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Potential inhalation pesticides exposure in South Italy: assessment of specific working scenarios

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Abstract:

Pesticides are widely used by agricultural workers to prevent or control pests and to maintain high product quality. Although many efforts have been made to minimize pesticide-related health problems, these chemicals remain a concern for health effects. The south of Italy is rich in vast lands used for crops, in open fields and greenhouses. The aim of this study was to evaluate potential exposure to pesticides of agricultural workers on Lamezia Terme (Calabria) and to identify possible hazard scenarios relating to harmful activities. The study was conducted during daily work activities without interfering with workers. Among all the products used, we focused the research on those containing at least one active substance (a.s.); imidacloprid and hexythiazox. Five different exposure scenarios were identified and monitored. The potential inhalation exposure was determined using a personal air pump and XAD-2/Glass Fiber Filter (OVS) sample tubes. The samples were analysed by a Perkin Elmer Gas Chromatograph with electron capture detection (GC/ECD).

Keywords: pesticides; imidacloprid; hexythiazox; scenarios; occupational exposure

Introduction

Pesticides are widely used in most sectors of the agricultural production. These substances are used for preventing, destroying, repelling, or mitigating any pest (insects, mice and other animals, unwanted plants (weeds), fungi, microorganisms). They contain at least one active substance and may contain other inert components including safeners and synergists. Occupational exposure to pesticides is related to increased risk of developing chronic diseases, neurological disorders and reproductive effects (Dich et al.1997; Weichenthal et al. 2010; Costa 2008). Respiratory symptoms and diseases have been associated with occupational pesticides exposures (Dowling and Seiber 2002; Mamane et al. 2015). The inhalation route should not be neglected because of the presence of airborne spray droplets or vapour resulting from the spray preparation; the application could be dangerous as the lungs can rapidly absorb the dissolved pesticides into the bloodstream (Ogg et al., 2012; Choi et al., 2013).

Although various database and modelling techniques as been developed in order to suggest the level of protection and the type of preventive measures to adopt during the worker's activities (Bańkowski 2013; Potapov 2005; Dosemeci et al.2002; Ross et al. 2002), the assesment risk for human health is not an easy process.Many variables must be considered: pesticides physico-chemical properties (volatility, water solubility, degradability), the toxicological properties of the active substances (harmfulness, irritability, toxicity), frequency and levels of exposure, physical status of the substances or mixtures (aerosol, solid, liquid or vapour), application techniques (manual launch, vehicle, cannon), working role and working environment (greenhouse features and/or open field), greenhouse coverings (plastic film, shade cloth, glass), area of application (m^2), crop height and microclimate conditions. So pesticide exposure assessment is not just a complex process: it is well accepted that pesticide operator exposure is scenario dependent (Lidong Cao et al. 2018).

The present study was to assess potential occupational inhalation exposure of agricultural workers in Lamezia Terme (Calabria) farms and where we have investigated five work different scenarios. The selected pesticides product, are those widely used in these farms, that contain active substances (a.s): imidacloprid and hexythiazox. Imidacloprid (Table 1) is a chlorinated compound. Structurally, it is classified as a chloronicotinyl nitroguanidine (NPIC Imidacloprid fact sheets, IRAC

2018). In WHO (2009) classification imidacloprid is in Class II, moderately hazardous. U.S. EPA (2005) has classified imidacloprid into Group E, 'evidence of non-carcinogenicity for humans'. Occupational exposure limits are unknown. EFSA (EFSA Journal 2013;11(12):3471) has delivered its scientific opinion at the request of the European Commission on imidacloprid. It concluded that some levels for acceptable exposure imidacloprid may not be protective enough to safeguard against developmental neurotoxicity. The current AOEL (acceptable operator exposure level) and ARfD (acceptable operator exposure level) of 0.08 mg/kg/bw/day should be lowered to 0.06 mg/kg bw/per day .Instead, both the WHO and NRA (National Registration Authority) set an ADI (acceptable daily intake) of 0.06 mg/kg bw/day.Effects of imidacloprid on human health depend on concentration, time, and frequency of exposure. Functionally, it belongs to the neonicotinoids family, a novel class of pesticides, chemically related to nicotine, that became commercially available in the early 1990's (Goulson, 2013) and which binds to insect cholinergic receptors, causing death at sufficient concentrations (Jeschke and Nauen,2008).These are selectively toxic to arthropods and relatively non-toxic to vertebrates. Imidacloprid have low toxicity via dermal exposure, but upon inhalation, its toxicity is variable, effects also depend on the health of a person and/or certain environmental factors. Imidacloprid dust is considered slightly toxic but the aerosol form is highly toxic. The human toxicity of imidacloprid affects the nervous system and thyroid lesions (Proença et al.2005; Kumar et al.2013).Signs of imidacloprid toxicity including drowsiness, dizziness, vomiting, disorientation and fever. Some cases of poisoning have been described in literature (Agarwal and Srinivas 2007; EPA 1995).

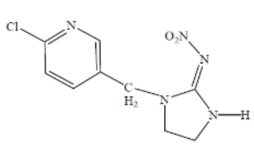
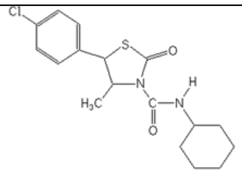
Common Name (IUPAC)	Chemical Structure	Activity
Imidacloprid ((EZ)-1-(6-chloro-3-pyridylmethyl)-Nnitroimidazolidin-2-ylideneamine)		insecticide
Hexythiazox (4RS,5RS)-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-1,3-thiazolidine-3-carboxamide		acaricide

Table 1: Imidacloprid and Hexythiazox.

Hexythiazox (table 1) is an acaricide that acts against egg, larval and nymph stage. It is classified in-group C of USEPA Cancer Classification: 'possible human carcinogen' based on a treatment-related increase in benign and malignant liver cancers in female mice and the presence of mammary gland cancers (fibro adenomas) in male rats (EPA 2009).The human toxicity excerpts are irritation eyes, nose, throat and skin. The main purpose of our study was to evaluate potential inhalation exposure during treatment with imidacloprid and hexythiazox, using personal samplers.

Materials and methods:

Work organization:

The research was conducted in Calabria (Southern Italy), in small family farms where the labor force is human labor; men dedicated to pesticides application and mainly foreign women to harvesting and leaf cleaning, activities. Table 2 shows the commercial formulations used.

Name	Characteristics	Class	Active substance
Nissorum	WP-wettable powder	Acaricide	Hexythyazox 10%
Difloron	water dispersible granules	aphicide-insecticide	lmidacloprid 200 g/l
Nuprid	water dispersible granules	aphicide-insecticide	lmidacloprid 200 g/l
Tenor	WP-wettable powder	Acaricide	Hexythyazox 10%

Table 2: Commercial formulation employed by workers

Each farm owns several plots of land and greenhouses, so that we could evaluate the exposure in the same farm, both in open field and in greenhouses even. These have different covers that can affect the exposure. So we also took this situation into consideration.All monitoring took place during real working time, in normal working activities and all subjects working in the days of monitoring were asked to participate, without interference.

All exposure experiments were conducted in April-May period at temperatures of 29–32 °C, relative humidity of 55–72%. For pesticide application, the spray solution was prepared by dispersing 90 ml of 17,1 % (200g/l) imidacloprid compounds and 60 gr of 10% hexythiazox in water; final concentration of active ingredient changes in relation to the volume of water in which the active ingredients are dissolved.

This working environment analysis brought out these five different main scenarios.

Scenario 1: workplace occupational exposure-greenhouse and open field:

The pesticide concentrations are highly dependent on working environment. The spreading activities were carried out in two different workplaces of the same farm: greenhouse and open field. The assessment of inhalation exposure was on worker engaged in mixing/loading and pesticide application, in open field and greenhouse. He used manual lance in both cases, applying an imidacloprid / hexythiazox mixture.

Scenario 2: occupational exposure and greenhouse covering:

The greenhouse provides the conditions to change the environment, in order to promote plant growth. The material that covers the greenhouse frame plays a key role in heat retention and in transmission of particular wavelengths of light. A variety of materials that can be used to cover a greenhouse. The most widely used in our area are polyethylene film (PE), shade cloth, rarely glass or solar panels. PE films are the most common and lowest cost type of covering material, for durability and for capacity to reduce heat loss, droplet formation and the amount of dust sticking to the film. Shade cloth is manufactured from knitted polyethylene fabric that does not rot, mildew or become brittle.

This helps protects plants and people from direct sunlight, improving light diffusion. These different covers create new exposition scenarios.

In order to examine the influence of the studied nets on occupational exposure, the worker was monitored during PE application with manual lance, under both covers. The greenhouses were treated with an imidacloprid / hexythiazox mixture.The monitoring was carried out between April and May. Temperature and humidity in plastic film and shade cloth covering, were monitorated.

Scenario 3: Subjects and exposure conditions, operator and support worker:

The different figures working agricultural holdings, are subject to different risks. In all farms visited, the single operator, runs a unit to spreading pesticides helped by other personnel, 'support worker' serving support roles. The certified applicator is a worker involved in activities related to the application of pesticides (operation of mixing/loading the products, emptying/cleaning and repairing the machinery after use, application product first-hand) while 'support worker' was a helper. This worker is in constant contact with pesticide even during all phases, but his role is underestimated.

Because in our farmer is often this way of working, it was necessary to evaluate the inhalation exposure for both figures.

Scenario 4: pesticide application equipment:

There are many ways of applying pesticides so that they are effective. The most common form of pesticide application is the use of mechanical sprayers. These convert a pesticide formulation, often containing a water/chemical mixture, into droplets, which can be large rain-type drops or tiny almost-invisible particles. This conversion is accomplished by forcing the spray mixture through a spray nozzle under pressure. The size of droplets can be altered using different nozzle sizes, or by altering the pressure under which it is forced, or a combination of both. The crops, the area size (ha), type of area to be treated (greenhouse or open field), pest and the weather conditions, the method of application called for on the label; also influence the choice of application equipment. Most commonly used pesticide application equipment in visitated farms, was hand operated (manual lance) and motorized / mechanical (air-blaster sprayers). We evaluated the worker's exposure during the of imidacloprid and hexythiazox application mixture with two different application system.

Scenario 5: application and re-enter:

Worker exposure can arise from also activities such as packaging, sorting and bundling. Maintenance activities, for example, may make it necessary for persons to re-enter treated areas relatively shortly after application e.g. for crop inspection activities or harvesting. The restricted-entry interval (REI) is the time immediately after a pesticide application, when entry into the treated area is restricted. Although it is indicated to re-enter the greenhouse after each treatment at least after hours, it is not so. Pesticides will have different re-entry intervals. That are established by considering the toxicity of the active ingredient, the rate and method of application including whether it is applied outdoors or in a confined space (such as greenhouses). Through environmental monitoring, we have evaluated how the persistence of a.s. after the application.

Analysis:

The potential inhalation exposure to imidacloprid and hexythiazox, was determined using personal air samplers (AirChek 2000) operating at a flow rate of 2 L/min equipped with an OVS sampling tube using XAD-2 sorbent (147/270 mg) as NIOSH 5602 method. The tube was located in the breathing zone of the operator and connected to the pump. Environmental monitoring is carried out using the same NIOSH 5602 method, placing the samplers in the environment at human height.

The samples were collected, transferred into polythene bags and transported to laboratory. The air sampling tubes were extracted in acetone, cleaned up using a Florisil column (NIOSH 3620) and analyzed by a Gas Chromatograph (Perkin Elmer Instruments, AutoSystem XL GC), with ECD (Electron Capture Detector). A PE Elite – 1701, 30m x 0.32mm x 0.25 µm column was used, and helium flow of 1.5 mL/min.
Concentrations of pesticides in air sample were used to calculate the real respiratory dose (RD) assuming a lung ventilation of 20 l/min (Fenske R.A. 1987)

The equation used for the calculation is the following:

RD (ug) = personal exposure (ug/m^3) × time of exposure (min) × lung ventilation (m^3/min)

GC operating conditions:

A calibration curve using standards of imidacloprid and hexythiazox was created for each compound. Calibration solutions were prepared at four concentrations ranging, from 0.2 to 50 ng/L by diluting a commercially available solution containing the analytes of interest. A calibration curve results by analysing each of the two calibration standards and fitting the data to a linear equation.

Reagent:

The pesticides standards compounds (imidacloprid, hexythiazox) were obtained from Sigma Aldrich in analytical standard purity grade (PESTANAL®). All solvents used for sample processing and analysis, were of GC grade. SPE extraction columns LC18 (500 mg, 6 mL) and XAD-2 sorbent tube were purchased from Sigma Aldrich.

Validation of the Analytical Method:

The validation of the proposed analytical method has been carried out in accordance with UNICHIM 179/1 "Guidelines for the validation of analytical methods in chemical laboratories", and in accordance with the technical standard UNI CEI EN ISO / IEC 17025: 2005 (General requirements for the competence of testing and calibration laboratories). The calibration curve was constructed with four concentrations of target compounds. Perkin Elmer Totalchrom Software was used for the integration of the areas of the chromatographic peaks generated by analysis of standard working solutions. The limits of detections (LOD) and limit of quantification (LOQ) were determined by considering 3- and 10-times ratio of signal to noise, respectively (IUPAC,1976). LOD for imidacloprid and hexythiazox were 0.21 and 0.17 ng/L, and LOQ for imidacloprid and hexythiazox were 0.5 e 0.42 ng/L respectively.

Recovery

Recovery experiments were performed to test sincerity of analytical method. In present study, the mean recovery values were acceptable: Imidacloprid 92.4 % R and hexythiazox 95.6 % R.

Results and discussion:

Scenarios 1: workplace occupational exposure-greenhouse and open field:

Mean of valid results obtained, was expressed as a Respiratory Dose (RD) of a worker in the two different workplaces (Figure 1). The plastic-clad structures or greenhouses may be prone to a higher concentration of pesticides relative to open field; because it is a literally a closed environment, that does not allow the exchange of air. Analyzing the data we find the presence of pesticides in the air, near the respiratory zone of the worker, in the open field too. Table 3 shows in detail the pesticides (imidacloprid +hexythiazox) concentrations in air samples of two worker monitored during daily working activity. The spray solution used was 0.18 g/l imidacloprid and 0.024 g/l hexythiazox.

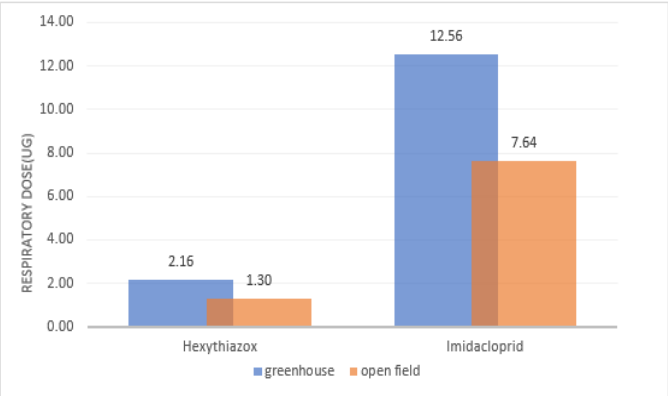


Figure 1: Occupational exposure differences: open field and greenhouse

n° personal air sampling (ug/m³)	Mean±SD	Median	Geometric mean	range(min-max)
Greenhouse (shade cloth)(n=10)	4,56 ± 4,43	3,33	2,99	0,75-15,98
Greenhouse (plastic film)(n=14)	5,30 ± 11,53	1,86	2,23	0,67-46,66
Open Field(n=4)	3,25 ± 2,26	3,01	2,36	1,02-5,97

Table 3: Exposure data of a two worker in working day activities in greenhouse and openfield.

Scenarios 2: greenhouse covering:

In greenhouse structures with a height of at least 4 meters, the natural passive ventilation becomes truly effective. Occupational exposition was assessed in greenhouses with a height of 4,5/5 meters and with two different covering, plastic film and shade cloth, We have compared 5 plastic film greenhouses and 3 shade cloth greenhouses measures (table 4), where the worker applied imidacloprid and hexythiazox by only hand lance (figure 2). Operator sprayed following their usual working routines; with the lance in right hand 30 cm above the top of the crop, swinging the lance from side to side. The area of greenhouse with plastic film is 3740 m², while the area of the greenhouse with shade is 2460 m². Table 5 shows the average temperature and humidity values. The applied concentrations in greenhouse shade-cloth covering, were 0.18 g/l imidacloprid and 0.024 g/l hexythiazox, in greenhouse PE film covering, were 0,0684 g/l imidacloprid and 0,0092 g/l hexythiazox (table 5). Despite the lower amount applied, the amount of substance to which the worker is exposed is greater in the greenhouse with the plastic film. Shade cloth is a material offering greater ventilation and a different expositional risk.

		RD(mg)		Concentration applied (mean) g/l	
Covering	mean area (m²)	Hexythiazox	Imidacloprid	Hexythiazox	Imidacloprid
plastic film (n=5)	3740	2,34	24,54	0,0092	0,0684
shade cloth(n=3)	2460	2,03	6,98	0,024	0,18

Table 4: Exposure data of a worker during pesticide application with lance in greenhouse.

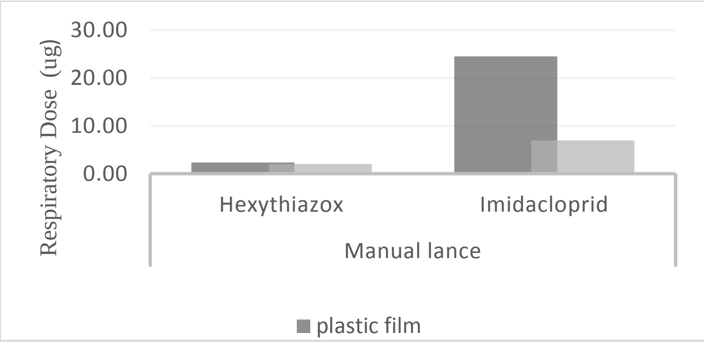


Figure 2. Assessment of occupational exposure in two different greenhouses covering: plastic film and shade cloth

	Temperature °C			**Humidity %**	
	mean ± SD	Range		mean ± SD	Range
Plastic film	21,95±3,80	19,27-27,32		58,27±1,33	56,38-59,21
Shade cloth	20,79 ±1,97	18,81-22,76		52,1±2,91	49,19-55,01

Table 5: Temperature and Humidity.

Scenarios 3: Subjects and exposure conditions:

During hand-lance application (hand-operated compression sprayer), operators were very exposed. Regularly one operator was with the lance above the top of crop swinging the lance and walking forward into the spray aerosol. The support operator helps the worker; he was exposed himself to pesticides during the application time, although he does not spread the pesticide directly. We have monitored both, considering them equally exposed, despite the apparent different roles (Figure 3). The applied mixture concentration during working day, was 0.13 g/l imidacloprid and 0.02 g/l hexythiazox.Results show the RD mean of the monitoring conducted in working day on two different workers (operator and support worker) during the manual application (figure 3a.).

Figure 3: Occupational exposure differences: operator and support worker

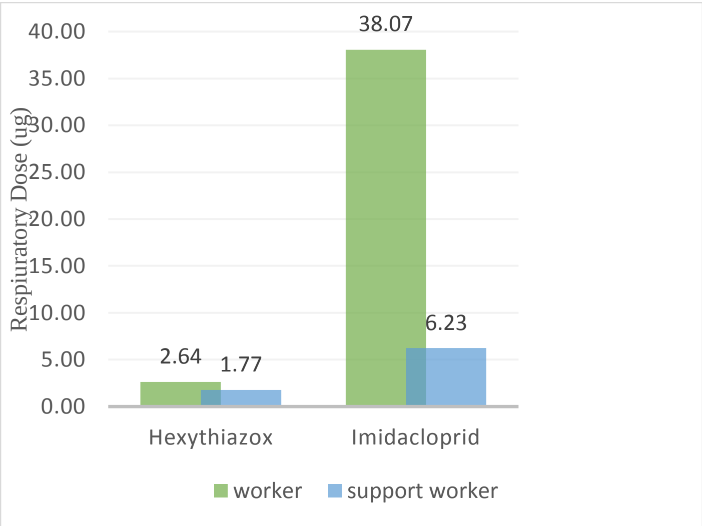


Figure 3a. Worker and support worker inhalation exposition during pesticide application.

Scenarios 4: pesticide application:

The level of pesticide exposure to the operator depends on the type of spraying equipment used. (https://monographs.iarc.fr/monographs-and-supplements-available-online/)

We found on farms, this principal system of pesticide application (figure 4):

a. system of a tank-vehicles attached to a garden hose connected to a lance used by operator

b. motorized sprayer, trailer-mounted, used to apply liquid pesticide mixture mostly to large areas (i.e. air-blast sprayers).

Figure 4. hand sprayer and air-blast application method

The tractor stood outside the greenhouse entrance while the spray operator held the spray lance at the end of a high-pressure hose connected to the tractor pump. The spray operator walked in plant rows spraying. He moved the lance continuously up and down, with the spray nozzle facing upwards, to the top of the plants, and with the nozzle spraying downwards, and back again.

We evaluated the worker's inhalation exposure to imidacloprid and hexythiazox during these different pesticides' application in greenhouse: air-blast sprayer and hand sprayer. Pesticides application by hand sprayer is usual, especially in little greenhouse. But the use of little spreading machines is also common in greenhouses when they are large enough to allow them. Normally a 50-60 cm lance is used, with a single nozzle and working pressures between 45 and 70 psig. Hand spraying with wide-area spray nozzles (when large areas need to be treated) is associated with greater exposure to the operator than narrowly focused spray nozzles.The application was done walking along one row and returning along the next one, spraying with the lance in right hand 30 cm above the top of the crop. When pesticides are applied with tractors the spray equipment is attached to a trailer.In this case, the operator is sitting at the wheel of the spraying machine, not cabled, and the product cloud is behind him.The applied mixture concentration during both applications, was 0.045 g/l imidacloprid and 0.006 g/l hexythiazox.The results (Figure 5) show a diverse occupational exposition.

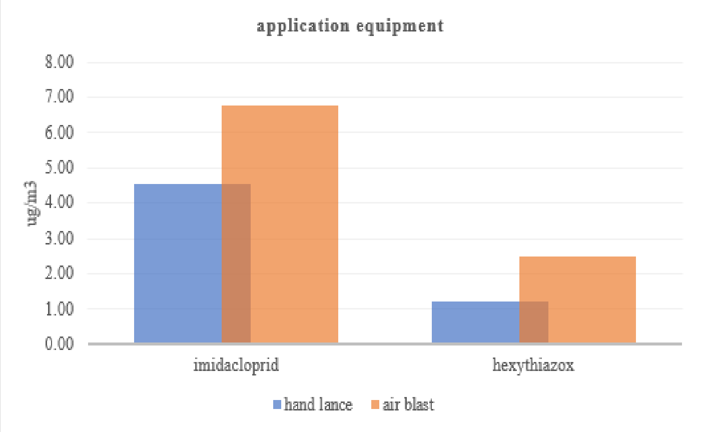


Figure 5: Assessment of occupational exposure during pesticides application with two different equipment: air-blast and hand sprayers.

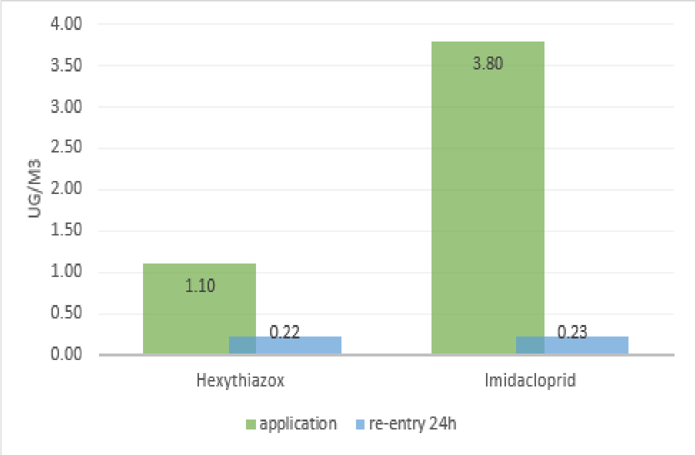


Figure 6: Imidacloprid and hexythiazox mixture, application and re-entry 24 h in greenhouse (plastic film)

Scenarios 5: application and re-enter:

One of the aspects often underestimated by the operators, but which instead may be relevant to health, is the moment in which one re-enters the crops to perform some operations such as thinning, harvesting, etc. Contrary to what we can thinking of this phase can also be an important source of exposure, especially if the so-called return time is not respected and personal protective equipment is not used. Imidacloprid and hexythiazox have a restricted-entry interval of 12 hours (EPA Registration No. 42750-117). Within 24 hours from pesticides treatment, an appreciable amount of the s.a. remains in air.A health risk evaluation of the observed levels of exposure after re-entry of greenhouses led to the conclusion that a health hazard may exist, especially after application of high rates of relatively toxic pesticides. The time between application and harvest, is called a "harvest interval," and it is necessary to allow pesticide residues to decrease to acceptable levels in crop. The re-entry time is the time interval that must pass from the last treatment to guarantee the operator the absence of exposure to product residuesstill present.This data should be present on the safety data sheet, even if it is currently for many products is not reported. So many activities are carried out in greenhouse even before the re-entry time.

We have done a environmental evaluation carried out in the same way as the personal one; with XAD vials, at man height, positioned in different points of the greenhouse, monitoring during application and left after the treatment. The applied mixture concentration was 0.045 g/l imidacloprid and 0.006 g/l hexythiazox. Figure 6 shows the mean of concentrations of imidacloprid and hexythiazox just after the application and after 24 hours.

In Figure 7 is shown the decay of the imidacloprid during application time,at 2h, at 4h, and at 24 h from the treatment. The treatment is done in a greenhouse shade cloth covering; after to 24h, the concentration of imidacloprid is very low compared to that present in the plastic covering. This shows once again how the greenhouse covering affects the exposure. Hexythiazox remains in the air; compared to the amount applied there is a 20% hexythiazox permanence.

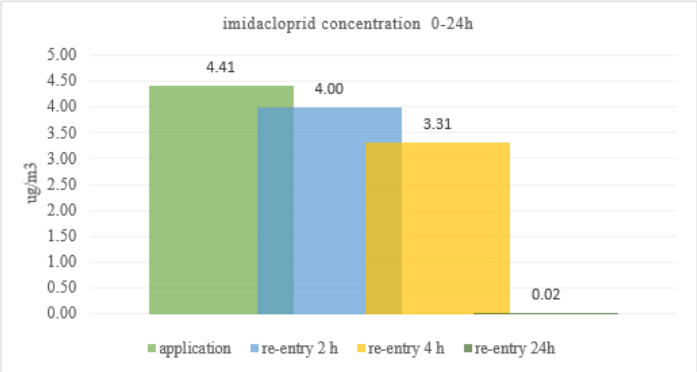


Figure 7: Imidacloprid application and re-entry after 2h, 4h, 24 h, in greenhouse (shade cloth)

Conclusion:

As regards potential inhalation exposure evaluation, the pesticide toxicology was studied, assessing active substance (a.s.) of pesticide products applied, the application equipment used (vehicle, manual), working areas (open field or greenhouse) and greenhouse features (plastic film, shade cloth). Risks associated with pesticide handling differ substantially for the different activities and from those experienced by agricultural re-entry workers.Many agricultural workers are exposed to chemicals on a daily basis. This field of work involves different exposure scenarios, each with its own risks to be assessed. In the course of this research work, we found the variability of this working environment; it becomes difficult to make an overall and uniform assessment. In the areas of the research, we have identified some scenarios, and we have evaluated the potential inhalation exposure in normal working conditions. The pesticides considered are those mainly used by examined companies and containing these active substances: imidacloprid and hexythiazox. The potential inhalation exposure was determined by active sampling, using GC-ECD for the instrumental identification and quantification. The results show the differences between the open field and greenhouse exposures. The little air circulation reduces the air movement in the greenhouse and increases the pesticide persistence time.

But it emerged that open-air exposure should not be underestimated; the application in the open field does not guarantee the absence of a risk of exposure. Moreover, the different types of greenhouse cover induce different exposure risks. The two covers most frequently encountered on farms (plastic film and shade cloth), give different results, so we cannot talk about greenhouse exposure in a univocal way. In addition, the roles of the workers are not strict; in small and medium-sized farms, the workers play several roles simultaneously. The figures involved in applying the pesticide are usually one or two men, appositely trained and the owner who is directly involved in this operation. These figures deal with handling of pesticides, including storage and diluting, mixing pesticides and cleaning of pesticide application equipment after use. They are often helped by other workers, not always formed, that not aware of the potential risks. The 'supporter worker' also works beside the operator, at the edge of the greenhouse or of open field, but since he does not directly take care of the pesticides application, he hardly ever wears the PPE. We have monitored and found that he is however, exposed to a not negligible amount of pesticide. In relation to the formulation of the product, to the areas treated and to the crops, different methods of pesticides application are possible. Therefore, pesticides spraying is often done using hand-operated sprayers. This lets you apply small amounts of pesticide to small areas, while for more extended areas the air-blast sprayers is used. Obviously, the application of pesticides by hand results in greater exposure both because there is greater proximity to the product, and because normally this application type takes place in a greenhouse, a closed environment. The application time with the lance is longer and the exposure becomes greater.

Re-entry intervals in greenhouse are set to protect people against poisoning by pesticides. If they enter a treated area too soon after application without proper protective equipment, workers may breathe vapours from a recent pesticide application, or some pesticide may be transferred to skin from plant treated. Even after the estimated time for re-entry, appreciable amounts of pesticide were detected in the air. Therefore, to minimize any unnecessary exposure to pesticides, always wear the appropriate personal protective equipment (PPE) as recommended on Safety Data Sheet (SDS) or product fact sheet and it is still good practice to wait until the product has dried before re-entering the treated area. If several pesticides are applied at the same time, the longest re-entry interval should be followed.

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