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RESEARCH ARTICLE

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Toxicological and ecotoxicological pressure due to pesticide use in Sancti Spíritus, Cuba

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Abstract

Aim of study: To quantify the toxicity and ecotoxicological pressure of pesticides in Sancti Spíritus province, Cuba, between 2011 and 2014.

Material and methods: A longitudinal descriptive study was designed for the study period, to identify potential risks to the environment and human health associated with the use of pesticides. In order to determine the toxicity and ecotoxicity of pesticide use, ΣSeq (Spread equivalents), POCER (Pesticide Occupational and Environmental Risk) indicator, and the Toxic Load (TL) methodology of the Plant Health Cuban Institute were used.

Main results: Corresponding to 62 chemical families, 124 active ingredients were applied in the province during the study period. Organophosphates, carbamates, pyrethroids, inorganic compounds (such as copper), dithiocarbamates, aryloxyphenoxypropionates, neonicotinoids, sulfonylurea, triazoles, and organochlorines predominated due to their frequency of use. Use of toxic-pesticides, lack of personal protection equipment amount others made workers, residents and applicators the toxicological modules with the highest risk of exposure. From the POCER results we found that aquatic organisms, persistence, and groundwater are the modules with the highest ecotoxicological pressure.

Research highlights: With the use of the POCER indicator as well as ΣSeq, a more accurate assessment of toxicity and ecotoxicity from certain pesticide can be done instead of the TL equation currently used in Cuba. In addition substitution of the most toxic pesticides by less toxic ones could help to reduce synthetic pesticide pressure on humans and the environment. This study can help to develop policies and management practices to reduce the hazards of synthetic pesticide use in Cuba.

Additional key words: organophosphates; endosulfan; POCER; ΣSeq indicator

Abbreviations used: : a.i. (active ingredient); POCER (pesticide occupational and environmental risk); PPE (personal protection equipment); RI (risk indices); TL (toxic load)

Authors' contributions: Conceived and designed the experiments: ELD, MH, ORR, PS. Performed the research: ELD, JDR. Analyzed the data: ELD, JDR, MH, ORR. Statistical analysis, drafting and critical revision of the manuscript for important intellectual content: ELD, MH, GDL, ORR, PS.

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Introduction

The use of pesticides worldwide has become a basic need for different crops to ensure quantity and quality in crop production. Pesticides have been a solution to fight against hunger and many diseases of humanity, allowing broad sectors of the population access to more high quality food (Räsänen *et al.*, 2015). The tendency to increase yields is argued in the pertinence of controlling diseases, insects, weeds and other organisms that can interfere with crop production (Leyva Morales *et al.*, 2014). Although its use favors production processes, it is also true that the inadequate use of synthetic pesticides, inappropriate timing of application, and their use in crops in which they have not been registered, make these pesticides a potential risk to human health and

the environment (Mesnage *et al.*, 2014; Dugger-Webster & LePrevost, 2018).

The increased use of pesticides can result in certain side effects in humans (Vryzas, 2018). There is no pesticide that lacks toxicity; they can result in acute poisoning once they are absorbed and accumulated in organisms (la Rosa *et al.*, 2014), and chronic damage can result from repeated exposure (Ventura *et al.*, 2016). For example, there are reports of teratogenic, carcinogenic and mutagenic diseases; damages to eyes, skin and mucous membranes; neurotoxic damage; damage to the immune system and lungs; and infertility (WHO, 2009; Mwila *et al.*, 2013; Botião *et al.*, 2014).

In Cuba, in order to increase the productivity of agricultural systems, technological packages have been introduced whose main component is the use of synthetic pesticides (Rosquete, 2011). In the province of Sancti Spíritus, where agriculture is the main economic sector, its management is in line with the rest of the country. As described by Damalas & Koutroubas (2018) in their studies on agricultural development in developing countries, the need to increase yields of priority crops in the province to reduce imports led to the use of synthetic pesticides.

Coupled with the use of synthetic pesticides, mainly in fruits and vegetables, there is a constant concern in the local population regarding the risk to human health and the environment reflected in various journalistic studies. There are currently no scientific studies that evaluate this risk pressure.

Toxicity and ecotoxicity studies are useful in monitoring environmental quality (Moermond *et al.*, 2016). Different methods and models have been developed and applied like the Dutch pesticide risk indicator (NMI 3), Danish pesticide load (PL) indicator, German pesticide risk indicator (SYNOPS), health risk indicator for operators (IRSA) and toxicity risk indicator for the environment (IRTE) (Strassemeyer & Gutsche, 2010; Kruijne *et al.*, 2012; Oussama *et al.*, 2015; Kudsk *et al.*, 2018) and software or programs like JOVA (Petersen *et al.*, 2013; Tollefsen *et al.*, 2016) and USEtox (Räsänen *et al.*, 2013; Nordborg *et al.*, 2017). An example of method is the criteria for reporting and evaluating ecotoxicity data (CRED) (Moermond *et al.*, 2016).

Derived from simplified quantitative models, the pesticide occupational and environmental risk indicator (POCER) (Vercruysse & Steurbaut, 2002), and the indicator based on the sum of the annual Spread equivalents (ΣSeq) (De Smet & Steurbaut, 2002), both developed at Ghent University (Belgium), stand as relevant options for the Cuban context. POCER assesses the risk for a large number of environmental modules, being one of the most dynamic and comprehensive models (Wustenberghs *et al.*, 2012). Five modules assessing the risk arising from occupational or other non-dietary exposure to agricultural pesticides, covering the four categories of persons, includes: risk to operators who apply the pesticides; risk to workers who may be exposed through re-entry activities such as harvest; risk to consumer; and risk to residents and bystanders who may be incidentally exposed during or after the pesticide applications. Seven modules covering different effects and environmental compartments assess the risk to the environment and include: persistence in the soil; risk of groundwater contamination; and acute risk to aquatic organisms, birds, bees and other beneficial arthropods, and earthworms. For each module, the risk is estimated by the use of risk indices (Vercruysse & Steurbaut, 2002).

The risk for modules concerning consumers and beneficial arthropods were not considered in this study. First, because the initial analysis obtained were very low and it was decided to study the risk of consumers through a probabilistic method, where detected residues in crops collected and the level of consumption of them are taken into account; the result from the probabilistic study is being reviewed. Second, because a 'No Data' response was obtained for many compounds of interest (*e.g.* ametryn, prometryn, triadimenol), due to the absence of necessary reference values, like the percentage of reduction of control capacity (RC), which affects the general analysis.

ΣSeq expresses the pressure on aquatic life that is produced by the use of pesticides (Fevery *et al.*, 2015). This indicator has been used since 1996 in the Flemish Government's (region in Belgium) environmental policy for a regional assessment of pesticide use (De Smet & Steurbaut, 2002). The use of each pesticide is weighted according to the differences in toxicity to aquatic organisms and the time of permanence in the environment (De Smet *et al.*, 2005).

In 1998 Cuba officially established the Environmental Law (González & Conill, 1999), in order to regulate sustainable agriculture. In addition, during the period 2007-2010, the Cuban Ministry of Science, Technology and Environment established a national environmental strategy, where by 2010, 80% of pest and disease control in the country should be done using natural products or biopesticides (Rosquete, 2011; Hernández & Pérez, 2012). However, there are no reports of compliance with this strategy to date. Similarly, there are no studies published in peer-reviewed journals or national information articles on the level of pesticide use in this territory or the evaluation of the toxicity and ecotoxicity due to the use of pesticides, and no indicators measuring such parameters were defined.

The constant concern for human health and environment in the local population was the basis to conduct this study, using the POCER and ΣSeq indicators to evaluate the toxicity and ecotoxicity instead of the level of toxic load (TL) according to the methodology of the Cuban Plant Health Institute. The goal is to determine the risks to human and environmental health that arise from the use of synthetic pesticides in the province of Sancti Spíritus. The study

concerns the years 2011 to 2014 and aims to identify the main pesticides causing pressure (unfortunately, usage data from more recent years were not available).

Material and methods

The province of Sancti Spíritus, constituted by eight municipalities, is one of Cuba's central provinces. Sancti Spíritus has a tropical climate, characterized by an average annual temperature of 24.3 ºC, average annual precipitation of 1,546.06 mm and 79.1% relative humidity recorded for the study period (National Bureau of Statistics and Information, 2015). Sancti Spíritus province has a varied agriculture, the main crops harvested being rice, tobacco, beans, roots, tubers (*e.g.* sweet potato), sugar cane, vegetables (*e.g.* tomato, cucumber, sweet pepper, onions), maize and fruits (*e.g.* papaya, guava, banana).

Operationalization of the variables

A database with all the pesticide use data registered in the accounting campaign strategy system of the Provincial Plant Protection Department during the study period was compiled. The use data per product were compiled according to their chemical family and biological function (per crop and year), as well as their toxicological reference values in humans and other terrestrial and aquatic organisms. The hazard classification criteria of the World Health Organization (WHO, 2009) were used.

Toxic load assessment

In the Cuban agricultural context, the indicator 'Toxic pollutant load' or simply 'Toxic load' (kg or L of active ingredient/ha) established by the Plant Protection Department of the Ministry of Agriculture of Cuba (Díaz, 2009) was used to give a measure of the general load on the environment resulting from the use of pesticides. To calculate the TL in priority crops, Eq. (1) was used. Analyzing Eq. (1) reveals that TL is a mere volume indicator and not at all a load indicator as meant by Kudsk *et al.* (2018). It has long and widely been acknowledged that quantities are not adequate proxies for assessing pesticide risk (Wustenberghs *et al.*, 2012). A similar equation was used to evaluate the contamination of drinking water by the use of pesticides in Vietnam (Chau *et al.*, 2015).

$$\mathrm{TL} = \mathrm{D} * \mathrm{a.i.}\,\% * \mathrm{NA} \quad (1)$$

where: TL=toxic load (kg or L of active ingredient/ha); D= dose (kg or L of commercial product/ha); a.i.% = active ingredient percentage in the commercial product; NA=number of applications = 1. It was calculated for each active ingredient per crop and year, showing the total in each case.

Toxicity and ecotoxicity assessment

In POCER, risk indices (RIs) for human health and for the environment are calculated as the ratio of predicted environmental concentration (PEC) to a toxicological reference value, as described by Vercruysse & Steurbaut (2002). After assessing the relevant risk parameters, the POCER calculations can be carried out by inserting the parameters (Eqs. 2-11) into the model, resulting in ten values, one for each of the human and environmental compartments (Claeys *et al.*, 2005). The calculated RI values are log-transformed, then a benchmarked between a lower and an upper limit are set, resulting in a dimensionless value between 0 and 1 for each compartment, where 0 indicates low risk and 1 indicates a high risk of exposure (Vercruysse & Steurbaut, 2002).

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

$$\text{Operator } RI_{operator} = \frac{IE_{operator}}{AOEL} \quad (2)$$

where IE = internal exposure during mixing/loading and application (mg kg^{-1} day^{-1}); AOEL = acceptable operator exposure level (mg kg^{-1} day^{-1}).

$$\text{Worker/Re-entry worker } RI_{worker} = \frac{DE * Ab_{de}}{AOEL} \quad (3)$$

where DE = dermal exposure (mg kg^{-1} day^{-1}); Ab_{de} = dermal absorption (–).

$$\text{Resident } RI_{resident} = \frac{DE * Ab_{de} + I * Ab_i}{AOEL} \quad (5)$$

$$\text{Aquatic organisms } RQ_{aquatic\ organisms} = \frac{PEC_{aqua\ org}}{minimum(norm_{aqua\ org})} \quad (6)$$

where $PEC_{aqua\ org}$ = predicted concentration in surface water (g L^{-1}); minimum ($norm_{aqua\ org}$) = lowest toxicity value of three groups of organisms (fish, *Daphnia*, and algae) (g L^{-1}).

The lowest of the following three quotients are used as the minimum ($norm_{aqua\ org}$): LC_{50} for fish/100; EC_{50} for Daphnia/100 and NOEC for algae/100.

$$\text{Birds } RI_{bird} = \frac{PEC_{bird} * 10}{LD_{50} * BW} \quad (7)$$

where PEC_{bird} = the estimated total daily pesticide intake (mg day^{-1}); LD50 = lethal dose for 50% of the population (mg kg^{-1} day^{-1}); BW = body weight (default = 0.01 kg).

Factor 10 is the criteria set by the uniform principles of the Commission of the European Communities established in 1994.

$$\text{Bees } RI_{bee} = \frac{AD}{LD_{50}*50} \quad (8)$$

where LD_{50} = lethal dose for 50% of the population (µg bee^{-1}).

$$\text{Earthworm } RI_{earthworm} = \frac{PEC_{soil}*10}{LC_{50}} \quad (9)$$

where PEC_{soil} = estimated concentration in the soil (mg kg^{-1}); LC_{50} = lethal concentration for 50% of the population (mg kg−1).

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

where DT_{50} = disappearance time for the first 50% of the pesticide (days).

$$\text{Groundwater } RI_{\text{groundwater}} = \frac{PCE_{groundwater}}{0.1} \quad (11)$$

where $PEC_{groundwater}$ = predicted concentration in the groundwater (µg L^{-1}); 0.1 = European drinking-water limit (µg L^{-1}).

Based on the fact that only the total amount of pesticides and areas cultivated for each crop are reported, per crop and year, the amount of each a.i. was divided by the area under cultivation to get a dosage value per ha (application rate). At the end in each case (crop and year), the sum of the final values of POCER was multiplied by the total hectares. In this way, it can be observed which crop's production has the greatest impact, at the territorial level, on human health and the environment.

For the toxicities modules, a group of assumptions was made. The assumptions were considered based on the results from a farmer survey study (Lopez *et al.*, 2020). First, IEoperator in Eq. (2) is strongly influenced by the use of protective clothing during mixing, loading and spraying. In this case, only a long-sleeved shirt, pants, boots and hat were considered protective clothing. Aerial spraying was considered for rice, as well as the tractor (open cabinet) for sugar cane. Second, for re-entry workers, similar to the operator scenario, no protective equipment like masks with or without filter, gloves, face- and/or eye shield was considered. For the resident module, there was no buffer zone considered because homes are within the farm and very close to the crops, and there is significant pesticide drift due to the use of a classic nozzle.

The sum of spread equivalents (ΣSeq) used in environmental policy in Flanders (Belgium) is an indicator of ecotoxicity that calculates the pressure from using pesticides for both agricultural and non-agricultural purposes (vector control) in aquatic organisms (De Smet & Steurbaut, 2002; Fevery *et al.*, 2015). ΣSeq was considered in this study since POCER considers that the exposure of aquatic organisms is mainly caused by the drift of pesticides, does not consider their ability to persist in the soil, and therefore ends in water bodies through surface runoff and leaching; parameters more in line with the current Cuban agricultural context. In addition, the variable minimum (normaqua org) is restricted to only three ecotoxicity values (LC_{50} for fish, EC_{50} for Daphnia and NOEC for algae), while MAC (maximum allowable concentration for aquatic life, mg L^{-1}) is determined on the basis of six different ecotoxicity values, allowing more accurate results. ∑Seq is calculated by the following equation:

$$\sum Seq = \frac{E*DT_{50}}{MAC} \quad (12)$$

where ∑Seq= Seq; E= annual use of pesticides (kg of a.i./ year); DT_{50}= degradation time of 50% of the a.i. in the soil (years).

The MAC values are calculated through dividing the lowest toxicity value (representative aquatic organisms, *i.e.* the acute or chronic toxicity to three trophic levels: $EC_{50algae}$, $NOEC_{algae}$, $LC_{50crustacea}$, $NOEC_{crustacea}$, LC_{50fish}, and $NOEC_{fish}$) by safety factor '10', as in Fevery *et al.*(2015).

Procedures for data processing

Data for all variables were summarized and tabulated. A group called 'vegetables' was created which includes tomatoes, onions, garlic, sweet pepper, cucurbitaceous vegetables, among others. The group 'grain' includes beans and corn; the 'roots and tubers' group is formed by sweet potato, malanga, and potato. 'Fruits' are a general group, taking into account coffee and banana, among others. The Statistical Package for Social Sciences (SPSS) program (v. 20) was used. Pearson correlations (p<0.01 and p<0.05) were used to evaluate the parametric correlation between TL values which POCER and ΣSeq indicator.

Results

Pesticide use in the province of Sancti Spíritus during the years 2011-2014

Fig. 1 shows that herbicides are the predominantly used pesticides, representing 63% of the total (1110 tons of a.i.). They are followed by fungicides (22%) and insecticides (15%). This is due to the fact that large land extensions have been used to grow crops such as sugar

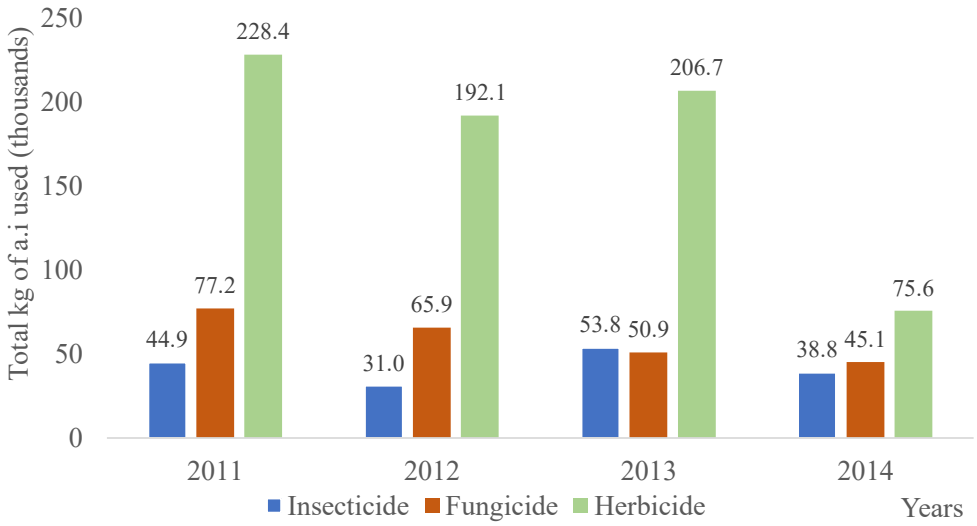

Figure 1. Total kilograms of active ingredients (a.i.) used in the Sancti Spíritus province per year. *Source*: Provincial Department of Plant Health accountant database of pesticide assigned to Sancti Spíritus province 2011-2014.

cane, rice and fruit trees, which requires large volumes of herbicides to control weeds. It is important to note that potato from the group 'roots and tubers' was planted only in 2011 and 2012. The country's economic strategy decided to stop planting potatoes in the province. On the other hand, potatoes were considered because they represented 73% and 33% respectively of the total amount of pesticide used in 2011 and 2012 in the group 'roots and tubers'. No data was found for sugarcane for 2014.

The absence of data for sugarcane for 2014 should not mean a problem to issue a conclusion in the general discussion at the end of the work if an average pressure equivalent to previous years is assumed. For the assumption, the following data were considered:

— The values of harvested area and production are similar to the average reported for the period 2011-2013 (Oficina Nacional de Estadística e Información de la República de Cuba, 2019, http://www.one.cu/).

— The stability between the annual values of kg of total a.i. (coefficient of variation of 8.7%).

— According to the national statistics of Cuba, the value of herbicide investment and its respective amount in tons for 2014 is equivalent to the average for the years 2011-2013 (ONEI, 2017).

During the study period the use of synthetic pesticides in Sancti Spíritus province showed a fairly constant use, as seen in Fig. 1 (except for 2014). These values are in contrast to the progressive reduction strategy of the crop protection policy promoted by the Cuban Ministry of Science Technology and Environment, where the aim is to reduce toxic pollutant load and its potential side effects in the environment and human health.

From the total amounts seen in Fig. 1, just sugar cane crop (scattered throughout the province) used 40% of total pesticides. Together with sugar cane, rice (24%) and tobacco (14%) used 78%. The results are in line with the main crops that are developed in the territory (rice, tobacco, vegetables, grains, sugar cane, and fruits).

In total, 124 a.i. (40 fungicides, 42 herbicides, and 42 insecticides) were used in agricultural activities during the study period, with a variable amount of their uses depending on the crop to which they were assigned. This a.i. corresponds to 62 chemical families. A similar amount (69 chemical families) were applied in other provinces of equal agricultural importance. The predominant chemical families are organophosphates, triazoles, sulfonylurea, pyrethroids, inorganic compounds (*e.g.* copper oxychloride), carbamates, dithiocarbamates, neonicotinoids, organochlorines, and aryloxyphenoxypropionate.

The a.i. most used during the study period was ametryn (215 tons, 19% from the total a.i. and 30% of the total herbicide used), followed by 2.4-D amine salt (165 tons, 14% from the total a.i. and 23% of the total herbicide used) and mancozeb (100 tons, 8% from the total a.i. and 36% of the total fungicide used). They were used in several crops, like sugar cane, rice and tobacco. There are six a.i., namely methyl parathion, methamidophos, methiocarb, methomyl, 1.3-dichloropropene and endosulfan, which are classified by WHO as extremely toxic (Ia) and highly toxic (Ib) to humans. In addition, 28 other compounds are in the category of moderately toxic (II). The 59% of the products show some degree of toxicity against bees; this constitutes an important environmental risk factor, as it can lead to declines in bee populations and the ecosystem services they perform. It is also shown that 80% of the pesticides are to some degree toxic to fish.

Evaluation of the toxic and ecotoxic load in Sancti Spíritus province

Studying pesticide pressure by calculating the (eco) toxic load has vital importance to understand the environment and human health risk. Once the more critical molecules are identified, actions can be proposed to eliminate them or to substitute them with less toxic compounds.

Fig. 2 shows that although herbicides were the pesticides most used in the province (as seen in Fig. 1), their pressure on humans and the environment was not always the highest. The TL values were different between biological functions. In 2011, for example, TL fungicide was significantly higher than TL herbicide due to potato cultivation, which reported the higher ratio kg a.i. per treated area (73.2 kg a.i. ha^{-1}), from 8 (tobacco 8.8 kg a.i. ha^{-1}) to 490 (corn 0.15 kg a.i. ha^{-1}) times higher than the other crops. The fungicides (*e.g.* mancozeb, chlorothalonil, copper oxychloride) represented 48% of the a.i. used this year. The herbicides (*e.g.* ametryn, glyphosate, EPTC) took the second position with 42%. In 2012 the ratio in potatoes decreased to 14.8 kg a.i. ha^{-1}, and in further years potato was not planted. Another observation is the TL trend, which decreased over time, although the consumption of a.i. remained fairly constant during the study period (Fig. 1), this is because the treated area of the crops increased (from 82.9 to 103.8 thousands of hectares), except sugarcane (from 58.7 to 28.3 thousands of hectares), thus causing a general progressive decrease in the ratio of kg a.i. used per treated area.

According to Eq. (1), TL only expresses the amount of a.i. (kg or L) applied per hectare, the particular toxicities for human health (NOAEL, AOEL…) and environment (DT_{50}, EC_{50}, NOEC, and LC_{50} values) are not taken into account, and hence the pressure of pesticide use is not very accurate. A simple substitution by another pesticide with a lower amount of a.i. will result in a decrease in the TL. However, if this new pesticide has higher toxicity and/or ecotoxicity, this will increase the pressure.

Fig. 2 also shows that POCER herbicide in 2011 is quantitatively greater than in the rest of the years. This is due to the cultivation of sugarcane, which has the largest treated area of all crops (41% in 2011), declining about half in 2012 (23%) and 2013 (24%). Sugar cane crop represented 75% of the total POCER herbicide pressure for 2011, the main a.i., due to its toxicity, being paraquat, hexazinone and diuron. In this work the ΣSeq for insecticide increased gradually due to endosulfan use, the a.i. with the higher Seq-factor (DT50/MAC = 1.2 * 10^8), 71 times higher than paraquat, the second ecotoxic a.i. (DT_{50}/MAC = 1.7 * 106). Endosulfan was used in corn (10 kg), beans (140 kg) and onion (280 kg) in 2011, in 2012 just in onion (296 kg), then for 2013 in tomato (175 kg) and onion (348). In 2014 tomato used 280 kg and onion 925 kg, onion being the crop that exerts the higher ecotoxic pressure on aquatic organisms. Unlike TL and POCER that decreased over the years, ΣSeq increased, its values are directly related to the use of endosulfan with an increase over the years.

With the use of POCER and ΣSeq indicators, taking into account the effect on both terrestrial and aquatic organisms, the pressure caused by a specific a.i. can be more accurately assessed. This is why, in both ΣSeq and POCER, insecticides exert significant pressure, with marked differences in the ΣSeq indicator case due to the use of endosulfan. The a.i. of the used insecticides negatively impacts the environment and human health.

Table S1 [suppl.] shows the trend over four years for the values of TL, ΣSeq, and POCER per the main group of crops. A positive Pearson's correlation shown in Table 1 is

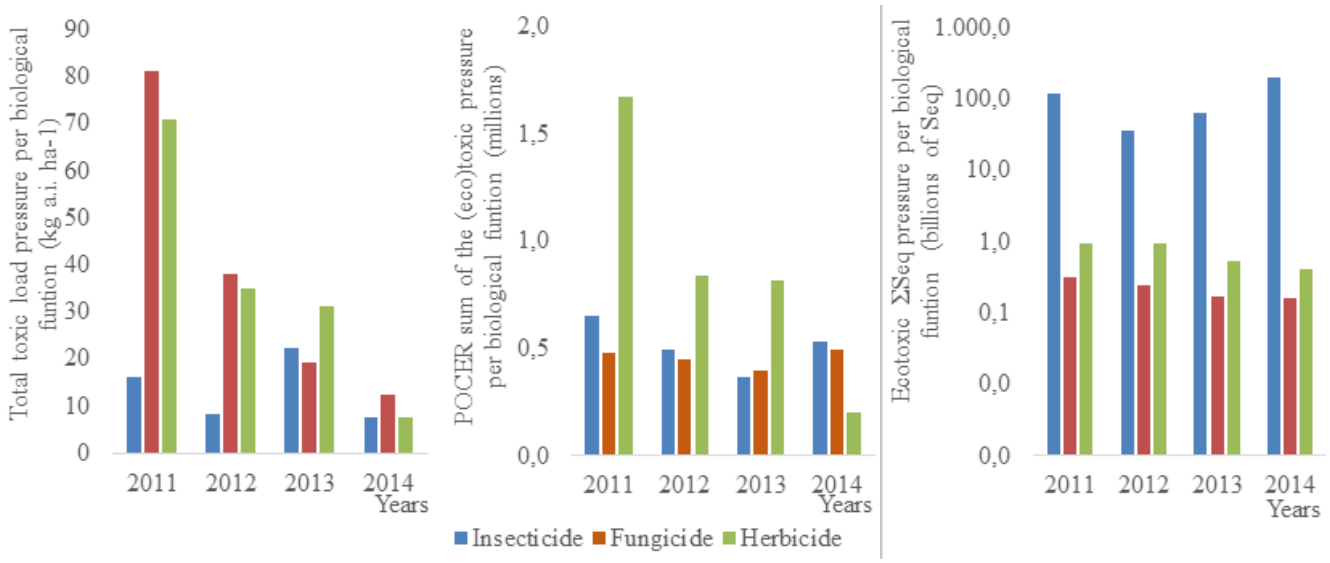

Figure 2. Total (eco) toxicity values per evaluated indicator corresponding to the sum over all crop groups based on the number of active ingredients used per biological family.

Table 1. General statistical analysis with and without the dependency of the years of the (eco) toxic parameters evaluated.

			Correlations			
			POCER Ecotoxic	**POCER Toxic**	**Toxic load**	**∑Seq**
POCER Ecotoxic		Pearson correlation	1.000	0.822**	0.468*	-0.010
		Sig. (2-tailed)		0.000	0.014	0.962
POCER Toxic		Pearson correlation	0.822**	1.000	0.613**	-0.050
		Sig. (2-tailed)	0.000		0.001	0.803
Toxic load		Pearson correlation	0.468*	0.613**	1.000	0.110
		Sig. (2-tailed)	0.014	0.001		0.585
∑Seq		Pearson correlation	-0.010	-0.050	0.110	1.000
		Sig. (2-tailed)	0.962	0.803	0.585	
Control variables						
Year	POCER Ecotoxic	Correlation	1.000	0.816	0.452	0.048
		Sig0. (2-tailed)		0.000	0.021	0.815
	POCER Toxic	Correlation	0.816	1.000	0.601	0.009
		Sig0. (2-tailed)	0.000		0.001	0.964
	Toxic load	Correlation	0.452	0.601	1.000	0.172
		Sig0. (2-tailed)	0.021	0.001		0.402
	∑Seq	Correlation	0.048	0.009	0.172	1.000
		Sig0. (2-tailed)	0.815	0.964	0.402	

*,**: Correlation is significant at the 0.05 and 0.01 level (2-tailed), respectively.

found between the POCER parameters Sum toxic (human toxicity) and Sum ecotoxic with and without dependency on the year. Also between POCER parameters and TL, a correlation was found. Only no correlation was found between the evaluated ΣSeq indicator with the TL and POCER parameters. This result may be due to the opposite described trend of the ΣSeq indicator.

As seen in Table S1 [suppl.], the order of the crops according to the level of pressure on human health and the environment will vary among the indicators. In general, the indicators evaluated point in the direction of sugarcane as the crop that exerts the largest pressure on human health and the environment followed by rice and fruits. Vegetables and grains follow in importance, especially on the ΣSeq where their values were higher than those exerted by sugarcane. Once the crops of higher pressure were identified, the benefit of using indicators such as POCER and ΣSeq instead of TL was that one also knows which modules from the environment and humans are most affected, in order to make decisions to reverse the pressure.

It should be mentioned that tobacco cultivation uses old and toxic compounds such as methamidophos, parathion methyl, diazinon, acephate and zineb, which are forbidden in the European Union. Its use was low, around 10%, there being other compounds based on the amount used that represented a higher pressure, but should not be neglected, and if possible replace them with less toxic compounds.

From a toxicological point of view, and taking into account the assumptions of the POCERs calculation, the non-use of personal protective equipment (PPE) by re-entry workers, and the negative consequences of not using a drift-reducing nozzle on residents during spraying activities, make these modules more risky than the applicator module. The ecotoxicological modules aquatic organisms, persistence, and groundwater were also most at risk, due to the use of old and persistent a.i. in the environment like endosulfan, parathion methyl, paraquat, ametryn amount others.

Analysis of individual hazardous active ingredients

Some developing countries maintain the use of a group of pesticides forbidden mainly in Europe and North America, and unfortunately Cuba is an example of these.

An example of the forbidden pesticides used is methamidophos (25.8 ton) which represented 15% of the total amount of insecticide used (half of this was used in 2011), mainly in rice, sweet potato, tobacco, grain, and vegetables. Other 5% are represented by endosulfan (3.5 ton), parathion-methyl (3.0 ton) and thiodicarb (1.7 ton). Some forbidden herbicides like ametryn (215.3 ton, 30% of the total herbicide used), prometryn, hexazinone, and paraquat (Table 2) were also used. Table 2 shows the main a.i. responsible for (eco) toxic pressure values. As can be seen, the quotient of DT50/MAC is very important for the Seq values. For example, while the endosulfan DT50 (0.236 year) is 23 times higher than methamidophos DT_{50} (0.01 year), MAC endosulfan value (2 10^{-9} mg L^{-1}) is 1350 times lower than methamidophos MAC value (0.0027 mg L^{-1}). Based on this, the ecotoxicological quotient of endosulfan is 31.8 million times higher than the methamidophos quotient. This example shows how small volumes of certain a.i. (such as endosulfan, paraquat and oxychloride copper) can exert greater pressure than others used in large volumes.

Similar results are also found for POCER. In this case, only the 2011 scenario is shown in Table 2, considered by the authors as the year that exerted the greatest (eco) toxicological pressure, due to the type of pesticides and quantity used. These can make the reduction of ecotoxic pressure easier since by eliminating or replacing a smaller amount of a.i. of higher (eco) ecotoxic pressure reductions can be achieved. To illustrate the previous approach, some examples such as those shown in Figs. 3 and 4 were developed.

Fig. 3 shows a scenario with average values of total ΣSeq reduced for each crop, based on the result from Table 2. For this purpose, the contributed values from the higher pressure a.i. per family (endosulfan, copper oxychloride and paraquat) were eliminated. In the case of tobacco, bifenthrin is used instead of endosulfan. Reductions in total ΣSeq values higher than 99% for crops using endosulfan (4.16 10^{+11} endosulfan ΣSeq over 5.58 10^{+11} of total ΣSeq), copper oxychloride (8.21 10^{+08} ΣSeq), and paraquat (1.40 10^{+11} ΣSeq) were achieved. In rice scenario, λ-cyhalothrin (5.33 10^{+07} over 3.77 10^{+08} of total ΣSeq), copper sulfate (1.25 10^{+07}), and ametryn (2.33 10^{+08}) were used instead of endosulfan, copper oxychloride and paraquat. The percentage of reduction of sugarcane crops is based only on the reduction of the use of paraquat (7.41 10^{+08} ΣSeq over 8.58 10^{+08} total ΣSeq) since insecticides and fungicides have not been assigned. As seen in Fig. 3, the percentage reduction in the group of roots and tubers was lower compared to the rest because potato, the crop

Table 2. Pressure values of the active ingredients more used and more (eco) toxic. To illustrate the POCER case the 2011 scenario was selected due to this year caused the higher pressure on the environment and human health.

	DT_{50}/MAC	Total kg a.i. used	Total ΣSeq	POCER sum (2011 scenario)					
				Vegetables	Grain	Rice	Fruit	Tobacco	Sugar cane
Endosulfan	117808219	3482	$4.10*10^{+11}$	$2.30*10^{+3}$	$2.08*10^{+4}$	NR	$7.35*10^{+3}$	NR	NR
Bifenthrin	193151	3254	$6.28*10^{+8}$	$4.89*10^{+3}$	$2.32*10^{+4}$	NR	NR	$5.79*10^{+3}$	NR
λ-Cyhalothrin	105023	527	$5.53*10^{+7}$	$2.80*10^{+2}$	NR	$2.90*10^{+4}$	NR	NR	NR
Parathion methyl	375	3049	$1.14*10^{+6}$	$1.42*10^{+4}$	$4.82*10^{+3}$	NR	1.78*10+^{4}	$1.41*10^{+2}$	NR
Methamidophos	4	25791	$1.05*10^{+5}$	$1.06*10^{+3}$	$3.11*10^{+3}$	$2.18*10^{+4}$	$1.09*10^{+4}$	$2.56*10^{+3}$	NR
Paraquat	1667659	1423	$2.37*10^{+9}$	$5.19*10^{+3}$	$1.49*10^{+4}$	$3.39*10^{+4}$	$1.88*10^{+4}$	NR	$1.81*10^{+5}$
Prometryn	2808	12803	$3.60*10^{+7}$	$8.79*10^{+2}$	NR	NR	$1.71*10^{+4}$	$5.54*10^{+3}$	NR
Ametryn	1408	215272	$3.03*10^{+8}$	$3.15*10^{+2}$	NR	$3.90*10^{+4}$	$6.28*10^{+3}$	NR	$7.76*10^{+4}$
Hexazinone	992	28452	$2.82*10^{+7}$	NR	NR	NR	NR	NR	$1.23*10^{+5}$
2,4-D Amine salt	0.01	165051	$1.85*10^{+3}$	NR	NR	$7.11*10^{+2}$	$7.66*10^{+1}$	NR	$1.52*10^{+4}$
Copper oxychloride	34247	24943	$8.54*10^{+8}$	$3.49*10^{+4}$	$2.03*10^{+4}$	NR	$1.89*10^{+4}$	$9.53*10^{+3}$	NR
Copper sulfate	769	21065	$1.62*10^{+7}$	NR	NR	$3.00*10^{+4}$	NR	NR	NR
Zined	10	31189	$3.27*10^{+5}$	$1.59*10^{+3}$	$1.07*10^{+2}$	NR	$9.80*10^{+3}$	$5.80*10^{+2}$	NR
Mancozeb	4	100229	$3.76*10^{+5}$	$9.03*10^{+3}$	$8.64*10^{+2}$	NR	$4.72*10^{+3}$	$9.28*10^{+3}$	NR

MAC= maximum allowable concentration for aquatic life (mg L^{-1}). NR = not reported

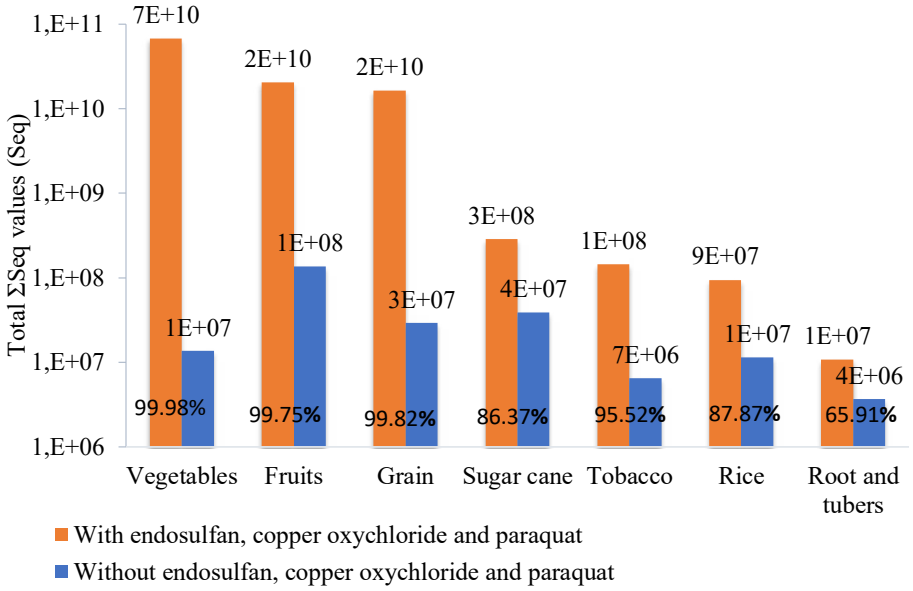

Figure 3. Percent of reduction of ΣSeq values from pesticides used per crop during the study period (with and without endosulfan, copper oxychloride, and paraquat). See in the text the exceptions made for tobacco and rice crops.

with the highest demand for pesticides in the group, was only cultivated in 2011 and 2012.

As POCER's objective is to evaluate the pressure, from low to high risk, exerted by a pesticide on each one of the evaluated modules, the decision-makers can either forbid the use of high risk a.i. (Table 2) or replace them with other a.i. that fulfill the same plant protection function with less pressure. In POCER results, (eco) toxicities from organophosphates and others like imidacloprid, bifenthrin and β-cyfluthrin were higher than coming from endosulfan in some scenarios. On the other hand, endosulfan remained in ΣSeq as one of the a.i. that received the highest score from the POCER's aquatic organisms module.

The organophosphorus compounds play an important role as a whole, due to their toxicities. As seen in Fig. 4, possible substitutes for the highest-scoring products are cypermethrin for parathion methyl, potentially reducing the risk by 50%. A mix of tebuconazole and triadimenol under commercial name Silvacur Combi® EC 30 reduce the risk exerted by copper oxychloride by 95%, and bispyribac-sodium reduce a 98% the risk exerted by the mix paraquat-diquat (Doblete® LS 20).

Discussion

Effects of pesticide use

Herbicides are the most used pesticides, mainly due to the development of monocultures in large areas of land, for example in cereal grains (Petersen *et al.*, 2013) and fruits, as is the case in this province. Cereals grains and fruits are the main crops of many countries that suffer from the highest pesticide load (Shil *et al.*, 2014; Chau *et al.*, 2015; Schreinemachers *et al.*, 2015; Böcker & Finger, 2016). The trend of the use of pesticides (slightly the same, considering also the lack of sugarcane data for 2014) shown in Sancti Spiritus for the study period is in correspondence with the national data shown in the statistical yearbook (ONEI, 2017) and not with strategies promoted to progressively reduce the use of synthetic pesticides (Rosquete, 2011). However, in other provinces, there was a sustained increase in the use of pesticides (Hernández & Pérez, 2012). The pressure of pesticide use is in correspondence with other tropical regions (El Salvador, Brazil, Taiwan, Cambodia, Tanzania, Vietnam…) (Cremonese *et al.*, 2014; Schreinemachers *et al.*, 2015). As can be observed, pesticides such as organophosphates, pyrethroids, carbamates, dithiocarbamates, neonicotinoids, and organochlorines used during the study period constitute an important risk to humans and the environment (Chau *et al.*, 2015).

Long-term environmental effects of pesticide use are worldwide alerted (Burgos, 2015; Mendonca *et al.*, 2016). Lethal and sublethal effects on wild and managed bees are well documented (Vázquez *et al.*, 2015; Fevery *et al.*, 2016; Hladik *et al.*, 2016). In aquatic ecosystems, pesticides constitute a potential threat to aquatic biodiversity (Levine & Borgert, 2018; Pérez *et al.*, 2018). The presence of highly toxic compounds can lead to a decrease in the number and varieties of fish, or alter phytoplankton communities, subsequently affecting other trophic levels (Altenburger *et al.*, 2013).

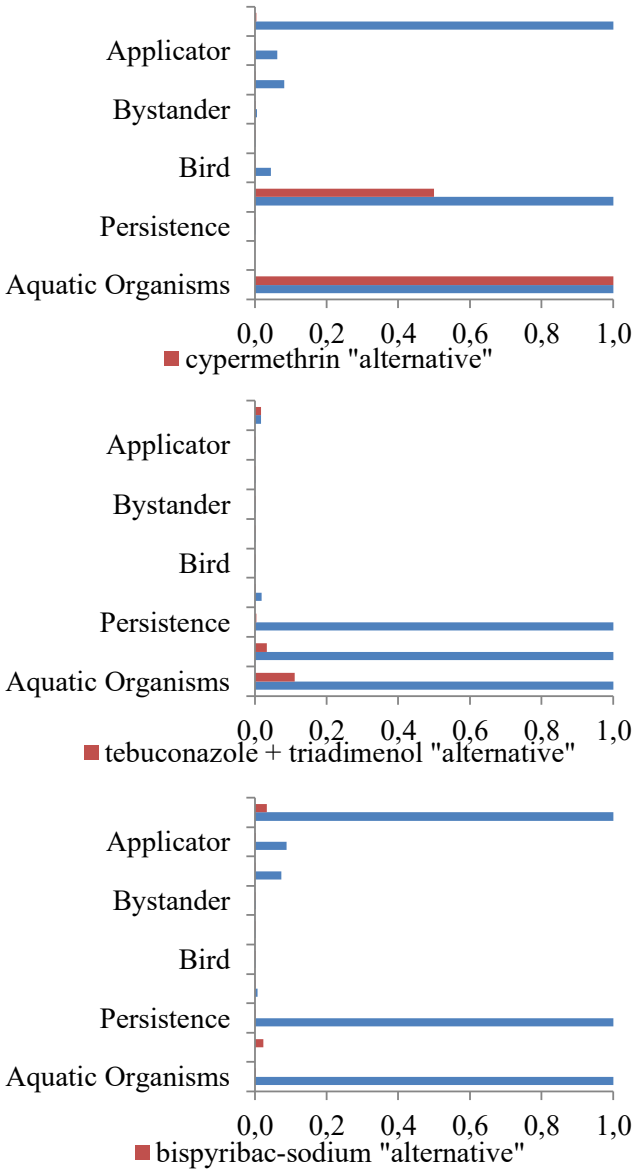

Figure 4. Proposal for substitution of high-risk pesticides to reduce the pressure evaluated in POCER's modules.

Toxic load associated risk

There is now a perception that pesticide use is increasing (Lopez *et al.*, 2020). This study showed that the total a.i. used in the studied period was slightly the same. What may peasant sector be misunderstood are the actions developed in biological control promoted by the country. Being increased the use of biological products and most of farmers do not distinguish differences between synthetic and biological products (Lopez *et al.*, 2020). In other provinces, crop production (rice, cucurbits, beans, sweet potato, and tomato) used amounts of pesticides similar to those reported in this study (Hernández & Pérez, 2012).

It is recommended that farmers become informed of the risks to which they are exposed, and the importance of using PPE and drift reduction nozzle in order to minimize pesticide exposure (Yarpuz & Bozdogan, 2016). At the same time, the government must be able to provide such PPE and nozzles, that today is not enough available. Afterward, their use should be mandatory. Examples of needed PPE that can well fit the tropics are: face masks with filters, eye protection glasses, and gloves. Its use would greatly help reduce the applicator's exposure. In addition, make extensible (only used today in some government enterprises) the use of tractors with closed cab equipped with interchangeable carbon filter and updated irrigation systems (drift reducing nozzles), similar to those used in aerial spraying on rice.

Another way to reduce risk is to use a.i. of lower toxicity (Morel, 2010). The FAO recommends in its Code of Conduct on Pesticides that pesticides of category Ia and Ib (WHO, 2009), and if possible Class II of human toxicity, should not be used in developing countries (OMS/FAO, 2014).

Regardless no so elevated values in POCER human modules were obtained compared with the POCER environmental modules, the POCER human modules only assess acute risk, not long term (chronic) risk. In this case is alerted that 45.7% of the total pesticides applied, present a category of possible, probable or human carcinogen and endocrine disruptor. From the 124 a.i. applied in Cuba, paraquat, methyl parathion, methamidophos and endosulfan are included in international conventions (PIC, COP, LRTAP), with the aim to eliminate or limit their use (UNEP/POPS/POPRC.5/10, 2009; FAO-PNUMA, 2016).

In both the EU and North America, 15 of these products still used in Cuba were banned (Roberts & Reigart, 2013; EFSA, 2017) because of damages to human health and biodiversity 10 years ago (Morel, 2010). In addition listed with a classification in cancer categories (possible, probable or human carcinogen) by USEPA, EU and the International Agency for Research on Cancer (IARC) is 41 a.i. Other 32 a.i. are potential endocrine disruptors in humans and wildlife (WHO, 2009), posing a risk for human health and the environment.

Ecotoxicity tests

From the total ΣSeq indicator obtained values, Sancti Spíritus increased ecotoxic output over time, from 118 billion Seq to 259 billion, in contrast to a developed country like Belgium which reduced the ecotoxicity values cause by pesticides. The province's ΣSeq values for 2011 were more than 10 times higher than those obtained by Fevery *et al.* (2015) for 2011 in Flanders (10.56 billion Seq). As they mentioned in their paper, the use of endosulfan was responsible for the high ecotoxicity values. Endosulfan represented in this study between 94.83% (beans in 2011) and 99.97% (onion in 2014) of the ecotoxicity indicator

outcome for the crops where it was used. It is necessary to eliminate the use of this insecticide, as was done in most developed countries (EFSA, 2017). An example of the positive change in ecotoxicity values when the use of endosulfan is eliminated is that experienced by the Flanders region in Belgium. When it was discontinued in 2012, its ΣSeq value decreased by 71% compared to the 2009 values (Fevery *et al.*, 2015). Like in this study, paraquat and copper oxychloride are also responsible for high pesticide pressure values in the province.

Several authors agree that, due to the persistence of some pesticides in soil and their ability to leach into groundwater and water bodies, aquatic organisms from the POCER indicator are the main modules at risk as a consequence of the use of highly toxic herbicides like paraquat and prometryn, as well as organophosphate insecticides (Bozdogan *et al.*, 2015; Fevery *et al.*, 2016; Yarpuz & Bozdogan, 2016). Also, in a citrus-growing region of Spain, the organophosphate chlorpyrifos followed by copper oxychloride were the most ecotoxic of the commonly applied pesticides for aquatic organisms (Cunha *et al.*, 2012).

As Fevery *et al.* (2015) mentioned and other authors before them too, 1 kg of certain pesticide can exert a different pressure than 1 kg of another pesticide. To quantify the risk of exposure to pesticides, it is necessary to weigh the use of pesticides to the toxicity coefficients for the various environmental compartments (Wustenberghs *et al.*, 2012; Fevery *et al.*, 2016).

The POCER indicator has already proven its usefulness in Belgium as well as in other European countries (Claeys *et al.*, 2005; Cunha *et al.*, 2012; Bozdogan *et al.*, 2015; Yarpuz & Bozdogan, 2016) as a tool of toxic pesticide reduction plans. POCER can be used as a decision-making tool for choosing alternative pesticides with respect to pressure on humans and the environment (Wustenberghs *et al.*, 2018). Coupled with economic models, the feasibility and effectiveness of policy measures, and the best practice on a farm level without jeopardizing profitability, can be evaluated (Vercruysse & Steurbaut, 2002; Wustenberghs *et al.*, 2018).

In summary, the study shows the suitability of POCER and ΣSeq as important tools for decision-makers as they help to reduce the toxicity and ecotoxicity pressure due to the use of pesticides. With the use of the POCER indicator as well ΣSeq, more accurate assessments of toxicity and ecotoxicity from pesticides can be done, compared to the TL equation currently used in Cuba. The toxic and ecotoxic pressure can be reduced by more than 50% by replacing the active ingredient. Those results are directly related to the reduction goals promoted by the national government. And will help in developing policies and management practices to reduce the hazards from pesticides by reducing the use of pesticides having the highest pressure on humans and the environment.

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