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Pesticides used in tobacco (*Nicotiana tabacum*) cultivation in Cuba: toxicological and ecotoxicological pressure*

Plaguicidas utilizados en el cultivo del tabaco (*Nicotiana tabacum*) en Cuba: presión toxicológica y ecotoxicológica

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Abstract

Introduction. Pesticides are used to control pests and diseases in agricultural activities. Improper handling of these products constitutes a potential risk to human health and the environment. **Objective.** To evaluate the toxicological (eco) pressure exerted by synthetic pesticides on tobacco (*Nicotiana tabacum*) crops in the province of Sancti Spíritus, Cuba. **Materials and methods.** A database of the Provincial Directorate of Plant Health for the period 2016-2019 was used, where the pesticides assigned to the province for this crop were taken and compared with those declared by the farmers interviewed. The dual indicator POCER was used to obtain the results, which determined the pressure exerted by the different pesticides and their harmful reference on humans and the environment. **Results.** The trend in pesticide consumption (summarized in tables and graphs) showed a decrease of 50 %, which corresponds to the country's crop protection policy. Farmers mentioned the use of authorised and unauthorized active ingredients in the surveys carried out. Sixteen high-risk active ingredients were detected in the samples analyzed. **Discussion.** Evaluation of the POCER results and analyzed samples shows that there is a high (eco)toxicological pressure on both the environment and human health exerted by a group of highly toxic active ingredients used in tobacco cultivation in the province. **Conclusion.** With the use of the dual indicator POCER, an evaluation of the toxicological (eco) pressure exerted by the synthetic pesticides used in the cultivation of tobacco (*Nicotiana tabacum*) in the province of Sancti Spiritus, Cuba for the study period is obtained.

Keywords: organophosphates, aquatic organisms, pesticide application equipment.

Resumen

Introducción. Los plaguicidas son utilizados para controlar las plagas y enfermedades en las actividades agrícolas. El manejo inadecuado de estos productos constituye un riesgo potencial para la salud humana y el medio ambiente. **Objetivo.** Evaluar la presión toxicológica (eco) ejercida por los plaguicidas sintéticos en los cultivos de tabaco (*Nicotiana tabacum*) en la provincia de Sancti Spíritus, Cuba. **Materiales y métodos.** Se utilizó una base de datos de la Dirección Provincial de Sanidad Vegetal para el período 2016-2019, donde se tomaron los plaguicidas asignados a la provincia para este cultivo y se compararon con los declarados por los agricultores entrevistados. Para la obtención de los resultados se utilizó el indicador dual POCER, que determinó la presión ejercida por los diferentes plaguicidas y su referencia nociva sobre el ser humano y el medio ambiente. **Resultados.** La tendencia del consumo de plaguicidas (resumida en tablas y gráficos) mostró una disminución del 50 %, que corresponde a la política de protección de los cultivos del país. Los agricultores mencionaron el uso de ingredientes activos autorizados y no autorizados en las encuestas realizadas, y en las muestras analizadas se detectaron dieciséis ingredientes activos de alto riesgo. **Discusión.** Al evaluar los resultados de POCER y muestras analizadas se observa que existe una gran presión (eco)toxicológica tanto para el medio ambiente como para la salud humana ejercida por un grupo de ingredientes activos de alta toxicidad utilizados en el cultivo de tabaco de la provincia. **Conclusión.** Con la utilización del indicador dual POCER, se obtiene una evaluación de la presión (eco) toxicológica ejercida por los plaguicidas sintéticos utilizados en el cultivo del tabaco (*Nicotiana tabacum*) en la provincia de Sancti Spíritus, Cuba para el período de estudio.

Palabras claves: organofosforados, organismos acuáticos, equipos de aplicación de plaguicidas

Introduction

More than half of the world's population has insufficient food consumption, even though food production has tripled since the II World-War. There is a shared responsibility to change this situation and to ensure that all people have access to healthy, quality food (Food and Agriculture Organization [FAO] et al., 2020; FAO, 2017). Humankind has developed agriculture as one of its main sources of food. Faced with this dilemma, high-yield agricultural production models have had to be implemented to supply the world market (Muller et al., 2017).

In order to achieve the aforementioned purpose, there are different strategies aimed at increasing food production, among which we can mention: new agricultural techniques, the use of improved seeds and the use of plant protection products, including pesticides. The application of pesticides on crops is becoming more and more important due to the expansion of intensive agriculture around the outskirts of cities, whose final destination is the domestic market (Rodríguez-Rojas & Peraza-Padilla, 2022; Sarkar et al., 2021).

It has been estimated that without the use of plant protection products (PPPs) a large amount of the world's food would be lost (FAO et al., 2020; FAO, 2017). It is necessary to state that the use of pesticides worldwide has become a basic necessity when applied to different crops (García Hernández et al., 2018; Leyva Morales et al., 2014). The tendency to increase is in function of controlling pests and diseases that can interfere with crop production (Sarkar et al., 2021).

Even if the use of these chemical products favors production processes, it is also true that their misuse, their application at inappropriate times and in crops in which they have not been registered, make these substances a potential risk for human health and for the environment in general, as they are generally toxic products, which can present residues in the final product that goes to the consumer and therefore diminish its quality (Lagos-Alvarez et al., 2022; Leyva Morales et al., 2017; Leyva Morales et al., 2014; López Dávila et al., 2022).

Although pesticides are indispensable in today's agriculture, the effect of pesticides on humans and the environment can be considerable (López Dávila et al., 2022; Sarkar et al., 2021). Pesticides are one of the main phytosanitary barriers and perhaps the biggest agricultural challenge of globalization. International regulations are becoming more stringent on a daily basis with regard to the maximum permissible limits of pesticides in different products for human consumption. The massification of food production has triggered an excessive use of pesticides, with serious implications not only for food residues, but also for the contamination of water sources, soil, air and other living beings (Sarkar et al., 2021; Singh et al., 2019).

The presence of chemical residues in food of agricultural origin has among its principles the various residues commonly known as environmental contaminants, which come from agrochemicals used in agriculture and livestock farming (Bastidas-Bastidas et al., 2014; Lagos-Alvarez et al., 2022). While pesticides have been a factor in the fight against many human diseases and hunger, the increased use of pesticides has led to the development of certain toxic diseases, making them a double-edged sword. They can produce acute or chronic poisoning, depending on the speed of absorption or repeated exposure to the pesticide (Blanco-Valdes et al., 2022; López Dávila et al., 2022).

It is important to note that organophosphates, dithiocarbamates, carbamates and pyrethroids have a marked action on the central nervous system. Some of these a.i. act reversibly, but others act irreversibly. Organophosphates bind irreversibly to acetylcholinesterase, preventing the degradation of acetylcholine in the environment, which produces nervous hyperactivity that ends with the death of the individual; they have high acute toxicity and are capable of causing neurotoxic effects in wildlife (Han & Wang, 2019; López-Dávila et al., 2020). Carbamates constitute a potential risk to human health because they are compounds capable of being absorbed by inhalation, ingestion and some even through the skin. Pyrethroids have a high speed of action against insect pests and can lead to adverse events that affect bacteria and even humans (Jardim et al., 2018).

Among the main adverse events caused by the aforementioned a.i. on humans, vomiting, headache, poor coordination, tremor, salivation, diarrhea and irritability to sound and touch, burning, itching and tingling progressing to numbness and moderate nervous manifestations have been observed. In addition, these compounds are highly toxic to bees, fish and aquatic arthropods. Therefore, it is important to use appropriate means of protection to avoid contact with the liquid and its vapours (López-Dávila et al., 2020; Tsakirakis et al., 2022). As well as respecting the dosage to be applied and the irrigation and distance regulations between the selected crop and the non-selected ones, as well as open water tanks (Vryzas, 2018).

In Cuba, in order to increase the productivity of agricultural systems, technological packages have been introduced in which the use of pesticides is the main component of the production system. The province of Sancti Spiritus is made up of eight municipalities, six of which produce tobacco (*Nicotiana tabacum*). The province has a varied agriculture, but the main crops harvested according to their importance are rice, tobacco, beans, roots and tubers, sugar cane, vegetables, onions and garlic, maize and fruits (Oficina Nacional de Estadísticas e Información [ONEI], 2019).

In the province of Sancti Spíritus, agriculture is one of the main sectors of the economy. In order to substitute imports, significant quantities of pesticides are being used on priority crops to increase yields. So far, no studies have been found on the consumption of pesticides in our environment. The risk they pose to human health and the environment has motivated to search for the main difficulties that may be associated with their use. Toxicity and ecotoxicological studies are variables to be monitored in environmental quality (Claus et al., 2021).

Several developed countries have developed different models capable of assessing the pressure, effect and impact of pesticides on man and the environment. In the pesticide reduction programme in Flanders, Belgium, a pesticide risk indicator was developed to assess pesticide risk reduction. The Pesticide Occupational and Environmental Risk Indicator (POCER), based on Annex VI of the 91/414/EC European Directive, consists of a module the risk to humans coming from occupational, non-dietary exposure and the risk to the environment. Each module is evaluated by the use of risk indices (Vercruysse & Steurbaut, 2002).

The POCER indicator has already proven its usefulness in Belgium and other countries (Bozdogan et al., 2015; Bueno & Da Cunha, 2020; Claeys et al., 2005; Cunha et al., 2012; Houbraken et al., 2016; Yarpuz-Bozdogan & Bozdogan, 2016) as a tool for the reduction of toxic synthetic pesticides. POCER can be used as a decision-making tool to choose alternative pesticides concerning the pressure on humans and the environment. It can also assess the impact of all pesticide applications related to a crop within a year and evaluate alternative cropping systems.

(Vercruysse & Steurbaut, 2002; Wustenberghs et al., 2018).

Due to the lack of studies that evaluate the pollutant pressure caused by synthetic pesticides of high (eco) toxicity used in tobacco (N. tabacum) cultivation in the province of Sancti Spíritus on human health and the environment, it is hypothesized that the use of an (eco)toxicological indicator as the POCER, will allow to evaluate this pollutant pressure, filling this gap, caused by the use of these pesticides.

This study aimed to evaluate the toxicological (eco-) pressure exerted by synthetic pesticides on tobacco (*Nicotiana tabacum*) crops in the province of Sancti Spíritus, Cuba.

Materials and methods

The conception of the study

A descriptive cross-sectional study was designed in the province of Sancti Spíritus, Cuba, between 2016 and 2019, in order to find out the possible risks to the environment and human health that may be caused by the use of pesticides in tobacco (*Nicotiana tabacum*) cultivation.

Operation of the variables

From a database that collect all pesticides assigned by the Provincial Plant Protection Department of Plant Health for tobacco cultivation, pesticides were classified by biological function, chemical nature, formulation and municipality. Data were also compiled for toxicological reference values no observed adverse effect level (NOAEL), acceptable operator exposure level (AOEL), acceptable daily intake (ADI), acute reference doses (ARfD), persistence in soil (half-life, DT_{50}), effect concentration (EC_{50}), no observed effect concentration (NOEC), lethal doses (LD_{50}) and lethal concentration (LC_{50}) in humans, terrestrial and aquatic organisms. The hazard classification criteria of the World Health Organization (World Health Organization [WHO], 2020) were used.

Data processing procedures

Data for all variables were summarized and tabulated. Absolute and relative frequencies (descriptive statistics) were calculated using the Statistical Package for the Social Sciences (SPSS) (v. 20) and expressed as percentages for each of the variable categories described. With the help of these analyses, better perception and connotation of the results are achieved to arrive at the conclusions of the research.

Toxicity and ecotoxicity assessment

In the Pesticide Occupational and Environmental Risk (POCER) indicator, risk indices (RIs) for human health and the environment are calculated as the ratio between the predicted environmental concentration (PAC) and a toxicological reference value, such as an acceptable operator exposure level (AOEL). After assessing the relevant risk parameters, POCER calculations can be performed by inserting the parameters (equations 1-10) into the model, which results in ten values, one for each of the human and environmental compartments (Vercruysse & Steurbaut, 2002). The calculated RI values are logarithmically transformed, and benchmarks are set between a lower limit and an upper limit, resulting in a dimensionless value between 0 and 1 for each compartment, where 0 indicates a low risk and 1 indicates a high risk of exposure (Vercruysse & Steurbaut, 2002; Wustenberghs et al., 2018).

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The total risk for human and environmental exposure is calculated in POCER by summing the values of the different components, assuming that all components are equally important. The risk to humans is therefore the sum of the risk to the applicator, the worker, the resident, and the bystander. The risk to the environment is calculated as the sum of the risk of persistence, leaching to groundwater, aquatic organisms, birds, earthworms and bees. The calculation formula for each module is described below:

Operator

Pesticide operators are the people who mix, load and apply pesticides (Tsakirakis et al., 2022). The exposure risk of pesticide operators in the POCER signaler is realized with the European Prophetic Operator Exposure Model (EUROPOEM). This model has been produced as a draft and the result is a Concrete Equation (CE) and is based on a database of relevant studies represented in European practices (Vercruysse & Steurbaut, 2002). Subsets of this database have been formed for generic exposure scenarios. The data are segregated into the categories of mixture, load and applicator, with further designation according to determinants such as type of formulation and type of application equipment. The pesticide operator risk index (Operator RI) is calculated with EUROPOEM in equation (1) as the quotient of the internal exposure, and the acceptable operator exposure level (AOEL).

Operator $$RI_{operator} = \frac{IE_{operator}}{AOEL} \quad (1)$$

Where, IE is the internal exposure during mixing/loading and application (mg/kg/day) and AOEL is the acceptable operator exposure level (mg/kg/day)

Worker

Workers interacting with the crop are exposed to contamination from pesticides that are still available on the crop after application such as exposure during re-entry tasks: harvesting, folding, etc. Dermal exposure through contact with crop foliage is considered the most important route of exposure during re-entry activities. There is a direct relationship between exposure, the application rate, and the degree of contact between crop and worker (Sarkar et al., 2021). During re-entry activities, inhalation exposure is very low compared to dermal exposure (Bozdogan et al., 2015; Sarkar et al., 2021), therefore, only the dermal exposure of the worker is calculated. For risk assessment, the internal exposure, calculated as the dermal exposure multiplied by the dermal absorption factor, is compared to the systemic AOEL. The internal exposure should be divided by the worker's human body weight (default = 70 kg) since the AOEL is expressed in (mg/kg/day). The risk index for workers is calculated in equation (2).

Worker/ Re-entry worker $$RI_{worker} = \frac{DE * Ab_{de}}{AOEL} \quad (2)$$

Where, DE is dermal exposure (mg/kg/day) and Ab_{de} is dermal absorption (-)

Bystander

In most cases, bystander exposure will occur through airborne contact during the application process (Cunha et al., 2012; Tsakirakis et al., 2022). At the moment, none of the internationally accepted models is available for bystander exposure assessment. In the POCER signaler, a bystander can interact with a dermal exposure and an inhalation exposure. If it is assumed that bystanders are located at a distance of 8 m downwind of the treated field,

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then there could be a dermal or inhalation uptake. The risk index for the bystander is calculated in equation (3).

Bystander $$RI_{bystander} = \frac{DE * Ab_{de} + I * Ab_i}{BW * AOEL} \qquad (3)$$

Where, DE is dermal exposure (mg/kg/day), I is inhalation exposure (mg/kg/day), AbI is Absorption by inhalation (–) and BW is body weight

Resident

The exposure of residents is practically negligible if it is assumed that they are not involved in any activity related to the use of pesticides on crops. There would have to be very specific climatic conditions (wind) and the dwelling would have to be very close to the treated field to consider any uptake by residents. The risk index for the resident is calculated in equation (4).

Residen $$RI_{resident} = \frac{DE * Ab_{de} + I * Ab_i}{AOEL} \qquad (4)$$

Where, DE is dermal exposure (mg/kg/day), I is inhalation exposure (mg/kg/day), AbI is Absorption by inhalation (–) and Abde is dermal absorption (–).

Aquatic organisms

For agricultural conditions in some countries, exposure to aquatic organisms is primarily caused by pesticide run-off (Bueno & Da Cunha, 2020). Another route of exposure is considered negligible as leaching. The Predicted Initial Environmental Concentration for aquatic organisms is calculated for a trench with a depth of 0.3 m and a width of 1 m. Since values are derivative (Vercruysse & Steurbaut, 2002). The factor 1000 is a conversion factor for the units. Equation 5 calculates the ratio of the Predicted Environmental Concentration (PEC) to the minimum standard for three groups of organisms (fish, daphnia and crustaceans). The safety factors used are defined in the Uniform Principles (Vercruysse & Steurbaut, 2002).

Aquatic organisms $$RI_{\text{aquatic organisms}} = \frac{PCsup_{\text{aquatic organisms}}}{minimum\ standard\,(_{\text{aquatic organisms}})} \qquad (5)$$

Where PEC superficial $_{\text{aquatic organisms}}$ is prediction of surface water concentration (g/L) and minimum standard ($_{\text{aquatic organisms}}$) is low toxicity values for three groups of organisms (fish, daphnids and crustaceans) (g/L).

Birds

Birds can be exposed to pesticides when collecting food in a treated field. There are three different worst-case scenarios for bird exposure. The first scenario is the daily feed intake taken by the birds assumed to be in treated crops. Small birds have a daily feed intake of 30 % of their body weight (Tassin de Montaigu & Goulson, 2020; Vercruysse & Steurbaut, 2002). The default weight of the bird is assumed to be 10 g. The average concentration in the crop immediately after spraying is estimated by multiplying the application rate (kg a.i./ha). The RI in birds is calculated in equation (6). Factor 10 is set by the Commodity Exchange Main Report).

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Birds $$RI_{bird} = \frac{PEC_{bird} * 10}{DL_{50} * BW} \quad (6)$$

Where PEC_{bird} is estimated total daily pesticide intake by birds (mg/day), LD_{50} is lethal dose for 50 % of the population (mg/kg/day) and BW is Body Weight (default = 0.01 kg).

Bees

The RI for bees only exists when pesticides are applied. In equation (7) the risk index for bees is calculated. Factor 50 is established by the Uniform Principles (Vercruysse & Steurbaut, 2002).

Bees $$RI_{bee} = \frac{AD}{LD_{50} * 50} \quad (7)$$

Where, AD is Application dose (g/ha), LD_{50} is lethal dose for 50 % of the population (µg/bee).

Earthworms

During pesticide applications, some of the application reaches the soil and may present a hazard to organisms such as earthworms. The risk index for earthworms is calculated with equation (8). Factor 10 is established by the main report of the Commodity Clearing House. For the initial estimation of the Estimated Pesticide Concentration in Soil (EPC in soil), it is assumed that the pesticide is concentrated in the first 5 cm of the soil. When pesticides are sprayed on crops, only a fraction reaches the soil below the plants and for the other types of treatments, it is assumed that the total pesticide dose reaches the soil (Gentil-Sergent et al., 2021).

Earthworms $$RI_{earthworms} = \frac{PEC_{soil} * 10}{LC_{50}} \quad (8)$$

Where, PECsoil is estimated concentration in the soil (mg/kg) and LC_{50} is lethal concentration for 50 % of the population (mg/kg).

Soil persistence

The persistence time of a pesticide indicates its persistence in soil. In Annex VI of 91/414/EC (European Commission, 2022) it is stated that no authorization of a plant protection product is granted if the DT50 of the pesticide in the soil is more than 90 days. In the Netherlands, no authorization is granted if the DT50 of the pesticide in the field is more than 180 days (Vercruysse & Steurbaut, 2002). These two principles are incorporated in the risk index for persistence in soil represented by the equation (9).

Persistence in soil $$RI_{persistence} = 10^{\left(\frac{DT_{50}}{90} - 1\right) * 2} \quad (9)$$

Where, DT_{50} is time in which 50 % of the pesticides disappear (days).

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Groundwater

According to the EU Uniform Principles, the concentration (a.i.) of a pesticide in groundwater should be less than 0.1 mg/l (Vercruysse & Steurbaut, 2002). The Pesticide leaching and accumulation model (PESTLA) is used to estimate the predicted Ambient Groundwater Concentration (AGW), i.e. the maximum concentration of a.i. in groundwater, for all types of applications. The risk indices for groundwater are calculated with equation (10). For spray applications, only a fraction of the sprayed pesticide reaches the soil due to interception by the crop. This fraction depends on the growth stage of the crop and its surface plant area. For the other types of application (seed and grain treatment, dipping and leaching) and powder applications directly on the field, 100 % of the a.i. reaches the soil (Cunha et al., 2012; Vercruysse & Steurbaut, 2002).

Groundwater $$RI_{groundwater} = \frac{PEC_{groundwater}}{0.1} \qquad (10)$$

Where, $PEC_{groundwater}$ is estimated pesticide concentration in groundwater (µg/L), and 0.1 is the European drinking water limit (µg/L) reference (Vercruysse & Steurbaut, 2002).

Integration of risk indices into total risk indicator values

For the integration of the risk indicators (RIs), the general method was developed by Beinat & van den Berg (1996). This method describes the extent to which a chosen index is exceeded as a dimensionless numerical value. First, a lower limit (LL) and an upper limit (UL) have to be established for the ten risk equations. Pesticides with a risk index value below the lower limit indicate a low risk 0, while when the upper limit is exceeded, a high-risk value of 1 is expected. Pesticides with a risk index below 1 fulfil the criteria formulated in the Uniform Principle of Directive 91/414/EC (European Commission, 2022). Secondly, the relative values of RI+, LL+ and UL+ are calculated by dividing respectively in equation 11 (Beinat & van den Berg, 1996).

$$X_{Transformed} = \log\left(1+\frac{1}{X}\right) \qquad (11)$$

Where, X is RI^{+}, LL+ and UL^{+}

Thirdly, the risk of a pesticide to the different components is related to the extent to which the lower limit of pesticide components is related to the extent to which the lower limit has been exceeded (Vercruysse & Steurbaut, 2002). The exceedance factor is calculated in equation 12.

$$EF = \left(\frac{X_{Transformed} - LL_{Transformed}}{UL_{Transformed} - LL_{Transformed}}\right) \qquad (12)$$

Exceedance factor (EF) values less than or equal to 0 are set to 0 and indicate low risk, EF values greater than or equal to 1 are set to 1 and indicate a high risk. Intermediate risk is found for values between 0 and 1. With the general method mentioned above, the pesticide risk to a particular component is expressed as a dimensionless value between 0 and 1. The total risk of a pesticide to man and the environment is calculated by summing the values of the ten components if it is assumed that all components are equally important. Thus, using the POCER indicator to calculate the total risk of a pesticide to man and the environment will provide a value from 0 to 10 (Vercruysse & Steurbaut, 2002).

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Tobacco samples analysis

Ten tobacco crop samples from farmers in the municipality of Cabaiguán were analyzed by gas and liquid chromatography to probe for the possible presence of trace pesticides. In this way, the traces could be correlated with the products applied. The municipality of Cabaiguán was chosen because it is the one with the highest tobacco production and pesticide consumption in this crop. Additionally, data from the interview with 50 farmers in the municipality of Cabaiguán from the doctoral study of López-Dávila et al. (2020) were used; to know what pesticides they used in the tobacco crop and to correlate them with those assigned in the study period.

QuEChERS

For the analysis of the tobacco leaves, the QuEChERS method was used. QuEChERS stands for Quick, Easy, Cheap, Effective, Rugged & Safe, which provides a highly beneficial analytical approach that simplifies the analysis of multiple pesticide residues in fruits, vegetables, cereals and processed products. The method comprises several simple analytical steps and will therefore be quick and easy to implement and is not susceptible to errors. QUEChERS offers high recoveries for a very wide range of pesticides belonging to different chemical classes (Huang et al., 2019; Musarurwa et al., 2019).

Procedure to analyze Tobacco crop samples

Two grams of the homogeneous sample are added in 50 ml Teflon centrifuge capped tubes. Milliq water is added until the sample weight is ten grams. Then 15 mL of acetonitrile (ACN) (from VWR Prolabo, Belgium) is added to the samples. To extract the contaminating co-extracts the following extraction salts (from Sigma Aldrich, Belgium) were added to the samples: 1.5 g NaCl, 1.5 g Na_3HCitrate dihydrate, 0.75 g Na_2HCitrate sesquihydrate and 6.0 g $MgSO_4$. The addition of these salts causes a reduction of the noise in the chromatograms. The samples are mixed for 5 minutes at 300 rpm. Then they are centrifuged for 5 min at 10 000 rpm in the centrifuge (Eppendorf, Belgium).

The solvent change is different in the samples for liquid chromatography with mass spectrometry detector (LC-MS/MS) compared to gas chromatography with electron capture detector (GC-ECD) (Claus & Spanoghe, 2020). In the case of LC-MS/MS 1 mL is taken from the top layer of the tube is added to a 10 mL volumetric vial; 9 mL of Milli-Q water is added to the 10 mL vial. This dilutes the sample 10-fold. The mixture is diluted well to obtain a homogeneous solution. A subsample of +/- 1.5 mL is pipetted into an LC-MS/MS vial. In the case of GC-ECD, 5 mL is taken from the top layer of the tube and added to an evaporation balloon. The solvent (ACN) is evaporated at the rotary evaporator (Buchi SL 200, Germany). 5 mL of hexane is added to the balloons. A subsample of +/- 1.5 mL is pipetted into a GC-ECD vial (López Dávila et al., 2020).

Analytical procedure

Samples are analyzed in the LC-MS/MS and GC-ECD. The working conditions are described below.

LC-MS/MS operating conditions

For operation in the MS/MS mode, the following parameters are set: curtain gas (N_2) at 7 bar; temperature 500

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°C (López Dávila et al., 2020).

Samples are analyzed in a Waters ACQUITY UPLC ™, equipped with a quaternary pump. The separation column, an Acquity UPLC BEH C18, 130 Å, 1.7 μm, 2.1 mm X 50 mm, is maintained at 40 °C. An automatic injector is arranged to inject 10 μl per sample. The phase mobile components are (A) Milliq water with 0.1 % formic acid and (B) acetonitrile with 0.1 % formic acid. The gradient used is adjusted to a flow rate of 0.4 mL/min of 98 % mobile phase A for 0.25 min. From 0.25 min to 7 min, a linear gradient to 98 % mobile phase B is used, which is maintained for 1 min. Then, a linear gradient of 98 % mobile phase A is used and maintained for 1 min. The assay is performed on a triple quadrupole system with electrospray ionization (detection mass spectrometer (Waters Xevo®

Table 1. Tandem mass spectrometry (MS/MS) transitions, applied collision energy and running time for the a.i. of pesticides used in tobacco cultivation in Sancti Spíritus province, Cuba, analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Cuadro 1. Transiciones de la espectrometría de masas en tándem (MS/MS), energía de colisión aplicada y tiempo de recorrido para los i.a. de los plaguicidas empleados en el cultivo de tabaco en la provincia de Sancti Spíritus, Cuba, analizados por cromatografía líquida con espectrometría de masas en tándem (CL-MS/MS).

Pesticide	Pesticide ion (m/z)	Fragment ion (m/z)	Ionization mode (/)	Cone voltage (eV)	Collision energy (eV)	Residence time (ms)
methomyl	163	88	ES	20	10	0.017
	163	106	ES	20	10	0.017
acephate	184.1	125.1	ES	11	18	0.052
	184.1	143	ES	11	8	0.052
pyrimethanil	200	82	ES	45	24	0.015
	200	107	ES	45	24	0.015
thiodicarb	355	87.9	ES	20	16	0.015
	355	107.9	ES	20	16	0.015
difenoconazole	406	111.1	ES	40	60	0.015
	406	251.1	ES	40	25	0.015
malathion	331	99	ES	20	24	0.013
	331	127	ES	20	12	0.013
dimethoate	230.1	125	ES	18	20	0.012
	230.1	199	ES	18	10	0.012
tebuconazole	308	70.1	ES	40	22	0.015
	308	125	ES	40	40	0.015
chlorpyrifos	349.9	97	ES	30	32	0.037
	349.9	198	ES	30	20	0.037
imidacloprid	256.1	175.1	ES	34	20	0.038
	256.1	209.1	ES	34	15	0.038
parathion	291.9	110	ES	30	33	0.017
	291.9	236	ES	30	14	0.017
diazinon	305	96	ES	31	35	0.017
	305	169	ES	31	22	0.017
propiconazole	342	69	ES	40	22	0.017
	342	159	ES	40	34	0.017
methamidophos	142	93.9	ES	28	13	0.163
	142	124.9	ES	28	13	0.163

*ES: electrospray. / ES: electropulverización.

TQD; Waters, Zellik, Belgium). The capillary needle is maintained at +2 kV. For operation in MS/MS mode, the following parameters are set: cut-off gas (N_2) at 7 bar; temperature 500 °C (López Dávila et al., 2020).

The A.I was monitored and quantified using multiple reactions monitoring (MRM). Two different m/z transitions were selected for each analyte. The MS/MS-transitions, ionization mode, cone voltage, and collision energy are given in Table 1. In general, the LOQ was set at 0.001 mg/kg and the LOD was 0.0003 mg/kg, respectively.

Gas chromatography with electron capture detection

Pesticides were analyzed on an Agilent Technologies 6890N gas chromatograph equipped with an Agilent Technologies 7683 Series automatic injector coupled to an electron capture detector (GC-ECD). Separation was performed on an HP-5MS capillary column (phenyl methyl siloxane 5 %, 30 m × 0.25 mm, 0.25 µm). The operating conditions were: initial column temperature 60 °C, then the oven temperature is increased at a rate of 20 °C/min until 150 °C. Subsequently, it is increased at a rate of 15 °C/min up to 250 °C, held for 2 min at 250 °C. Next, it is increased at a rate of 30 °C/min up to 270 °C and held constant for 10 min at 270 °C. Finally, it is increased at a rate of 30 °C/min to 280 ° and held for 11 min. The injector and detector temperature are maintained at 200 °C and 250 °C, respectively. Helium is used as carrier gas at a flow rate of 1.1 mL/min and injections are made in the cut-off mode at a ratio of 52.7:1 (López Dávila et al., 2020). The retention times of the active ingredients of interest analyzed in the GC are chlorothalonil 11.4 min., captan 13.6 min., endosulfan 14.2 min. and cypermethrin 24.2 min. To evaluate selectivity, individual solutions were injected, followed by a mixture of the normal solutions, and a mixture of the formulated a.i. Blank tests were performed (n=3) following the analytical extraction method to check for the absence of interference peaks under the same conditions regarding degradation products, impurities and matrix effects. Accuracy is evaluated with the placebo recovery method (European Commission Directorate General for Health and Food Safety [DG SANTE], 2021). The tobacco reference is injected at a concentration (100 μL x 10 mg/L) to determine the recovery at the maximum amount of expected a.i. The samples are analyzed under the same conditions and the ratio of the calculated amount to the expected amount expressed as a percentage is

Table 2. Limit of detection (LOD), the limit of quantification (LOQ) and coefficient of determination (R^2) of the active ingredients used in tobacco cultivation, tested by gas chromatography with electron capture detector (GC-ECD). Sancti Spíritus, Cuba. 2016-2019.

Cuadro 2. Límite de detección (LD), límite de cuantificación (LC) y coeficiente de determinación (R^2) de los principios activos empleados en el cultivo de tabaco, analizados por cromatografía de gases con detector de captura de electrones (GC-ECD). Sancti Spíritus, Cuba. 2016-2019.

Active ingredients	(LOD (μg L^{-1})	LOQ (μg L^{-1})	R^2
chlorothalonil	0.003	0.01	0.9998
alachlor	0.003	0.01	0.9998
endosulfan	0.003	0.01	0.9994
bifenthrin	0.003	0.01	0.9995
λ-cyhalothrin	0.003	0.01	0.9999
cypermethrin	0.003	0.01	0.9982

used to evaluate the recovery.

The linearity of each a.i. was determined through linear regressions of the calibration curve for five concentration levels between 0.004 and 0.1 mg/kg for each a.i. and the coefficient of determination (R^2) was calculated. Repeatability is evaluated by the coefficient of variation (CoV) of the measurement of each standard

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concentration and each a.i. The limits of detection (LOD) and quantification (LOQ) shown in Table 2 are determined by the method of the $t_{99sLLMV}$ (Bernal, 2014).

Tobacco recoveries are systematically low and need to be corrected. The values obtained of percent recoveries of the a.i. analyzed by GC-DCE and LC-SM/SM were corrected for recoveries below 70 % or above 120 %. The dilution made during sample preparation is also taken into consideration in the calculations. Pesticide recoveries in tobacco were generally low due to interference or matrix effects (DG SANTE, 2021; López-Dávila et al., 2020).

Table 3. Main questions of the survey on risk perception and pesticide use among tobacco (*Nicotiana tabacum*) growers in the province of Sancti Spiritus, Cuba.

Cuadro 3. Principales preguntas de la encuesta sobre percepción del riesgo y uso de plaguicidas realizada a cultivadores de tabaco (*Nicotiana tabacum*) de la provincia de Sancti Spíritus, Cuba.

Question
Socio-demographic background of study farmworkers
What is your: age/gender/education level/family members / total hectares / Did you receive training to apply pesticides?
Knowledge and use of pesticides
Where do you store pesticides? Do you follow the label instructions?
What outfit do you usually wear during pesticide application?
How close to a water body, do you spray pesticides?
Pesticide application
What do you mainly spray against? / Which products do you use?
How many hours do you work in the field?
How many pesticides do you apply per month?
What type of equipment do you use to apply pesticides?
Farmers' risk perception of human and environmental health by the use of pesticides
What is the field re-entry interval? What is the pre-harvest interval?
Do you consider that the use of pesticides can affect your health & the environment? / What health risks are related to working with pesticides?

Description of the applied survey

Fifty farmers representative of the municipality of Cabaiguán (farmers of average yields across all tobacco cooperatives in the region and references for the municipality's tobacco growers' association) were interviewed. A summary of the main questions is described below in Table 3. It should be clarified that to the author's knowledge, and to the absence of bibliographical evidence of any previous study, this is the first study on the subject to be carried out not only in the municipality under study but in the country as a whole.

Results

Generalities of the active ingredients (a.i.) used in the province of Sancti Spíritus on the tobacco crop in the

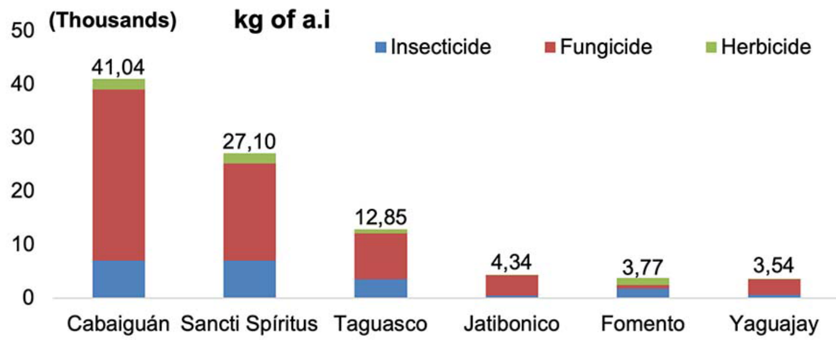

Figure 1. Total consumption of active ingredients (kg) in tobacco (*Nicotiana tabacum*) cultivation by the municipality in the period studied. Source: Pesticide allocations to the province of Sancti Spíritus, Cuba. 2016-2019.

Figura 1. Consumo total de materias activas (kg) en el cultivo de tabaco (*Nicotiana tabacum*) por el municipio en el periodo estudiado. Fuente: Asignaciones de plaguicidas a la provincia de Sancti Spíritus, Cuba. 2016-2019.

period 2016-2019

In this first part, aspects of the a.i. used in the period studied are presented with the support of a database of all the pesticides assigned by the Provincial Department of Plant Protection of Plant Health in the province of Santi Spíritus for the tobacco crop. Figure 1 shows the total consumption of a.i. for tobacco cultivation by the municipality in the period studied.

Just the municipalities of Cabaiguán and Sancti Spíritus, shown in Figure 1, are responsible for 74 % consumption of the a.i. allocated to the province. The above results are following the agricultural strategy of the province, where together Taguasco, Sancti Spíritus and Cabaiguán are the provincial municipalities with the greatest potential for tobacco cultivation and represent 87 % of the total a.i. used in the period studied. The municipalities of Trinidad and La Sierpe are not shown, since they are not part of the provincial strategy for tobacco cultivation. In addition to the provincial strategy, there is a historical geographic-cultural tradition where Trinidad is identified in coffee and livestock productions and La Sierpe in livestock and rice productions considered the second rice pole in the country.

The most used chemical family at the municipal level, as shown in Figure 1, are fungicides, followed by insecticides and finally herbicides, except for Fomento, which consumed more insecticides. One possible reason why more insecticides than fungicides were used in Fomento is that the main crop in this territory is coffee, which is susceptible to attack by Broca (*Hypothenemus hampei*), which is controlled with insecticides. At the provincial level, 66.2 tons of fungicides, 20.4 tons of insecticides and 5.29 tons of herbicides were used, representing 71%, 22% and 7%, respectively of the total consumed (91.9 t).

In general terms, shown in Figure 2, in the period studied the trend in pesticide consumption showed a 50 % decrease. This result corresponds to the policy of phytosanitary protection of crops in the country, where the trend is to reduce the toxic pollutant load and its possible effects on the environment and human health. In particular, herbicides and insecticides have a not very noticeable trend, since their a.i. values used are relatively low, not so for fungicides, which have a higher a.i. use per unit area.

Results of the interview with farmers

A total of 67 a.i. were declared by the farmers in the interview and 45 % of these did not coincide with those

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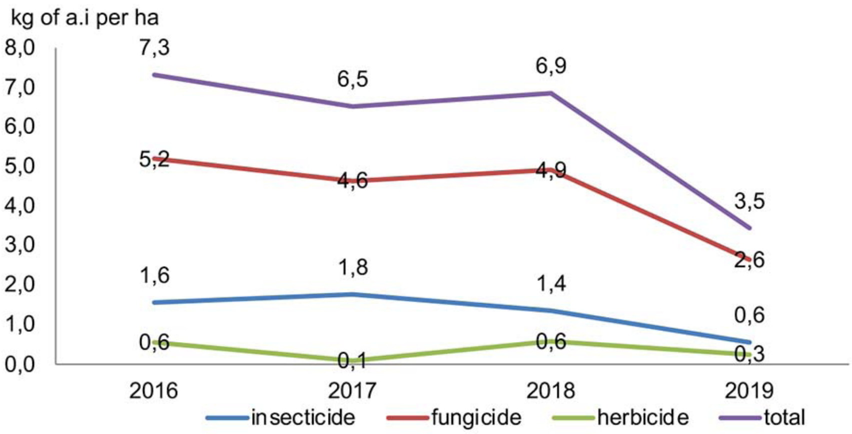

Figure 2. The trend of pesticide consumption in tobacco (*Nicotiana tabacum*) cultivation in Sancti Spíritus province, Cuba, period 2016-2019. Pesticide allocations to Sancti Spíritus province, Cuba. 2016-2019.

Figura 2. La tendencia del consumo de plaguicidas en el cultivo del tabaco (*Nicotiana tabacum*) en la provincia de Sancti Spíritus, Cuba, período 2016-2019. Asignaciones de plaguicidas en la provincia de Sancti Spíritus, Cuba. 2016-2019.

Table 4. Main active ingredients and chemical families declared by farmers and assigned by the Provincial Department of Plant Health for the cultivation of tobacco (*Nicotiana tabacum*) in Sancti Spíritus, Cuba. 2016-2019.

Cuadro 4. Principales materias activas y familias químicas declaradas por los agricultores y asignadas por el Departamento Provincial de Sanidad Vegetal para el cultivo de tabaco (*Nicotiana tabacum*) en Sancti Spíritus, Cuba. 2016-2019.

Declared by the farmers			**Assigned at Provincial Level**		
Active Ingredient	**Frequency**	**%**	**Active Ingredient**	**Frequency**	**%**
2,4-D	3	2.5	acephate	19	7.9
acephate	4	3.3	cypermethrin	8	3.3
bifenthrin	5	4.1	diazinon	7	2.9
cypermethrin	4	3.3	folpet	8	3.3
chlorothalonil	4	3.3	fosetyl aluminium	8	3.3
difenoconazole	3	2.5	imidacloprid	7	2.9
fosetyl aluminium	3	2.5	mancozeb	22	9.1
imidacloprid	6	4.9	methomyl	7	2.9
mancozeb	8	6.6	novaluron	10	4.1
tebuconazole	6	4.9	thiodicarb	6	2.5
Chemical Family	**Frequency**	**%**	**Chemical Family**	**Frequency**	**%**
phenoxy acetic acid derivative	2	1.7	aryloxyphenoxypropionates	6	2.5
aryloxyphenoxypropionates	4	3.3	benzoylurea	18	7.5
avermectins	2	1.7	carbamates	16	6.6
carbamates	3	2.5	inorganic compound	6	2.5
inorganic compound	3	2.5	dithiocarbamates	29	12
dithiocarbamates	3	2.5	phthalimide	9	3.7
neonicotinoids	4	3.3	neonicotinoids	7	2.9
organophosphates	5	4.2	organophosphates	45	19
pyrethroid	5	4.2	pyrethroid	16	6.6
triazoles	4	3.3	triazoles	8	3.3

assigned by the Provincial Plant Health Department. If we take into account that the list of a.i. assigned by the Provincial Department of Plant Health shows only 59 a.i., it can be affirmed that the farmers interviewed used additional a.i. not assigned to the tobacco crop. The use of a.i. in non-assigned crops constitutes one of the main risks for the health of the crop as well as for the applicator and agricultural worker, in addition to the risk of pest resistance.

Of the most commonly used a.i., as shown in Table 4, five (acephate, cypermethrin, fosetyl aluminium, imidacloprid and mancozeb) are common among those declared by farmers and those assigned by plant health. The correspondence between the 10 most used chemical families declared by farmers and those assigned by plant health is even greater (Table 4).

The predominant chemical families are carbamates, triazoles, aryloxyphenoxypropionates, organophosphates, neonicotinoids, inorganic compounds (copper oxychloride), dithiocarbamates and pyrethroids. A total of 39 chemical families were reported by farmers while Plant Health assigned only 35. A similar situation exists in the case of chemical families to that found in the a.i., a higher number of chemical families is reported by farmers.

A decentralized analysis by the municipality shows that the a.i. methyl parathion (Ia) and methomyl (Ib) extremely and highly hazardous respectively to humans were used in five of the six municipalities that cultivated

Table 5. List of active ingredients banned for use by the EC and the USA.

Cuadro 5. Lista de ingredientes activos cuyo uso está prohibido por la CE y los EE.UU.

Active ingredient	Prohibited in EC and/or USA	Restricted	Cancerogenic	Endocrine disruptor
acephate	x			
bifenthrin		x		x
captan			x	
β-ciflutrina	x			
chlorothalonil			x	
deltamethrin		x		x
diazinon	x			x
diquat	x			
phenthoate				x
fluazifop-p-butyl	x			
glyphosate		x		
glufosinate - ammonium		x		
imidacloprid		x		
lufenuron		x		
malathion		x		x
maneb		x	x	x
methamidophos	x			
methomyl	x			x
paraquat	x			x
parathion methyl	x			
quizalofop -p- ethyl		x		
tetraconazole			x	
tiodicarb	x			
zineb	x	x		x

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tobacco. Except for methamidophos (Ib) which was only used by the municipality Sancti Spíritus in 2016. Within the moderately toxic (II) classification for humans we can say that, at the provincial level, the insecticides that were used in the period studied are the most harmful pesticides to human health.

There is a group of a.i. used in the study period that presents a certain degree of toxicity (extremely and/or highly hazardous) to man and the environment. Some of this a.i. are no longer used, or their use is restricted in some countries, mainly Europe and North America. The Table 5 list the a.i. assigned by the Provincial Plant Health Department that are prohibited for use and/or restricted by the European Food Safety Agency and the Environmental Protection Agency of the United States of America.

As can be seen, about 19 % of the a.i. assigned are prohibited for use. A similar percentage is restricted for use. Additionally, the a.i. considered as possible or probable carcinogens and/or endocrine disruptors appear, mainly organophosphates, pyrethroids and dithiocarbamates. By analyzing these results can be seen that the tobacco crop can be considered a crop of moderate toxicity and ecotoxicity based mainly on the assigned compounds and the effect they have on human health and the environment.

To assess the possible risk of toxicity to humans and ecotoxicity to fish and bees, a characterization of the a.i. used in the period under study is given below.

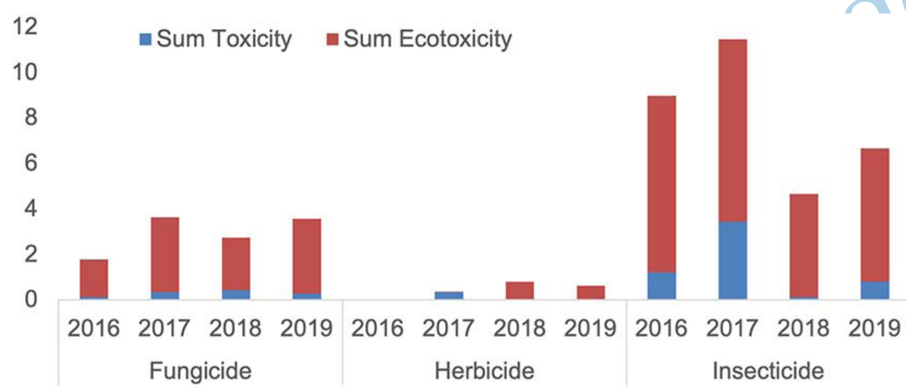

Figure 3. The trend of toxicity and ecotoxicity by biological function of the products used in tobacco (Nicotiana tabacum) cultivation in Sancti Spíritus, Cuba, over the study period. 2016-2019.

Figura 3. La tendencia de la toxicidad y la ecotoxicidad por función biológica de los productos utilizados en el cultivo del tabaco (Nicotiana tabacum) en Sancti Spíritus, Cuba durante el período de estudio. 2016-2019.

Toxic and ecotoxic assessment of tobacco cultivation

The study of the toxic and ecotoxic pressure of pesticides is of vital importance in understanding the risk to the environment and human health. Once the most critical molecules are identified, actions can be proposed to eliminate them or substitute them with less toxic compounds, thus reducing their negative pressure on the environment and human health. When analyzing Figure 3, it can be seen that, although fungicides were the most used pesticides in the province (Figure 1), their pressure on humans and the environment was not the highest.

The active ingredients of insecticides (organophosphates, carbamates, pyrethroids, neonicotinoids) are used to affect the environment and human health to a greater extent than the main chemical families of fungicides and herbicides. This is why, at POCER, insecticides exert significant pressure. POCER calculates the risk of the total intended pesticide use. While during the study period, there was a general slight trend towards decreasing

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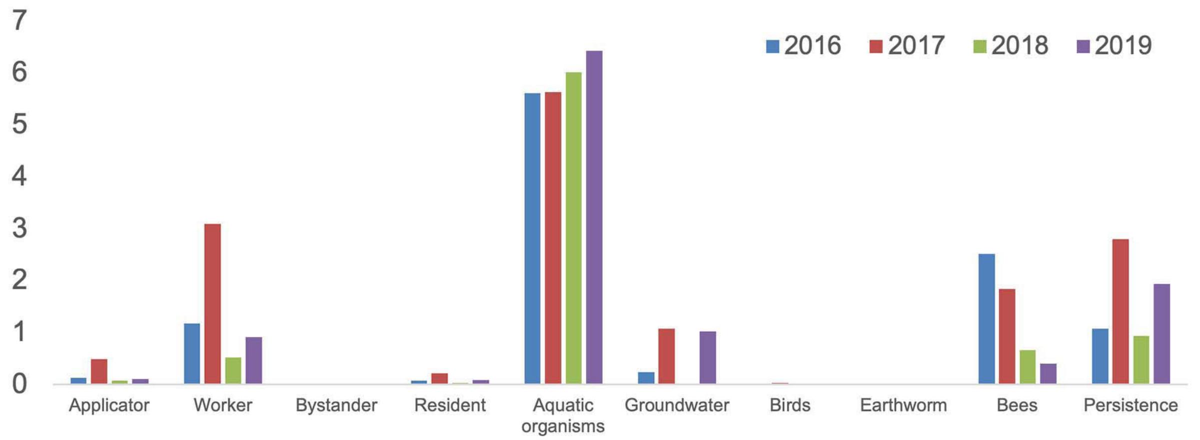

Figure 4. The Sum of toxic and ecotoxic pressure calculated by POCER of the products used in tobacco (*Nicotiana tabacum*) cultivation in Sancti Spíritus, Cuba, for the period 2016-2019.

Figura 4. La sumatoria de la presión tóxica y ecotóxica calculada por el POCER de los productos utilizados en el cultivo del tabaco (*Nicotiana tabacum*) en Sancti Spíritus, Cuba, para el período 2016-2019.

kilograms of a.i. per hectare (Figure 1), the POCER indicator shows contrary results. In general, the toxic and ecotoxic pressure of both fungicides and herbicides showed a tendency to increase. Insecticides showed an irregular decrease, but their values remained quantitatively higher than the pressures exerted by fungicides and herbicides.

The ecotoxic pressure is quantitatively higher than the toxic pressure on the man. The results shown in Figures 3 and 4 (below) show this approach. On many occasions, human beings do not perceive the damage that synthetic pesticides can cause to the environment and it is precisely in this environment where the natural pest controllers that cause losses and damage to crops coexist. Pesticides become double-edged weapons because when a man uses highly eco-toxic pesticides, he controls the pest and at the same time eliminates its natural controller, leaving him

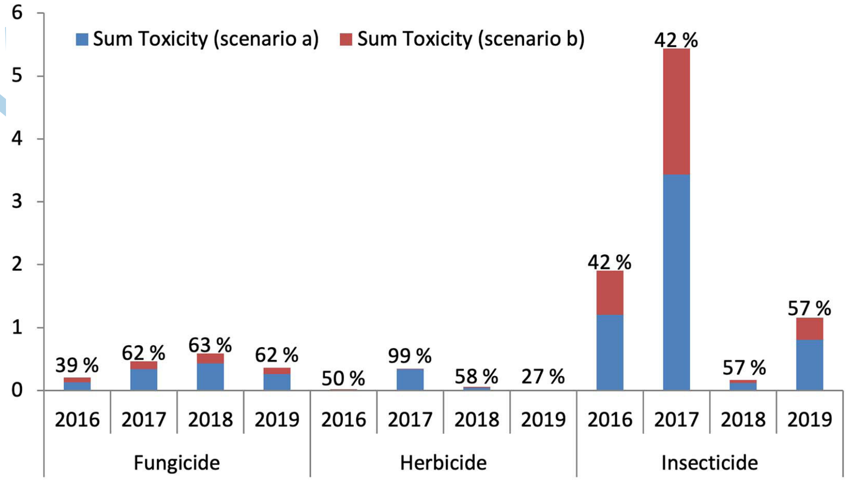

Figure 5. Use of the POCER indicator in the evaluation of the benefit of personal protective equipment (scenario b) to the reduction of toxic pressure (scenario a) of the products used in tobacco (*Nicotiana tabacum*) cultivation for the period 2016-2019 in Sancti Spíritus, Cuba.

Figura 5. Utilización del indicador POCER en la evaluación del beneficio de los equipos de protección personal (escenario b) para la reducción de la presión tóxica (escenario a) de los productos empleados en el cultivo de tabaco (*Nicotiana tabacum*) en el periodo 2016-2019 en Sancti Spíritus, Cuba.

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dependent on synthetic chemicals for the control of future pests.

Correct use of personal protective equipment (PPE) such as gloves, masks and respiratory filters can reduce risk rates in treated field re-entry activities. A practical example of these benefits is shown in Figure 5 when the POCER indicator was used to model two scenarios, the first ¨a¨ without PPE and the second ¨b¨ with the appropriate PPE depending on the activity carried out. This figure shows that the sole use of the appropriate means of protection can provide a reduction of more than 50 % of the pressure exerted on human health in most scenarios.

The objective of POCER is to evaluate the pressure, from low (0) to high risk (1), exerted by a pesticide in

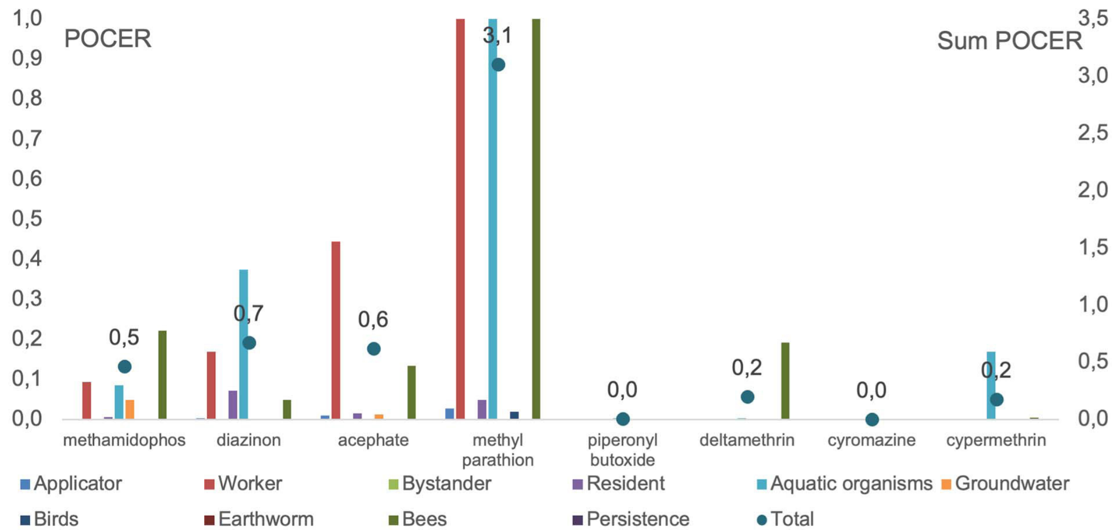

Figure 6. Active ingredients of insecticides with the highest and lowest toxic (eco-) pressure on the environment and human health evaluated with POCER. Sancti Spíritus, Cuba.2016-2019.

Figura 6. Ingredientes activos de insecticidas con mayor y menor presión tóxica (eco) sobre el medio ambiente y la salud humana evaluados con POCER. Sancti Spíritus, Cuba. 2016-2019.

each of the evaluated modules. With this information, decision-makers can decide whether to prohibit the use of certain high-risk products or replace them with others that fulfill the same phytosanitary function, but with less risk. Figure 6 is an example of the above. As can be seen, organophosphates (methamidophos, diazinon, acephate, methyl parathion) present the highest values of polluting pressure and also exert pressure on a greater number of modules, compared to other i.a. used in pest control for tobacco cultivation. With the simple substitution of use of this chemical family, an important reduction of the contaminant pressure would be observed (Figure 6).

Chemical residues

In total, 16 different a.i. were detected (Tables 6 & 7). A minimum of six a.i. was detected in the samples

Table 6. Active ingredients were detected in tobacco (*Nicotiana tabacum*) leaves sampled from farmers interviewed. Sancti Spíritus, Cuba. 2016-2019.

Cuadro 6. Ingredientes activos detectados en las hojas de tabaco (*Nicotiana tabacum*) recolectadas en los agricultores entrevistados. Sancti Spíritus, Cuba. 2016-2019.

	Active ingredients (mg/kg)							
Sample	**cyper-methrin**	**tebucona-zole**	**captan**	**chlorothalonil**	**metamidofós**	**pyrimethanil**	**valifenalate**	**difenoconazole**
Tobacco 1	< LC			0.01	< LD	< LC	< LC	
Tobacco 2	0.04				< LD			
Tobacco 3	0.04		0.41	0.01	< LD	< LC		<LD
Tobacco 4	< LC		0.41	0.02	< LD	< LC		
Tobacco 5	< LC		0.41	0.01	< LD			
Tobacco 6	0.10	< LC	0.41	0.01	< LD	< LC		
Tobacco 7	0.01	< LC		0.01	< LD		< LC	< LC
Tobacco 8	0.02	< LC	0.41	0.01	< LD	< LC	0.05	
Tobacco 9	< LC			0.01	< LD			
Tobacco10	0.07		0.41	0.01	< LD			
% recovery	48.4	57.0	56.4	54.3	50.2	40.9	45.2	46.9
(CV)	(5.72)	(2.32)	(14.0)	(8.67)	(10.36)	(8.62)	(12.5)	(14.0)

Table 7. Continuation of the active ingredients detected in the tobacco (*Nicotiana tabacum*) leaves sampled from the farmers interviewed. Sancti Spíritus, Cuba. 2016-2019.

Cuadro 7. Continuación de los ingredientes activos detectados en las hojas de tabaco (*Nicotiana tabacum*) recolectadas en los agricultores entrevistados. Sancti Spíritus, Cuba. 2016-2019.

	Active ingredients (mg/kg)							
Sample	**parathion methyl**	**thiodicarb**	**ethoxysulfuron**	**fipronil**	**chlorpyrifos**	**thiamethoxam**	**endosulfan**	**phenthoate**
Tobacco 1					<LC		0.04	<LD
Tobacco 2	0.11		<LC	< LC	<LC		0.12	<LD
Tobacco 3	0.06		<LC	0.02	<LC		0.09	<LD
Tobacco 4	0.05			0.03	<LC		0.06	<LD
Tobacco 5					<LC	0.077	0.05	<LD
Tobacco 6					<LC		0.04	<LD
Tobacco 7		0.03			<LC		0.10	<LD
Tobacco 8					<LC		0.09	<LD
Tobacco 9					<LC		0.04	<LD
Tobacco 10					0.02		0.04	<LD
% recovery	42.0	42.0	49.6	49.3	49.4	54.3	47.5	53.7
(CV)	(11.8)	(3.79)	(13.1)	(6.05)	(10. 6)	(9.63)	(6.66)	(6.93)

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analyzed. Eight a.i. (30 %) and 10 (30 %) were the highest frequencies. Up to 12 a.i. were detected in one sample. Forty-five per cent of these residues were quantifiable. Tebuconazole and chlorothalonil do not appear on the Cuban list of pesticides authorized for use in tobacco cultivation. Actions such as these, where certain a.i. are used on unauthorized crops, are examples of how pest resistance can develop. Four a.i. of the 16 detected (thiodicarb, methyl parathion, methamidophos and endosulfan) are banned for use in Europe. Endosulfan is also listed in Annex III of the Rotterdam Convention as a chemical banned or restricted for use. This shows Cuba as an example of how developing countries maintain the use of some banned products.

Discussion

The authors of the study recommend increased supervision, advice and vigilance on the part of phytosanitary signalers, to reduce infractions and incorrect use of chemicals, which could cause severe damage to crop production and the farmer's health. A similar amount (41 chemical families) was applied in other provinces of equal agricultural importance in the country (Pérez-Consuegra, 2018).

One a.i. (methyl parathion) is classified by WHO (2020) as extremely hazardous (Ia). Two others a.i. (methomyl and methamidophos) are classified as highly hazardous (Ib). In addition, another 16 compounds (27 %) are in the moderately hazardous category (II). The 37 % of the products show some degree of toxicity to bees; this constitutes an important environmental risk factor, which can lead to a decrease in bee populations and thus to a decrease in the role of bees in ecosystems (Claus et al., 2021; Claus & Spanoghe, 2020; Fevery et al., 2016). It is also observed that 71 % of the a.i., have some degree of toxicity in fish.

In agreement with the results obtained in Figure 4, several authors agree that, due to the persistence of some pesticides in the soil and their capacity to leach into groundwater and water bodies, aquatic organisms of the POCER indicator are the main modules at risk as a consequence of the use of highly toxic herbicides such as paraquat and prometryn, as well as organophosphate insecticides (Bozdogan et al., 2015; Fevery et al., 2016; Yarpuz-Bozdogan & Bozdogan, 2016). In addition, in a citrus region of Spain, chlorpyrifos (organophosphates) were the pesticides most commonly found to be ecotoxic to aquatic organisms (Bueno & Da Cunha, 2020; Cunha et al., 2012). It is known from previous studies that about 90% of the farmers in the province do not use PPE (López-Dávila et al., 2020).

Communication about risks and the use of PPE is facilitated by pictograms on pesticide labels (Bagheri et al., 2021; FAO & WHO, 2020). It is recommended that farmers be informed about the toxic risks to which they are exposed, in addition to knowing the importance of using PPE to minimize exposure to pesticides and prevent damage to health (Damalas et al., 2019; Yarpuz-Bozdogan & Bozdogan, 2016).

Since the tobacco plant is not considered human food or animal feed, there are no Maximum Residue Limit values available in Cuba, nor in the North American or European Environmental Protection Agency (López Dávila et al., 2020). In 1979, the Cuban delegation to the Code of the Committee on Residual Pesticides pointed out the need to discuss the question of pesticide residues in tobacco crops, as this was part of an important item in international trade (FAO, 1979). Today, there are maximum residue level guidelines for most of the a.i. used in tobacco leaf production from the Center for Cooperative Research in Tobacco Science and Technology (CORESTA) (Papenfus, 2017); but many values vary between countries and there remains a need to gain recognition by international regulatory bodies and authorities such as EFSA and U.S. EPA.

Some a.i. found in the tobacco leaf samples have the same mode of action (methamidophos, chlorpyrifos, phenthoate and methyl parathion, plus cypermethrin; tebuconazole and difenoconazole) and should be alternated with a.i. with another mode of action to prevent the development of resistance (Fungicide Resistance Action Committee, 2018; Inseticide Resistance Action Committee, 2019).

It is relevant to note that the a.i. found in the tobacco leaves sampled, six a.i. (endosulfan, ethoxysulfuron, fipronil, thiamethoxam, chlorpyrifos and phenthoate) were not assigned to the tobacco crop. Once again, facts like this demonstrate the negligence and bad agricultural practices developed by certain farmers who use certain chemical formulations in non-assigned crops, which increases the risks of creating resistance to pests and compromises the health of the crop, the environment and human health. Endosulfan, chlorpyrifos and phenthoate, together with cypermethrin, methamidophos and chlorothalonil are the most prevalent in the samples analyzed. Future actions should be taken to raise farmers' awareness of these bad practices and to control more systematically the pest control products used by farmers in tobacco cultivation as well as in the rest of their crops.

Conclusions

The interview with local farmers revealed the incorrect use of pesticides not assigned to tobacco cultivation. With the use of the POCER indicator, more precise analyses of the toxicity and ecotoxicity of synthetic pesticides can be carried out. Elimination or substitution of higher-pressure active ingredients with lower-pressure ones will significantly reduce the risk exerted on human health and the environment. A wide range of pesticides is detected in samples collected from tobacco. The authors consider that phytosanitary control should be increased in rural areas to avoid the inappropriate use of synthetic products on unauthorized crops. In addition to increasing the knowledge of agricultural producers through a training system aimed at the rational use of pesticides in production systems. Likewise, promote the implementation of Integrated Pest Management and Ecological Pest Management programs.

References

Bagheri, A., Pirmoazen, S., & Allahyari, M. S. (2021). Assessment of farmers' understanding of the pictograms displayed on pesticide labels. Environmental Science and Pollution Research, 28(14), 17812–17825. https://doi.org/10.1007/s11356-020-11821-w

Bastidas-Bastidas, P. J., Leyva-Morales J.B., Barraza-Lobo, A. L., Olmeda- Rubio, C., Pineda-Landeros, J. M., & Ramírez-Reyes. (2014). Evaluación de los datos de monitoreo sobre niveles de residuos de plaguicidas en hortalizas cultivadas en el valle agrícola de Culiacán, Sinaloa, México (período: 2011 – 2013). In L. O. Orozco Hernández, L. E. Garay Martínez, & M. R. Torres Vitela (Eds.), Investigaciones en Inocuidad de Alimentos (pp. 20–23). Prometeo Editores S.A. de C.V. http://congresoinocuidad.com/documentos/Investigaciones%20en%20Inocuidad%20de%20Alimentos.pdf

Beinat, E., & van den Berg, R. (1996). Euphids, a decision support system for the admission of pesticides. PBL Netherlands Environmental Assessment Agency. https://bit.ly/3BJVrmG

Bernal, E. (2014). Limit of detection and limit of quantification determination in gas chromatography. In X. Guo (Ed.), Advances in gas chromatography (pp. 57–81). INTECH. https://doi.org/http://dx.doi.org/10.5772/57341

Blanco-Valdes, Y., Cartaya-Rubio, O. E., & Espina-Nápoles, M. (2022). Efecto de diferentes formas de aplicación del Quitomax® en el crecimiento del maíz. Agronomía Mesoamericana, 33(3), Artículo 47246. https://doi.org/10.15517/am.v33i3.47246

Bozdogan, A. M., Yarpuz-Bozdogan, N., & Tobi, I. (2015). Relationship between environmental risk and pesticide application in cereal farming. International Journal of Environmental Research, 9(3), 1047–1054. https://dx.doi.org/10.22059/ijer.2015.992

Bueno, M. R., & Da Cunha, J. P. A. R. (2020). Environmental risk for aquatic and terrestrial organisms associated with drift from pesticides used in soybean crops. Anais Da Academia Brasileira de Ciências, 92(suppl. 1), Article e20181245. https://doi.org/10.1590/0001-3765202020181245

Claeys, S., Vagenende, B., De Smet, B., Lelieur, L., & Steurbaut, W. (2005). The POCER indicator: A decision tool for non-agricultural pesticide use. Pest Management Science, 61(8), 779–786. https://doi.org/10.1002/ps.1062

Claus, G., Pisman, M., Spanoghe, P., Smagghe, G., & Eeraerts, M. (2021). Larval oral exposure to thiacloprid: Dose-response toxicity testing in solitary bees, Osmia spp. (Hymenoptera: Megachilidae). Ecotoxicology and Environmental Safety, 215, Article 112143. https://doi.org/10.1016/j.ecoenv.2021.112143

Claus, G., & Spanoghe, P. (2020). Quantification of pesticide residues in the topsoil of Belgian fruit orchards: terrestrial environmental risk assessment. Pest Management Science, 76(10), 3495–3510. https://doi.org/10.1002/ps.5811

Cunha, J. P., Chueca, P., Garcerá, C., & Moltó, E. (2012). Risk assessment of pesticide spray drift from citrus applications with air-blast sprayers in Spain. Crop Protection, 42, 116–123. https://doi.org/10.1016/j.cropro.2012.06.001

Damalas, C., Koutroubas, S., & Abdollahzadeh, G. (2019). Drivers of personal safety in agriculture: A case study with pesticide operators. Agriculture, 9(2), Article 34. https://doi.org/10.3390/agriculture9020034

European Commission Directorate General for Health and Food Safety. (2021). Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. https://www.eurl-pesticides.eu/userfiles/file/EurlALL/SANTE_11312_2021.pdf

European Commission. (2022). Regulations - Commission Delegated Regulation (EU) 2022/643. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R0643

Fevery, D., Houbraken, M., & Spanoghe, P. (2016). Pressure of non-professional use of pesticides on operators, aquatic organisms and bees in Belgium. Science of the Total Environment, 550, 514–521. https://doi.org/10.1016/j.scitotenv.2016.01.123

Food and Agriculture Organization of the United Nations. (1979). Report of the eleventh session of the Codex Commities on Pesticide Residues. https://www.fao.org/3/ac313e/AC313E01.htm

Food and Agriculture Organization of the United Nations. (2017). The future of food and agriculture: trends & challenges. https://www.fao.org/3/i6583e/i6583e.pdf

Food and Agriculture Organization of the United Nations, International Fund for Agricultural Development, United Nations International Children's Emergency Fund, World Food Programme, & World Health Organization. (2020). The state of food security and nutrition in the World: Transforming food systems for affordable healthy diets. https://doi.org/https://doi.org/10.4060/ca9692en

Food and Agriculture Organization of the United Nations, & World Health Organization. (2020). Guidelines for personal protection when handling and applying pesticides - International Code of Conduct on Pesticide Management. http://www.who.int/neglected_diseases/vector_ecology/resources/9789240000223/en/

Fungicide Resistance Action Committee. (2018). Fungicides sorted by mode of action. https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2022--final.pdf?sfvrsn=b6024e9a_2

García Hernández, J., Leyva Morales, J. B., Martínez Rodríguez, I. E., Hernández Ochoa, M. I., Aldana Madrid, M. L., Rojas García, A. E., Betancourt Lozano, M., Perez Herrera, N. E., & Perera Rios, J. H. (2018). Estado actual de la investigación sobre plaguicidas en México. Revista Internacional de Contaminación Ambiental, 34(Esp. 01), 29–60. https://doi.org/10.20937/RICA.2018.34.esp01.03

Gentil-Sergent, C., Basset-Mens, C., Gaab, J., Mottes, C., Melero, C., & Fantke, P. (2021). Quantifying pesticide emission fractions for tropical conditions. Chemosphere, 275, 130014. https://doi.org/10.1016/j.chemosphere.2021.130014

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Gentil-Sergent, C., Basset-Mens, C., Renaud-Gentié, C., Mottes, C., Melero, C., Launay, A., & Fantke, P. (2022). Introducing ground cover management in pesticide emission modeling. Integrated Environmental Assessment and Management, 18(1), 274–288. https://doi.org/10.1002/ieam.4482

Han, T., & Wang, G. (2019). Peroxidase-like activity of acetylcholine-based colorimetric detection of acetylcholinesterase activity and an organophosphorus inhibitor. Journal of Materials Chemistry B, 7, 2613–2618. https://doi.org/10.1039/c8tb02616e

Houbraken, M., Bauweraerts, I., Fevery, D., Labeke, M. Van, & Spanoghe, P. (2016). Pesticide knowledge and practice among horticultural workers in the Lâm Đ ồng region, Vietnam. Science of the Total Environment, 550, 1001–1009. https://doi.org/10.1016/j.scitotenv.2016.01.183

Huang, Y., Shi, T., Luo, X., Xiong, H., Min, F., Chen, Y., Nie, S., & Xie, M. (2019). Determination of multi-pesticide residues in green tea with a modified QuEChERS protocol coupled to HPLC-MS/MS. Food Chemistry, 275, 255–264. https://doi.org/10.1016/j.foodchem.2018.09.094

Insecticide Resistance Action Committee. (2019). Mode of action classification scheme. https://https//www.irac-online.org/documents/moa-classification/

Jardim, A. N. O., Brito, A. P., van Donkersgoed, G., Boon, P. E., & Caldas, E. D. (2018). Dietary cumulative acute risk assessment of organophosphorus, carbamates and pyrethroids insecticides for the Brazilian population. Food and Chemical Toxicology, 112, 108–117. https://doi.org/10.1016/J.FCT.2017.12.010

Lagos-Alvarez, Y. B., Díaz-Ramírez, L. M., Melo-Velasco, J. M., & Hurtado Bermudez, J. J. (2022). Residuos de plaguicidas en mora (Rubus glaucus Benth.) en el Valle del Cauca, Colombia. Agronomía Mesoamericana, 33(2), Artículo 47538. https://doi.org/10.15517/am.v33i2.47538

Leyva Morales, J. B., García de la Parra, L. M., Bastidas Bastidas, P. de J., Astorga Rodríguez, J. E., Bejarano Trujillo, Jorge Cruz Hernández, A., Martínez Rodríguez, I. E., & Betancourt Lozano, M. (2014). Uso de plaguicidas en un valle agrícola tecnificado en el noroeste de México. Revista Internacional de Contaminación Ambiental, 30(Esp. 3), 247–261.

Leyva Morales, J. B., Martínez Rodríguez, I. E., Bastidas-Bastidas, P. de J., & Betancourt Lozano, M. (2017). Plaguicidas altamente peligrosos utilizados en el valle de Culiacán, Sinaloa. In F. Bejerano González (Ed.), Los plaguicidas altamente peligrosos en México (1ª ed., pp. 197–207). Red de Acción sobre Plaguicidas y Alternativas en México, A. C. https://www.rapam.org/wp-content/uploads/2017/09/Libro-Plaguicidas-Final-14-agst-2017sin-portada.pdf

López-Dávila, E., Torres, L. R., Houbraken, M., Laing, G. D., Romero, O. R., & Spanoghe, P. (2020). Knowledge and practical use of pesticides in Cuba. Ciencia Tecnologia Agropecuaria, 21(1), Article e1282 https://doi.org/10.21930/rcta.vol21_num1_art:1282

López Dávila, E., Houbraken, M., De Rop, J., Wumbei, A., Du Laing, G., Romero Romero, O., & Spanoghe, P. (2020). Pesticides residues in tobacco smoke: risk assessment study. Environmental Monitoring and Assessment, 192, Article 615. https://doi.org/10.1007/s10661-020-08578-7

López Dávila, E., Martínez Castro, Y., & Romero Romero, O. (2022). Características y consecuencias adversas a la salud humana de agroquímicos usados en la agricultura cubana. Revista Cubana de Salud Pública, 48(Suplemento especial

(Revisiones), Artículo e2810. http://www.revsaludpublica.sld.cu/index.php/spu/article/view/2810

Muller, A., Schader, C., El-Hage Scialabba, N., Brüggemann, J., Isensee, A., Erb, K. H., Smith, P., Klocke, P., Leiber, F., Stolze, M., & Niggli, U. (2017). Strategies for feeding the world more sustainably with organic agriculture. Nature Communications, 8, Article 1290. https://doi.org/10.1038/s41467-017-01410-w

Musarurwa, H., Chimuka, L., Pakade, V. E., & Tavengwa, N. T. (2019). Recent developments and applications of QuEChERS based techniques on food samples during pesticide analysis. Journal of Food Composition and Analysis, 84, Article 103314. https://doi.org/10.1016/j.jfca.2019.103314

Oficina Nacional de Estadistica e Información. (2019). Oficina Nacional de Estadistica e Información.

Papenfus, H. D. (2017). CORESTA Guide N°19. Responsible use of Crop Protection Agents (CPAs) in tobacco leaf production. Cooperation Centre for Scientific Research Relative to Tobacco. https://bit.ly/3RQPTwe

Pérez-Consuegra, N. (2018). Alternativas a los plaguicidas altamente peligrosos en América Latina y el Caribe. Asociacion Cubana de Técnicos Agrícolas y Forestales. https://ipen.org/sites/default/files/documents/alternativas_pap_v_final_16_enero_19.pdf

Rodríguez-Rojas, A., & Peraza-Padilla, W. (2022). Uso de Beauveria bassiana en el control de tecla [Strymon megarus (Lepidoptera: Lycaenidae)] en piña (Ananas comosus (L.) Merr.). Agronomía Mesoamericana, 33(3), Artículo 48235. https://doi.org/10.15517/am.v33i3.48235

Sarkar, S., Dias Bernardes Gil, J., Keeley, J., Mohring, N., & Jansen, K. (2021). The use of pesticides in developing countries and their impact on health and the right to food. European Union. https://doi.org/10.2861/28995

Singh, A., Dhiman, N., Kar, A. K., Singh, D., Purohit, M. P., Ghosh, D., & Patnaik, S. (2019). Advances in controlled release pesticide formulations: Prospects to safer integrated pest management and sustainable agriculture. Journal of Hazardous Materials, 385, Article 121525. https://doi.org/10.1016/J.JHAZMAT.2019.121525

Tassin de Montaigu, C., & Goulson, D. (2020). Identifying agricultural pesticides that may pose a risk for birds. PeerJ, 8, Article e9526. https://doi.org/10.7717/peerj.9526

Tsakirakis, A. N., Kasiotis, K. M., Glass, C. R., Charistou, A. N., Anastasiadou, P., Gerritsen-Ebben, R., & Machera, K. (2022). Sequential indoor use of pesticides: Operator exposure via deposit transfer from sprayed crops and contaminated application equipment. Applied Sciences, 12(8), Article 3909. https://doi.org/10.3390/app12083909

Vercruysse, F., & Steurbaut, W. (2002). POCER, the pesticide occupational and environmental risk indicator. Crop Protection, 21(4), 307–315. https://doi.org/10.1016/S0261-2194(01)00102-8

Vryzas, Z. (2018). Pesticide fate in soil-sediment-water environment in relation to contamination preventing actions. Current Opinion in Environmental Science & Health, 4, 5–9. https://doi.org/10.1016/j.coesh.2018.03.001

World Health Organization. (2020). The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 2019. https://bit.ly/3S5oZjQ

Wustenberghs, H., Fevery, D., Lauwers, L., Marchand, F., & Spanoghe, P. (2018). Minimising farm crop protection pressure supported by the multiple functionalities of the DISCUSS indicator set. Science of the Total Environment, 618, 1184–1198. https://doi.org/10.1016/j.scitotenv.2017.09.211

Yarpuz-Bozdogan, N., & Bozdogan, A. M. (2016). Pesticide exposure risk on occupational health in herbicide application. Fresenius Environmental Bulletin, 25(9), 3720–3727.