concentration of 0.0738 mg/kg dry matter at the surface of the F area. At 14 days after the application of the pesticide in all sampling areas, chlorpyrifos-methyl concentrations were below the limit of detection of the method (<0.02 mg/g dry matter).

Both at the surface and in depth of the sampling area covered with vegetation the soil contains clay with relatively homogeneous density was and an adsorption capacity mostly between 80 and 100%. The adsorption capacity of various soils depends on the humus percentage.

Given that the pesticide was identified in few samples of soil covered with vegetation (15% in surface samples and 25% in in-depth samples) we will further discuss only about the presence of chlorpyrifos-methyl in the sampling areas with soil without vegetation.

The figures below (see Figure 51) show the chlorpyrifos-methyl concentrations in the soil taken from the 5 sample areas without vegetation, at two depths (0-5 and 30 cm), after applying the pesticide chlorpyrifos-methyl. What is obvious is the very marked decrease in the concentration of pesticide in the first 7 days and in the 14 days after spraying. When detailing the development of the concentration of the pesticide in the soil from seven days after spraying until the end of the experiment (133 days after spraying) there is another sudden drop in the concentration of the pesticide in the time frame 14-21 days after spraying, so that later decline is gradual and relatively uniform.



Days after pesticide use	A 0-5cm	A - 30cm	B - 0-5cm	B - 30cm	C - 0-5cm	C - 30cm	D - 0-5cm	D - 30cm	E - 0-5cm	E - 30cm
0 (30 minutes)	5.249	0.17228	5.3691	0.0503	3.7409	0.1107	1.2089	0.1153	3.3362	0.0529
7	0.4023	0.0747	0.1851	0.0856	0.4703	0.1027	0.4048	0.0612	0.2262	0
14	0.1013	0.0364	0.3448	0	0.4208	0	0.1346	0	0.1011	0
21	0.0347	0	0.0526	0	0.1035	0	0.1161	0	0.0515	0
49	0.1781	0.0359	0.0894	0.0359	0.086	0.0316	0.0842	0.0324	0.0377	0
133	0.0329	0.0247	0.0296	0.0293	0.0302	0.0313	0.0317	0.0311	0.0315	0

Figure 51: Concentration of chlorpyrifos-methyl in soil without vegetation layer (sampling period: 0-133 days after spraying)

In Figure 53 are the average values and the standard deviation of the chlorpyrifos-methyl concentrations in the soil at various time intervals, after the application of the pesticide, pointing out the progressive decrease in the first 3 weeks after application, followed by a slight increase at

49 days, and then decrease. The decrease during the 49-133 days period, in the 30 cm soil, was less important, meaning a certain degree of retention of the pesticide in the soil.

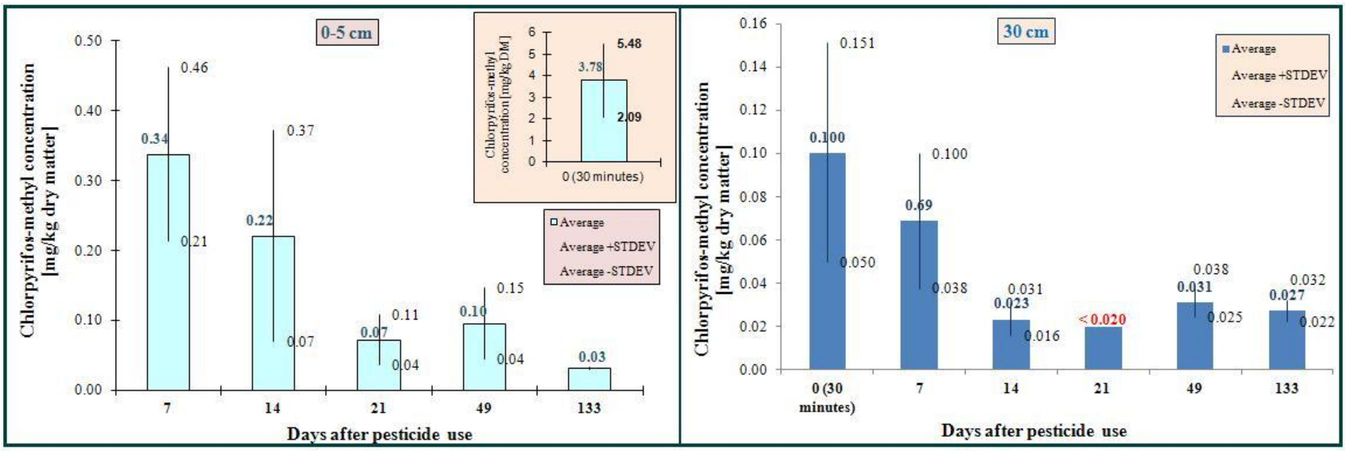


Figure 53: Average concentrations +/- SD of chlorpyrifos-methyl in soil (0-5 cm and 30 cm) after spraying pesticide

When plotting the time evolution of the average concentrations of chlorpyrifos-methyl on surface soil and in depth, we can observe the relative parallel evolution of the concentrations in the surface and in depth of the soil, mainly after 14 days of application. Returning to the period of 0-14 days of application it is worth mentioning that the massive drop in pesticide concentrations in the surface soil is less reflected in the soil at 30 cm depth, as the soil structure (clayey soil of various kinds) is slowing the migration of the pesticide (retention).

Accordingly, the difference between the concentrations of chlorpyrifos-methyl at 0-5 cm and 30 cm were statistically significant ($p < 0.05$) up to 49 days after the application of the pesticide, showing higher concentrations at the surface than in depth. At 133 days after pesticide spraying, these differences were not statistically significant ($p = 0.14$), showing a tendency of the pesticide concentrations to equalize at the two depths.

Most of the chlorpyrifos-methyl sampled at 0.5 cm soil depth, was degraded within 7 days after application, from the average concentration of 3,781 mg/kg dry substance to 0.338 mg/kg dry substance (concentrations 11 times lower than that measured 30 minutes after application) ($t = 4.53$, $p = 0.01$); at 133 days, the average concentration of chlorpyrifos-methyl was 120-fold less than the one measured 30 minutes after application ($t = 4.95$, $p = 0.008$).

Average concentrations of chlorpyrifos-methyl soil samples taken from a depth of 30 cm after 30 minutes (day 0) from the application of the pesticide were 0.10 mg/kg dry substance, and reached a concentration 1.6-fold lower (0.07 mg/kg dry substance) in the 7 days after the application of the pesticide and less than 3.3 times to 133 days.

Figure 55 shows the degradation rate of the pesticide chlorpyrifos-methyl according to the time of sampling. A value of zero is regarded as the starting concentration (i.e. the concentration of the pesticide in the soil within 30 minutes). It is noted that for the maximum concentrations measured, the decrease during the study period was more pronounced than for the average and minimum concentrations.

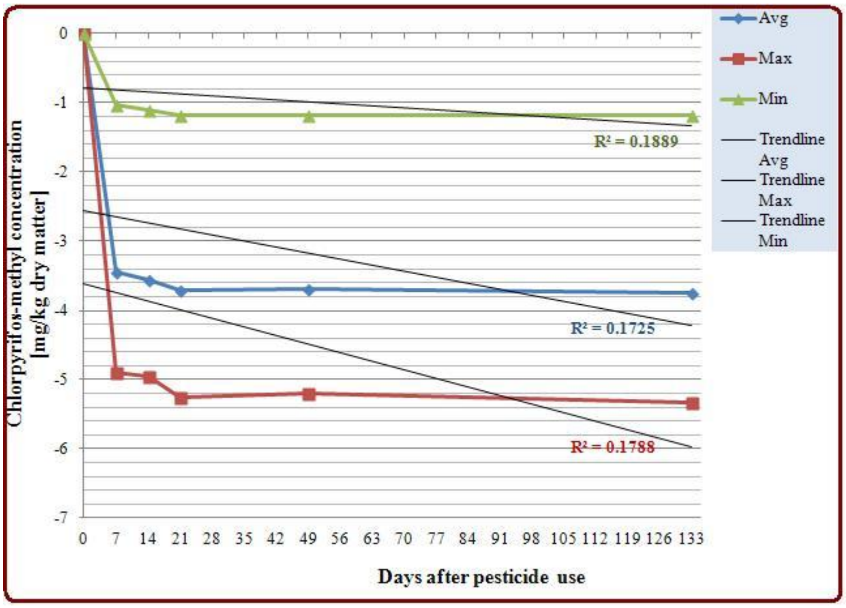


Figure 55: Degradation rate of the pesticide chlorpyrifos-methyl in 133 days

According to the FAO, in 2000, the pesticide chlorpyrifos-methyl is classified as easily degradable (DT50 <20 days) (FAO 2000). In our study, although the first measurement was made after a week, the estimated half-life is less than 7 days probably about three days.

1b. Vegetation

Sampling of vegetation was started on day 21 of the experiment, until day 203. Please note that collection of the vegetation sample at 21 days, was dictated by the classification of the pesticide chlorpyrifos-methyl as easily degradable (DT50 <20 days) (FAO, 2000). Last harvest of vegetation was conducted in April, with the harvesting corresponding to the zero moment of application of the pesticide for the next harvest. The results showed that there are differences between the concentrations of clorpyrifos-methyl measured in the tissue of unwashed vegetables compared to the concentration in the tissue of washed vegetables, concentrations of the latter case are lower, which means that most of the pesticide remains in the plant tissue. Statistical differences between unwashed and washed vegetables are not statistically significant (p 0.07) in terms of pesticide concentrations, confirming retention in plant tissue. A special feature to note is that the vegetation harvested 21 days after spraying has a significant amount of chlorpyrifos-methyl per area (+0.08 mg/kg dry matter) compared to the next collection periods, where the differences are very small. Another particularity is that at 133 days chlorpyrifos-methyl was found only on the surface of plants, this is explained by the fact that, at that time, the vegetation was completely dry.

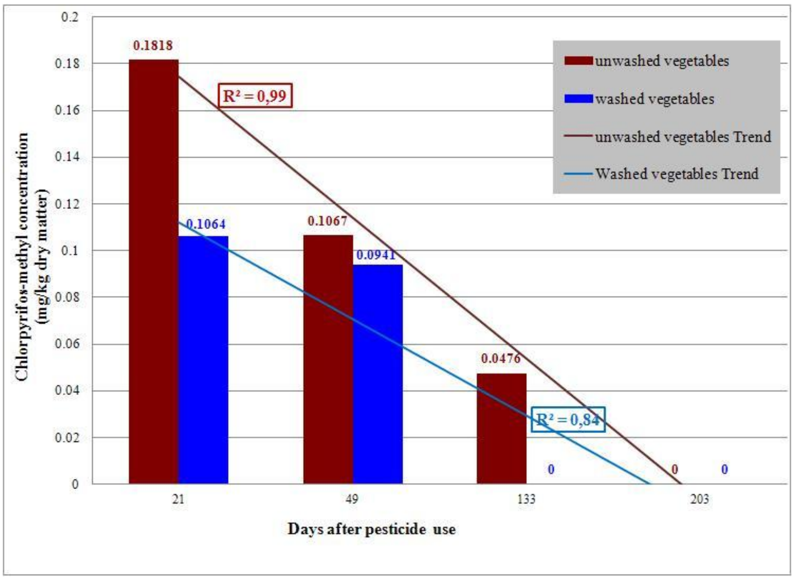


Figure 56: Concentration of chlorpyrifos-methyl in the washed and unwashed vegetation (sampling period at 21-203 days after pesticide spraying)

From figure 56 we can observe the decrease in the concentration of chlorpyrifos-methyl inside and outside of plants, as well as the fact that at 203 days after spraying, the pesticide was below the detection limit of the method.

2. Factors affecting the concentrations of chlorpyrifos-methyl in soil

Chlorpyrifos-methyl concentrations depend also on the soil properties (pH, moisture content, structure, and so on).

Soil samples taken at 0.5 cm below soil surface had a lower density (from 0 to 1.08 g/cm3) compared to the soil samples collected from 30 cm depth (1.08 to 1.12 g/cm^3).

In figure 57 we can see a reduction in the concentration of the chlorpyrifos-methyl correlated with an increase in the density of soil samples collected at 0.5 cm depth only for the time of harvest at 7, 14 and 21 days. For the samples collected from 30 cm deep, this relationship is evident only 30 minutes after spraying.

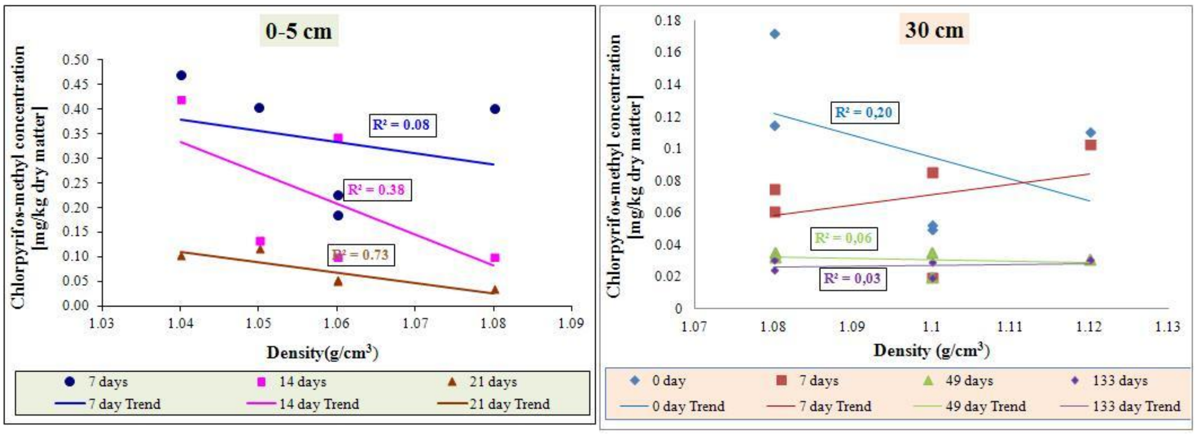


Figure 57: Regression line between the concentration of the chlorpyrifos-methyl and the density of the collected soil at 0-5 cm and 30 cm depth

In this experiment (225m2) I have met four different types of clay soil: silty clay, clay, sandy clay and clay silt sand, on a relatively small area.

The concentrations of the chlorpyrifos-methyl vary depending on the types of soil (clay, sand, and silt).

The soils with high clay content have higher concentration of pesticide in the soil for longer time periods, while soils with higher sand content have lower concentrations of chlorpyrifos-methyl in the case of surface soils sampled at 7, 14 and 21 days after pesticide spraying. In the case of the surface soils, with high clay content, collected at 0, 49 and 133 days after spraying of the pesticide, there was a lower concentration of the pesticide. Soils with higher sand content have higher concentrations of chlorpyrifos- methyl. At 30 minutes after spraying concentrations in the soil increase in relation to the increase in the sand content of the soil. The relationship begins to reverse over time so that in 133 days we can speak of a balance between pesticide concentrations in soils with different percentages of clay and sand.

30 minutes after spraying, the pesticide enters sandy soils quickly, so that pesticide concentration increases in soils with higher sand content. Soils with high clay content have lower concentrations because they it is more difficult for the pesticide to cross the clay layers.

The relationship between the concentration of the chlorpyrifos-methyl and the type of soil is similar, also at 30 depth cm, to that described for the surface soil during 7-21 days after spraying. At 14 and 21 days, the concentrations of chlorpyrifos-methyl were lower than the method detection limit (<0.02 mg/kg dry matter), so that the substance is measured again at 49 and 133 days after spraying, albeit at concentrations very similar to the detection limit.

A negative correlation was found for the soil samples taken from a depth of 30 cm, between the concentration of chlorpyrifos-methyl and the proportion of the clay, but there is a positive correlation with the percentage of silt content in soil, i.e. soils with a high percentage of clay have lower concentrations of chlorpyrifos-methyl and a higher percentage of silt have higher concentrations of pesticide due to the higher permeability of silt.

Another factor that comes into mobility and degradation of substances is pH. Table 28 shows the pH of the soil samples in the field experiment we conducted. Due to the measurements made before applying the pesticide, we can characterize analyzed soils as slightly acidic with a pH between 5.8 at the surface, to 5.87 in depth. During the course of the experiment, the soil pH had a tendency to decrease, so that at 133 days, the pH of the surface soil had a mean value of 5.01 and a value of 5.07 at 30 cm depth, with soils maintaining their acidic features. Increase in the pH of the soil at 21 days compared to 14 days is due to external factors (e.g., precipitation), and not due to the pesticide itself, with regard to the tendency to decrease during the whole experiment.

The experiment shows the presence of the pesticide in the soil to adjust the pH thereof.

Water content (W_{H2O}) per mass of soil samples was between 6.91 and 32.47%. Positive Bravais-Pearson correlation between the humidity and the concentration of chlorpyrifos-methyl wasfound in soil samples collected only 133 days at 0.5 cm and 30 cm depth (r = 0.52, r = 0.85)

The soils were analyzed for nitrogen content from 0.11 to 0.15%, thus they can be classified as having low and average nitrogen content, according to Lăcătuşu R., 2000. During the experiments carried out, we observed no changes in the concentration of the pesticide according to the total N (0.11 to 0.15%), adsorption capacity (60-90%) and organic matter (2-5%) in the soil, because we did not have differences of these indicators in the soil samples analyzed.

3. Calculation of the exposure chlorpyrifos-methyl from soil

Chlorpyrifos-methyl is classified as a non-carcinogenic organophosphinic substance, which inhibits the activity of acetylcholinesterase. Acceptable Daily Intake (ADI) of chlorpyrifos-methyl is 0 to 0.01 mg/kg body weight (bw) established at the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), where the compound was evaluated in 1975, 1991 and 1992 (WHO, 2009b).

Based on the lowest, average and highest chlorpyrifos-methyl concentrations measured in soil at 0-5 cm and 30 cm depth, the daily intake by ingestion and skin contact in children and adults, during the experiment, was calculated. Children were selected from the 1-6 years for oral exposure and the 1-11 years age group for dermal exposure, knowing that until the onset of adolescence, these populations have an increased susceptibility to toxic substances. Pica disease (ingestion, due to an appetite, of non-nutritive substances such as clay, ice, sand, paint walls, etc.) is specific to young children, and the reason why I chose the 1-6 years age group forthe calculation of exposure dose. In the case of dermal exposure we chose 20 days/year exposure duration, both for children and adults.

Exposure surface soil (0-5cm)

The exposure dose was calculated for oral intake of soil and for each sampling time of the experiment, at 30 minutes, 7, 14, 21, 49 and 133 days. In the case of oral exposure to concentrations in soil measured 30 minutes after spraying, which are the highest concentrations, estimated oral dosage in children varied between 1.51E-05 mg/kg/day and 6.71E-05 mg/ kg/day and the integral 1,73E-06 mg/kg/day and 7.67E-06 mg/kg/day in adults. If the children suffering from Pica disease are exposed to the concentrations in soil specified above (30 minutes after the application) oral exposure dose ranges between 3.78E-04 mg/kg/day and 1.68E-03 mg/kg/day. Each exposure considered lead to a daily exposure dose lower than that of the ADI. In children with the Pica disease, we calculated the highest exposure doses, ranged between 3.78% and 16.8% of the ADI, which starts to become significant in terms of health risks.

Under the same conditions of exposure, dermal exposure dose ranges between 2.01E-07 mg/kg/day and 8.93E-07 mg/kg/day for children and 6.68E-08 mg/kg/day and 2.97E-07 mg/kg/day for adults.

Cumulative exposure (oral and dermal) is higher in children than in adults, and it represents a percentage of the ADI ranging between 1.53E-01 mg/kg/day and 6.80E-01 mg/kg/day and between 1.80E-02 mg/kg/day to 7.97E-02 mg/kg/day, the contribution of these routes of exposure is virtually insignificant in the presented computing scenario.

Calculated exposure doses, both oral and dermal and also cumulated, dropped in adults and children according to the concentrations considered at 7, 14, 21, 49, and 133 days after the application of the pesticide. Thus, in the case of children, for the measured concentrations of the pesticide in soil, in the last stage of the experiment (133 days), oral dose of exposure is between 3.70E-07 mg/kg/day and 4.11E-07 mg/kg/day, as opposed to adults where the same daily dose ranged from 4.23E-08 mg/kg/day and 4.70E-08 mg/kg/day. This time too, children with the disease Pica recorded the highest exposure doses ranging between 1.03E-05 mg/kg/day and 9.25E-06 mg/kg/day, which is about 0.09% to 0, 1% of the ADI, which decreases health risks enormously.

Regarding dermal exposure, it was within the limit of the same order of magnitude, both in children and adults (E-09), with differences between 3.28 mg/kg/day and 3.65 mg/kg/day.

Depth soil exposure (30 cm)

Oral exposure dose was calculated also for pesticide concentrations that reached the organism through the soil with concentrations equivalent to 30 cm. Referring to oral exposure to pesticide concentrations measured 30 minutes after application, the estimated oral dose in children varied between 6.29E-07 mg/kg/day and 2.15E-05 mg/kg/day and the integral 7,19E-08 mg/kg/day and 2.46E-06 mg/kg/day in adults. Same as in the case of concentrations in the surface soil, the dermal route exposure dose was less than the oral dose, ranging between 8.37E-09 mg/kg/day and 2.87E-07 mg/kg/day for children and 2.78E-09 mg/kg/day and 9.52E-08 mg/kg/day for adults. If the children with Pica disease are exposed to the specified soil concentrations (30 minutes after the application) oral exposure dose ranges between 1.57E-05 mg/kg/day and 5.38E-04 mg/kg/day. Each exposure considered led to a daily exposure dose much lower than the ADI, but this time also, children with Pica disease receive the highest daily dose, which is between 0.15% and 5.38% of the ADI.

Dermal exposure dose ranges between 8.37E-09 mg/kg/day and 2.87E-07 mg/kg/day for children, compared to 2.78E-09 mg/kg/day and 9.52E-08 mg/kg/day for adults.

Cumulative exposure (oral and dermal) is higher in children than in adults and in the case of the concentrations measured at 30 cm, it represents percentages of the ADI between 0.006% and 0.2% in children and between 0.0007% and 0.03% in adults.

Daily exposure doses calculated on both routes of exposure and cumulatively, decreased in adults and children according to concentrations considered at 7, 14, 21, 49 and 133 days after pesticide application. Thus, in the case of children, for the measured concentrations of the pesticide in soil, in the last stage of the experiment (133 days), the oral dose of exposure ranged between 2.50E-07 mg/kg/day and 3.91E-07 mg/kg/day, In contrast to the adult where the same daily dose ranged between 2.86E-08 mg/kg/day and 4.47E-08 mg/kg/day. Children with the Pica disease have registered the highest exposure doses, ranging between 6.25E-06 mg/kg/day and 9.78E-06 mg/kg/day, which is about 0.06% to 0.1% of the ADI, thus the health risk is extremely low.

Regarding dermal exposure, it was within the same order of magnitude, both in children and adults, showing (E-09) differences between 2.22 mg/ kg/day and 3.48 mg/ kg/day. ADI contribution of this route of exposure is the concentration measured at 133 days 0.0000049% and 0.000054%, which becomes insignificant in terms of human health risks.

Aggregation of oral and dermal exposure leads to exposure doses, which expressed as a percentage are of the same magnitude as oral exposure (E-04).

In the soil samples collected from a depth of 30 cm, we can see larger differences between the estimated daily intake and ADI, compared to the soil samples collected from a depth of 0-5 cm. Daily intakes of oral and dermal exposure at 21 days, showed a sharp decline, because at that time, the concentration of chlorpyrifos-methyl in soil samples was below the limit of detection of the method. Knowing that pesticides can enter the bloodstream through the stomach more easily than through skin (Nesheim et al., 2008), we also observed in our case that for the same concentrations of pesticide in soil, we have a higher oral exposure dose than a dermal one.

Summarizing what we have discussed above, we can say that highlighting the fate of pesticides is essential for making decisions on their use. In our study, as cited by literature, we have shown that clay type soil (four varieties) influences the retention and transport of pesticides in soil and it led to the possibility of identifying it for a long period of time, 133 days, even if the time half-life (DT50) in our study was very short. Also, the persistence of chlorpyrifos-methyl in soil over 133 days was favored by the slightly acidic pH of the soils with I worked with, otherwise noted in the literature. Literature estimates a potential to develop new pesticides, from microbial derivatives, that are effective, safe and have a low environmental risk. Associating them with precise application techniques, can thereby reduce the intake, generating an effective method to reduce the transport and emissions and avoid accumulation resistance in the target organism. (Lo'pez-Periago et al., 2008), which is also an effective way of decreasing health risks to people exposed to the place and time of application as well as those exposed to the drift.

In terms of identifying the pesticide in plants, our study showed long persistence, both on the surface and inside the vegetable plant tissue. The absorption of the pesticide tin the plant foliage will vary depending on the chemicals and plants, and may be greatly influenced by environmental conditions and aids. For a given chemical substance, absorption varies greatly depending on plant species and at present there is a no simple method to quickly assess the surface permeability of a plant leaf (Liu & Wang, 2007). The vegetation type investigated by us it was a composite, the aim of our study was not to show the differences between plant species from the field of the experiment. Even if the samples have been mixed, the retention level of the surface of plant and vegetable tissue has been identified and has been tracked for a long period of time, approximately 6 months after pesticide application.

CONCLUSIONS

The experiment conducted on previously unused agricultural land by spraying the Reldan 22 EC insecticide (chlorpyrifos-methyl) showed different behaviors of this pesticide in soil, depending on the soil vegetation cover.

The presence of pesticides in the soil stripped of vegetation was detectable at 133 days from the time of application. In this case, the half-life (DT50) was less than 7 days. The concentration of the chlorpyrifos-methyl at 7 days was 11 times less than in the case of the surface soil and only 1.6 times more than in the in-depth soil.

The concentration of chlorpyrifos-methyl in the soil without vegetation showed significant differences ($p < 0.05$) at 0-5 and 30 cm until 49 days after the application of the pesticide, which suggests an increased degree of retention at the surface of the soil in question. After this period of time there is a trend to equalize concentrations (surface-depth).

At 133 days, the concentrations of chlorpyrifos – methyl, sampled from depths of 0-5 cm and 30 cm, showed no significant differences.

Pesticide was found in relatively few soil samples covered with vegetation (15% in surface samples and 25% in in-depth samples). Measured concentrations ranged from 0.035 to 0.0738 mg/kg DM in the surface soil and from 0.036 to 0.062 mg/kg DM in in-depth soil, showing retention at vegetation level and in the surface layer of the soil.

In this experiment, the long persistence of chlorpyrifos-methyl is due to the clay soil type (high density), slightly acidic (pH), which favors retention (adsorption), thus slowing the degradation process, particularly in in-depth soil.

The differences measured between chlorpyrifos-methyl for the unwashed and the washed plants that we analyzed, exhibit a concentration of pesticide in the plant tissue.

In the period of 21-133 days after the application of the pesticide, the difference between the measured concentrations of pesticide for the unwashed vegetation compared to the washed one, gradually decreased; the most significant difference was observed at day 21. Presence of chlorpyrifos-methyl in detectable concentrations was reported including at 133 days on the dry vegetation from the land where the experiment took place.

At 203 days after spraying, the newly grown vegetation layer has shown no detectable amounts of pesticide content, which means that the probability of pesticide retention in soil is low.

Considering the default soil ingestion intake, the oral exposure dose calculated using the ATSDR toxicological model is 1-2 orders of magnitude higher than the dose of exposure by the dermal route, regardless of the considered concentration of the pesticide in the soil.

Calculated exposure dose oral and/or dermal concentration decreases accordingly to the concentrations accounted for.

Oral and dermal exposure doses, as well as the calculated cumulative dose, are significantly lower than the acceptable daily intake for adults and children.

Regardless of the pesticide concentration in soil, children reach an exposure dose expressed as a percentage of the acceptable daily intake by an order of magnitude higher than adults.

The exposure doses was calculated daily for children with Pica disease, in their case, only this route of exposure making up to about 16.8% of the acceptable daily intake.

The most exposed group is children with Pica disease, those who ingest the greatest amount of soil (standard intake 5000 mg / day).

13. BIOMONITORING AND BIOMARKERS OF EXPOSURE – ORGANOPHOSPHATE PESTICIDES

Observational studies show human urine as the main body fluid selected for biomonitoring, the advantages of using it in comparison with blood are due to the fact it is non-invasive and easily collected and quantitatively accessible (Wessels et al., 2003). On the other hand the disadvantages are that urinary biomarkers are relatively short and highly variable from the point of view of concentration.

Biomonitoring of pesticide exposure involves measurement of an exposure biomarker, which can be a pesticide(s), (its) metabolite(s) or reaction product(s) in biological environments, such as urine, blood or blood components, breath, hair or nails and tissues (Barr et al., 2006, Ngo et al., 2010). Organophosphate metabolites have relatively short half-life and are excreted primarily in the urine (Adgate et al. 2001).

In our experimental model we studied the dispersion of chlorpyrifos-methyl in air and its soil degradation, related to the spraying process, we also proposed measurement of 3,5,6-trichloro-2-pyridinol (TCP) in urine (a chlorpyrifos-methyl metabolite) as a biomarker of exposure for persons with and without protective equipment.

Materials and Methods

Urine samples were collected from three individuals who participated in both experiments: the one who performed the spraying, and two people who stood outside the field during spraying. The participants were equipped differently in the two experiments, as follows:

¬ dispersion of pesticide in the air by spraying: the three people wearing full protective equipment (mask, goggles, gloves, overalls with hood and boots). Morning urine samples were collected at 12, 36 and 60 hours after spraying of the pesticide.

¬ pesticide degradation in soil after the spraying process: the three persons were not wearing protective type equipment: mask, goggles, gloves and hood. Urine samples were collected in the morning prior to spraying, and after 12 hours, 36 hours and 60 hours after spraying (the morning urine samples).

Urine samples were collected in the morning in special containers, disposable set with airtight lid to prevent spills, evaporation and contamination of the urine specimen, and were transported to the laboratory in an isothermal box immediately after sampling.

Urine samples were frozen until analysis.

Urine samples were analyzed by the method of Phung et al., which is based on qualitative determination of the metabolite 3,5,6-trichloro-2-pyridinol (TCP) (Phung et al., 2012) which consists in: 1.5 ml of the enzyme was added on 2 ml of urine (8000 β - glucuronidase units dissolved in acetate buffer, 0.2 M concentration). Samples were incubated for 3h at 37 °C in a water bath. 6.5 ml ultrapure water and 1 ml saturated sodium sulfate solution were added and the samples stirred after each stage using a vortex type stirrer. The pH was adjusted to 2 by the addition of 4 drops of concentrated HCl. The samples were extracted with 2 ml of 30% MTBE/hexane by shaking for 2 minutes and centrifugation for 10 minutes at 3000 rpm. The extraction was repeated 3 times. The organic phase was removed with a Pasteur pipette, 3 ml of 0.25 M NaOH and 1 ml of a saturated solution of sodium sulfate were added. The samples were stirred for 2 minutes. The aqueous phase was removed with a pipette, the pH was adjusted to 2 by the addition of 4 ml of 0.5 M HCl and extraction was repeated 3 times without centrifugation step. The organic phase from all stages was dried with anhydrous sodium sulfate, concentrated with a gentle stream of nitrogen to a volume of 1 ml and stored at 4 °C, until analysis by GC-MS.

GC-MS analysis (qualitative determination/ compounds identification) was performed using the same parameters as in the soil samples. Analysis was performed by selected ion monitoring (SIM) using m/z 169 as the target ion and two qualifying ions (m/z 197 and 171).

Results and Discussion

The analysis of the urine samples, from the three subjects who performed the experiment of dispersion of chlorpyrifos-methyl in the air, did not reveal the presence of the metabolite TCP in any case (12, 36 and 60 hours after exposure), mentioning that they were wearing protective equipment.

Unlike this situation, in people who have performed the pesticide degradation experiment in the soil and were not wearing full protective equipment metabolite, TCP was identified.

The person who performed the spraying (male, 65 years old), had features of TCP presence in urine, visible in the chromatogram below. The maximum elimination was done in the first 12 hours of exposure and decreased further to 36 hours, followed by the second removal step of 60 hours, with TCP concentration greater than that of 36 hours.

The two others participants in the experiment were exposed to the pesticide under similar conditions. Even so, they showed different ways of urinary elimination of the compound.

For the second person (male 34 years) chromatogram shows a maximum disposal 12 hours of exposure followed by a less marked between 36 and 60 hours when the removal of TCP was still increased.

For the third person participating in the experiment, elimination increased from 12 to 36 hours, when there was the highest concentration of TCP and decreased thereafter to 60 hours after exposure.

The time period to maximum excretion of urinary metabolites of organophosphate pesticides depends on the route of intake (Meuling et al., 2005; Garfitt et al., 2002). It was observed that maximum excretion occurs 6 to 24 hours later for dermal exposure, compared to oral exposure, largely because of differences in the specific intake following exposure routes.

The late maximum elimination in the people participating in our experiments is associated to dermal exposure as otherwise noted among volunteers carrying out activities that allow extended contact time with a treated surface (Krieger et al., 2000) up to 48 hours after skin exposure. Long maximum excretion times suggest that chlorpyrifos pesticide may be retained by the skin and may remain systemically available for long periods of time (Meuling et al., 2005). We believe that in people with elimination peaks at 36 and 60 hours of exposure in our experiment, this may be due to skin retention of the applied compound. For the third person participating in the experiment, for which elimination was gradual with a peak at 12 hrs, this possibility is unlikely.

CONCLUSIONS

Level of TCP removal was increased in persons directly exposed to the spraying process.

Metabolite presence in the urine of the three involved persons has been identified even at 60 hours of exposure.

Maximum excretion of TCP differed as points in exposure time, suggesting both differences in the primary route of exposure (inhalation and dermal) and also individual particularities related to the absorption, metabolism and elimination of the pesticide.

TCP as a biomarker is a good way of assessing exposure even in the absence of direct measurement of the concentration of the parent substance (chlorpyrifos-methyl) in the air during the spraying process.

14. AWARENESS OF THE POPULATION ON THE CORRECT AND SAFE USE OF PESTICIDES AND ON ALTERNATIVE METHODS OF PLANT PROTECTION

Misuse of pesticides is often the result of ignorance, which can only be fought through education and training. It is important that all staff, even the distribution chain is aware of the dangers, and the use of the products that they sell. They should send appropriate instructions emphasizing the danger when using pesticides without taking precautions for both the environment and the population. The more toxic a pesticide, the more the user needs to receive adequate instructions and there is a greater responsibility for the supply distributor. If people in rural areas who use pesticides knew the risks these pesticides involve, they were more careful about how they keep, prepare and apply pesticides, protective equipment, and wash the waste disposal and packaging. Another way is to adopt an integrated pesticide management program, with emphasis on non-chemical strategies to combat crop pests, such as the removal of diseased plant parts, crop rotation which can disrupt the life cycle of pests and biological control, such as the use of insect predators.

Particular attention, moreover, should be given to rural areas as the health of the population in these areas is poor and makes inhabitants more vulnerable to toxic substances, especially since it is usually dependent on agriculture as the main source of livelihood and income.

Leaflets containing basic information have been developed and distributed for the purpose of public awareness in the town of Sâncraiu (expressed in accessible language, in Romanian and Hungarian languages) on the correct and safe use of pesticides and plant protection alternatives to replace chemical compounds.

15. GENERAL CONCLUSIONS

Crop protection by broad and intense use of pesticides became common in conditions of increasing needs for food and agricultural production, with more severe effects on society as a whole, than the agricultural sector in which it operates.

In order to identify issues related to the use of pesticides, a study was conducted in a rural population group in the town of Sâncraiu, Romania.

The questionnaire-based study showed that the representative sample population from the town, showed exposure to pesticides, mainly due to unmechanized agricultural activity, 30 to 100 days per year, this time frame including preparation, implementation and maintenance of spray equipment.

Pesticides with moderate toxicity are the most commonly used group of pesticides. Over their lives, some of the respondents used currently obsolete pesticides (DDT, Regent).

During the application of the pesticides, interviewees do not use protective equipment, while the personal hygiene measures and equipment are limited and insufficient.

A small number of subjects reported affirmative symptoms associated with pesticide use namely skin irritation, eye irritation, headaches, frequent chest discomfort and other symptoms such as nausea, vomiting, headache and fatigue. Smoking is a factor of error in association with pesticide exposure respiratory complaints.

The experimental model of the distribution of the pesticide chlorpyrifos-methyl during the spraying process has shown that there were no significant differences in the vertical dispersion of the concentrations, but significant differences were found in dispersion concentrations on the horizontal at 0 m, 2 m and 5 m, respectively, influenced also by wind speed.

Following this experimental exposure, daily intake of chlorpyrifos-methyl calculated by inhalation was 80.4% of the acceptable daily intake, thus being below the protection values for human health.

Due to deviation, the exposure dose and hence the risk implied to health may be greater at ends of the spraying area, persons on close lands may be involuntarily exposed to airborne concentrations even higher than those the person who sprayed is subject to; on the other hand, spray drift contributes to soil contamination outside the target area.

The experiment conducted on previously unused agricultural land by spraying the insecticide Reldan 22 EC (active substance chlorpyrifos-methyl) showed different behaviors depending on its soil vegetation cover.

The presence of the pesticide in the soil stripped of vegetation was detectable also at 133 days from the time of application, with a half-life (DT50) less than 7 days.

Chlorpyrifos-methyl concentrations in the bare soil were significantly different ($p < 0.05$) at 0-5 and 30 cm until 49 days after the application of the pesticide, which suggests high degree of retention on the surface of soil in question, occurrences after this period of time tend to equalize concentrations (surface-depth).

Pesticide has been found in a relatively small number of samples of soil covered with vegetation, showing measured concentrations of vegetation and retention in the surface layer of the soil.

In this experiment, the long persistence of chlorpyrifos - methyl is due to clay soil type (high density), slightly acidic (pH), which favors retention (adsorption) slowing the degradation particularly in in-depth soil.

The pesticide chlorpyrifos-methyl has concentrated in plant tissue and maintained concentrations up to 49 days; at 133 days the presence of the pesticide was never identified in plant tissue (vegetation washed).

In the period of 21-133 days after the application of the pesticide, the difference between the measured concentrations of the pesticide in the unwashed vegetation

compared to the washed one, gradually decreased. The presence of detectable concentrations of chlorpyrifos-methyl have been reported including 133 days after spraying on dry vegetation on the land where the experiment took place.

Human exposure to chlorpyrifos-methyl, even at low concentrations, may be present over a long period of time due to the contamination of soil and vegetation.

Surface soil compared with soil depth, is a significant source of exposure and the most plausible source for human exposure.

At 203 days after pesticide spraying, the new vegetation has not showed detectable amounts of pesticide content, which means that the probability of pesticide existence in soil is low.

Considering the default soil ingestion intake, the oral exposure dose calculated by the ATSDR toxicological model is 1-2 orders of magnitude higher than the dose of exposure by the dermal route, regardless of the considered concentration of the pesticide in the soil.

Regardless of the pesticide concentration in soil, children reach a dose of exposure expressed as a percentage of the acceptable daily intake by an order of magnitude higher than adults. The calculated oral, dermal and cumulative exposure doses are significantly lower than the acceptable daily intake for adults and children.

The exposure doses were calculated daily for children with Pica disease and, in their case, only the oral exposure makes of up to about 16.8% of the acceptable daily intake.

The presence of the biomarker of exposure to chlorpyrifos-methyl (3,5,6-trichloro-2-pyridinol-TCP) was traced to people who have been involved as volunteers during our experiments.

The level of urinary excretion of 3,5,6-trichloro-2-pyridinol (TCP) was increased in the person directly exposed to the spray, compared with the other two. All three, however, showed the presence of the metabolite in urine, even 60 hours after the exposure event.

Maximum removal of 3,5,6-trichloro-2-pyridinol (TCP) differed as a moment in time to the moment of exposure, suggesting differences in the way both main exposures (inhalation and dermal) and individual particularities related to absorption, metabolism and excretion of the pesticide.

3,5,6-trichloro-2-pyridinol (TCP), as a biomarker, is a good way of assessing the exposure even in the absence of direct measurements of the concentration of the parent substance (chlorpyrifos-methyl) in the air during the spraying process.

Evolution of chronic morbidity in adults and children in the town Sâncraiu show that organophosphate pesticide exposure can contribute to extra illnesses or worsening of existing ones.

Existing pathology in children is dominated by chronic respiratory diseases and asthma, the latter in particular has had oscillating high values in the absence of registration of the cases. The seasonal nature of asthma morbidity (Quarter III and IV) suggests nature/infectious component in children, as this season the circulation of respiratory virus strains and reentry of children in different collectives, are the major causative factors of the disease.

Both in children and in adults, acute conditions were dominated, from the incidence point of view, by those related to the upper respiratory tract, lower respectively, with significant decreasing trends in both population groups.

The group of conditions that dominate morbidity in adults is cardiovascular disease, followed by chronic respiratory disease.

The increasing trend in the incidence/frequency of chronic diseases of all organs and systems in adults, including tumor specific age falls in the spectrum of old age and is mainly due to aging.

Component specific allergic diseases (rhinitis, dermal, urticaria and erythema) have evolved in parallel, almost overlapping frequency in adults and children, in the second and third quarters of the year.

Alcohol and tobacco use is relatively moderate in the community currently under investigation and may increase vulnerability to toxic substances (pesticides), especially as chronic respiratory and cardiovascular pathology is stated as common.

Exploration of respiratory function showed that the respiratory status of the subjects included in the study, who use pesticides, may be affected more so as most commonly used applicators are in the form of spray devices; estimated lung age (specific variable) was higher in those exposed to pesticides and smoking compared to those not exposed, who do not display any difference.

In order to make sensible decisions on the authorization and use of pesticides, it is essential to know their behavior.

Persistence of pesticides or harmful metabolites in soil and plants is a permanent and cumulative environmental risk and, ultimately, a risk to human health.

Knowing the behavior of pesticides in the environment, their health effects, especially the means of protection, including individual protection, must be subject of educational activities among rural communities that are gradually becoming suppliers of local and regional food.

16. ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

- This study is **a novelty at national level** as it brings together **complex assessment data regarding pesticide exposure in agricultural farms**, to estimate health effects.

- This paper investigated the **health of the rural community** under study regarding both data on the **structure and evolution of morbidity** and **personal statement and examinations assets** (exploration of respiratory function).

- It is **national news** (to our knowledge, there are no existing published data) to **study the spray drift** of a moderate toxicity **pesticide (organophosphate) in field conditions**.

- This study is also **national news** (to our knowledge, there are no existing published data) on the **development of an experimental model for the study of degradation/transfer in soil and plants of an organophosphate pesticide in field conditions**.

- **It is the first national study** (to our knowledge there are no published data from similar studies) that measures **biomarkers of exposure to chlorpyrifos-methyl** (metabolite 3,5,6-trichloro -2-pyridinol-TCP) **in the urine of human subjects**.

- It is the **first national study** (of our knowledge there are no published data from similar studies) that calculated the **exposure dose** from data obtained in experimental models regarding the concentrations of chlorpyrifos-methyl in the air and soil and estimating the effects based on **the reference dose**.

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