RISK ANALYSIS AND ASSESSMENT

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I. INTRODUCTION

Mexico relies heavily on agriculture as one of its main sources of income for its population (SADER, 2018). These crops face challenges such as plagues and infection for microorganisms such as bacteria, fungi and viruses, which cause crucial agricultural loss. This is usually fought with exorbitant quantities of agrochemicals, also known as pesticides. The constant use of chemicals causes irreversible environmental damage and health issues to organisms that have been in contact.

Health and food safety issues have become paramount. The use of chemicals and their residues produce health problems. That includes acute intoxication, chronic intoxication, and neurobehavioral and carcinogenic effects. Most producers in developing countries do not allow pesticides to decompose before they send fruits and vegetables to the market. Most consumers do not wash these agricultural products before consumption, which has led to the accumulation of pesticide residues (Abubakar *et al*., 2020).

Agrocapsi seeks to eliminate these problems by controlling *P. capsici*, but also by taking into account environmental and health risks. To ensure those goals were achieved, Agrocapsi developed a Risk Analysis and Assessment (hereinafter referred as "RAA") that discusses pesticides, its classification, usage, disposal and environmental impact (Nguyen *et al.*, 2022).

The elaboration of Agrocapsi consists of peptide recombination in 2 different bacteria strains, *Escherichia coli BL21* and *Escherichia coli HT115*. The use of biological strains were analyzed through an analytical method of production. This examination takes into consideration strain transformation and possible effects of strain during peptide production. The aforementioned is intended to improve the quality, reliability and safety, as well as to increase customer satisfaction.

II. PRODUCT CLASSIFICATION

A pesticide is a substance that helps to prevent, control or eradicate pests. Among which are insects or microorganisms that cause damage to crops or are vectors of diseases, and plant species that interfere with crop growth (FAO, 2006). Pesticides are applied for three main reasons:

1. To control diseases during crop establishment and development.
2. To increase the productivity of a crop and reduce crop damage that affects the commercial value of the crop.

3. To improve the storage period and quality of plants as well as harvested products (Mahanty *et al*.,2017).

Pesticides can be classified in agreement with its biological activity, formulation, route of entry, chemical nature, and toxicity (Table 1).

Table 1. Types of classification

Classification	Description
Biological activity	The product is a fungicide because its target pest is *Phytophthora capsici*, an oomycete that causes wilt.
Formulation	The formulation of the Product is a liquid composed of 3 active ingredients: DrsB1, a dermaseptin, and PcOSM, a like-osmotin protein. To this end, we aimed to silence the genes coding for the RXLR effector proteins through small interfering RNA (siRNA). The nanocapsule will be composed of chitosan. Chitosan is a biodegradable compound formed of glucosamine and N-acetylglucosamine, which, thanks to its properties, presents a high biocompatibility, biodegradability, low immunogenicity, solubility in water and neutral pH.
Route of entry	The route of entry of the Product is by contact. The Product will be released through nanocapsules and absorbed by the plant through its roots (ANASAC, 2001).
Chemical nature	It is an organic resource because it is a peptide from a plant and a frog (Abubakar *et al*., 2020)
Toxicity	The peptides DrsB1 and PcOSM, as well as the siRNA RXLR1 have been reported to have no negative effect on mammalian cells and plants (Geetha *et al*., 2021; Khademi *et al.,*2020; Mani & Manjula, 2010). In turn, a great variety of enzymes have been identified coming from organisms as much prokaryotes as from animals, plants and yeasts releasing amino sugars that are capable of degrading chitosan (Mendoza *et al.*, 2016). After analyzing the technical data sheets of the compounds of the nanocapsule, the outcoming

	conclusion was that the Product has a toxicity level 4, being level 5 the lowest degree of toxicity and level 1 the highest (Xu *et al*., 2014).

It is important to present the classification according to the coloration band from the highest to the lowest degree of toxicity (Table 2). According to **NOM-232-SSA1-2009** which establishes the requirements for the packaging, packing and labeling of products labeling of technical grade products for agricultural, forestry, livestock, gardening, urban, industrial and domestic use. The Product's toxicity is **level 4**. Agrocapsi requires a certain caution degree, as there is the possibility of mild intoxication either by ingestion, skin contact or inhalation.

Table 2. Symbols and warning pictograms.

	Categories				
Pictograms	**1**	**2**	**3**	**4**	**5**
Oral danger	 Mortal if ingested	 Mortal if ingested	 Acute toxicity if ingested	 Harmful if swallowed	May be harmful if swallowed
Cutaneous danger	 Mortal if there is skin contact	 Mortal if there is skin contact	 Acute toxicity if in contact with skin	 Harmful if there is skin contact	May be harmful if there is skin contact
Inhalation	 Mortal if inhaled	 Mortal if inhaled	 Acute toxicity if inhaled	 Harmful if inhaled	May be harmful if inhaled
Warning word	Danger	Danger	Danger	Caution	Caution
Pantone color	Red (199·C)	Red (199·C)	Yellow (101·C)	Blue (293·C)	Green (347·C)

III. POTENTIAL RISKS

Producers, agricultural engineers, and field workers are frequently exposed to these pesticides due to the work processes inherent in products such as products. Exposure to pesticides increases the likelihood of adverse reactions and consequent poisoning. There are three different types of poisoning (Table 3).

Table 3. Different types of poisoning

Acute poisoning	Subacute poisoning	Chronic poisoning
Short-term exposure and rapid toxic absorption, single or multiple doses in a period not exceeding 24 hours. Clinical manifestations may include systemic effects (nausea, bradycardia, myosis) or localized (dermatitis).	Frequent or repeated exposures during several days or weeks.	Repeated exposures during a long period of time. The signs of intoxication manifest due to the accumulation of the toxic components in the body with each exposure

It is important to stress that there is no risk without a user, so it is necessary to take correct precautions in different working conditions. The greatest exposure occurs when the biocide is concentrated or when the user is in close proximity to the product, such as handling the concentrated product, opening containers, weighing the product, mixing, loading equipment, spraying, or re-entering the treated product area. It is important to wear appropriate clothing, gloves and masks.

Therefore, it is necessary to understand and address the exposure routes of products entering the human body (Table 4).

Table 4. Different exposure routes.

Exposure routes	What to do
Dermal	Occurs through the skin due to the physicochemical properties of the pesticide. These can be absorbed and enter the body. The person should remove clothing and immediately wash off pesticide residues with soap and water.
Oral	May be ingested. The most common route is hand-to-mouth contact with contaminated hands while smoking or eating. Some presenting symptoms are vomiting, severe abdominal pain, and diarrhea. Patients should receive immediate medical attention from a physician at their place of residence or health center.

Inhalation	Pesticides are in contact with air laden with dust particles, gasses, and vapors or through the inadequate use of masks. In the event of this intoxication, it is recommended that the person's shirt be unbuttoned and he/she should be taken to the nearest medical center.
Eye	Pesticides may come into contact with eyes through dust, gas and vapor. Sometimes when the concentrate is poured into a liquid, it can splash and cause serious and deep injury. It can also be produced by hand-eye contact. If pesticide gets in your eyes, rinse immediately with water for 10 minutes. Take the person to the nearest health center immediately

In the event of any type of poisoning, call 911, SINTOX and ATOX. These are some of the medical service plans that provide free 24-hour emergency advice related to recognizing symptoms, administering first aid, and administering antidotes. It is important not to self-medicate and to follow the guidelines set by each organization.

Figure 1. National phone numbers of organizations that handle poisonings.

Other factors that affect the severity of poisoning include: age, sex, body temperature body weight, and ambient temperature, pesticide concentration, route of exposure, duration of exposure, nutritional and health status, stage of pregnancy, and genetic factors. In conclusion, it is recommended that workers must be at least 18 years old, preferably avoid hiring pregnant women or people with chronic illnesses (due to conditions to which workers may be exposed), and have employer-provided insurance for medical expenses.

IV. CONTAINERS AND PACKAGING

It is essential to define the sanitary specifications of the containers and packaging to be used to contain the product. For this purpose, it is necessary to take into account the requirements and characteristics of **NOM-232-SSA1-2009**. It is also necessary to consider the International **Code of Conduct on the Distribution and Use of Pesticides**, adopted in

1985 by the FAO Conference, whose function is to serve as a reference especially until countries establish adequate infrastructures for pesticide regulation.

According to the **NOM-232-SSA1-2009**, the product shall not show any deformation on its structure and must include a security seal. If the package does not bear such a seal, it must have a closing system in which it is visible that the package was opened before. Also, it shall be designed to withstand pressure and be airtight, bearing a full label corresponding to the packed product (Table 5). Only good-quality containers should be accepted.

Table 5. Label format for pesticides for agricultural and forestry use

Central part of the label.	In the upper part of this part the legend "AGRICULTURAL USE" or "FOREST USE", as appropriate and as indicated in the registration, shall be printed in letters of a minimum size of 2% of the height of the central part of the label.
	With a size not exceeding 25% of the central area of the label, the logo of the registration holder and/or of the product and the commercial name of the product must be printed, preferably indicating the percentage of the active ingredient and the abbreviations corresponding to the type of formulation.
	Under the text "PERCENTAGE COMPOSITION", indicate the percentage by weight of the active ingredient(s).
	In the line immediately below each of the active ingredients, the equivalent in grams per liter of products at 20 °C or 25 °C must be expressed.
	Unique registration number of the product issued by the Ministry of Health and net product content.
	Printed or affixed lot number (including date of manufacture, year, month, day, in pairs of digits) and expiration date of the product.
	If the product is manufactured or formulated in national territory, the legend "MADE IN MEXICO" must be printed.
	The pictograms, danger phrases and signal words, as appropriate.
Left side of the label	The phrase "STOP, READ THE LABEL BEFORE USING THE PRODUCT" in letters of the following size and highlighted in bold type.
	Under the heading "PRECAUTIONS AND WARNINGS FOR USE", the appropriate protective equipment for handling the product during the preparation of mixtures and the loading of application equipment; safety equipment required during the use or application of the product must be indicated.

	Include the legend "IN CASE OF INTOXICATION, TAKE THE PATIENT TO THE PHYSICIAN AND SHOW HIM THIS LABEL".
	Under the heading "FIRST AID", point out the immediate measures to be taken in case of overexposure to the product and those to be avoided.
	Under the heading "RECOMMENDATIONS TO THE PHYSICIAN", chemical group of the product, antidotes, when available, and specific treatment.
	Under the heading "ENVIRONMENTAL PROTECTION MEASURES", include the following legends in list form: "DURING PRODUCT HANDLING, AVOID CONTAMINATION OF SOILS, RIVERS, LAGOONS, STREAMS, DAMS, CANALS OR WATER RESERVOIRS BY NOT WASHING OR POURING PESTICIDE RESIDUES OR EMPTY CONTAINERS INTO THEM."
	Report adverse effect on terrestrial and aquatic flora and fauna
Right side of the label.	The text should indicate the recommended use of the product. Examples of these legends: "USE ONLY ON THE CROPS AND PESTS RECOMMENDED HERE".
	Add "INSTRUCTIONS FOR USE".
	Indicate the following legend. “TO PREVENT THE DEVELOPMENT OF RESISTANT POPULATIONS, ALWAYS RESPECT THE DOSES AND FREQUENCIES OF APPLICATION".

Having established the foregoing, AGROCAPSI label results (Figure 2).

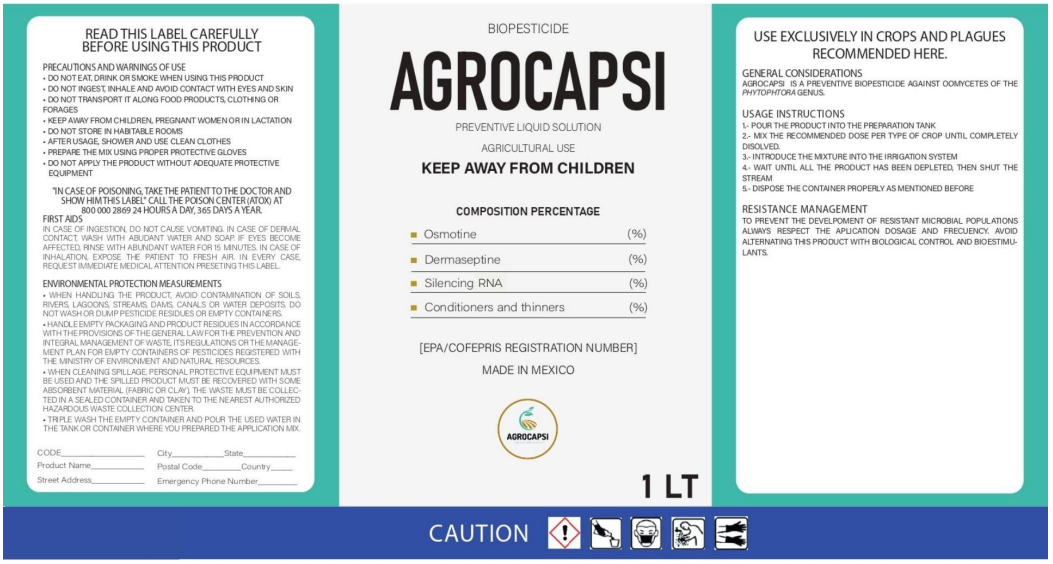

Figure 2. Labeling of Agrocapsi.

V. EQUIPMENT AND MEASURES TO ENSURE PERSONAL PROTECTION

The article 1.5 of the International Code of Conduct on the Distribution and Use of Pesticides, adopted by the FAO Council (2006), states that the standards of conduct set forth are:

- Encourage responsible commercial application practices.
- Guarantee the safe handling and use of this Product.
- Encourage practices that promote the safe and effective use of pesticides.
- Ensure that the pesticides are used effectively to improve agricultural production and health of humans, animals, and plants.

To achieve proper use of the fungicide, adequate protection equipment is required during handling and use of the Product. Due to the degree of toxicity level 4, it is essential to count with available protective equipment sets (rubber gloves, masks, overalls, long-sleeved shirts) in good condition.

As well established and adequate procedures for cleaning clothes and protection equipment after its use should be provided. These sets, along with the replacement filters, should be stored aside and separated from phytosanitary products, in a well ventilated area (FAO, 2006). In addition to the above, it is important to mention that some of the substances are biodegradable and the others have no impact on the water or soil's pH.

VI. TRANSPORT

When transporting pesticides, it is important to mention that they should not be transported along with food or other goods that might get contaminated, such as toys or clothes. In the same way, they should not be transported next to the driver or in the vehicle or tractor's cabin (ONU, 1996). Some principles to be considered are:

- Do not transport open containers or containers with losses.
- The driver must be informed about the cargo's content (pesticides) and receive instructions regarding the measures to be taken in case of emergency (crash, fire or spill). Likewise, the product's safety data sheets must be provided to the driver.
- The cargo must be inspected at regular intervals during transport.
- In case of spillage, it must be cleaned up immediately.
- Containers made of paper, cardboard or other water-soluble materials must be protected from rain with a waterproof cover.

VII. WASTE DISPOSAL

Pesticide containers contain residues of the products. It is important to take appropriate measures for the proper disposal of containers to avoid their use for unauthorized purposes. Pesticides should not be discharged into bodies of water because wastewater treatment plants are not designed to remove all toxic chemicals. Pouring them down sinks, toilets and sewers pollutes and is against the law. It is necessary to read the label in the "Storage and Disposal" section or take them to a hazardous waste collection center when

specified. After emptying a pesticide container, rinse it properly for recycling. Wear protective clothing, chemical-resistant gloves, and safety glasses when doing this.

For their correct disposal, it is necessary that the producer washes them 3 times and takes them to CAT. This organization receives them and classifies them as clean or contaminated. If they are clean, they are compacted and recycled (Michemberg & Mohamad, 2016).

VIII. ENVIRONMENTAL IMPACT ANALYSIS

Our product is classified as a biopesticide because of its active ingredients. It does not contain components that require special attention, such as persistent organic pollutants ("POPs"). Persistent organic pollutants are pollutants that cause long-term physiological effects (Del Puerto *et al.,* 2014).

Agrochemicals are often applied in such a way that overdosing occurs; this is done to ensure that the active component is administered in adequate amounts where it is most needed. More than 90% of pesticides sprayed are lost to the environment or do not reach their intended target, increasing costs and contributing to pollution. In turn, the continued use of pesticides allows the development of resistance by target organisms, as well as cross-resistance to other vigorous components of some pesticides. This could lead to more aggressive pests (Abubakar *et al*., 2020).

According to environmental degradation processes, pesticides have different levels of residuality. It is recommended to use pesticides with low residuality in a production program with excellent agricultural practices to reduce the danger of the commodity having these residues, which usually have some degree of toxicity to humans and animals (Garcia & Rodriguez, 2012). The peptides DrsB1 and PcOSM, as well as the siRNA RXLR1 have been reported to have no negative effect on mammalian cells and plants, making them harmless (Geetha *et al.*, 2021; Khademi *et al*.,2020*;* Mani & Manjula 2010).

Since the fungicide formulation is based on biologically active molecules and must be applied to crops in small doses, all biological, climatic and physicochemical factors associated with the disease to be treated must be taken into account when preparing the ingredients and ensuring their correct application. The objective is for the active component to reach the target biological site where it can begin to act as a pesticide, interact with it and reach its target. To this end, neither the degradation of the formulation prior to application nor the time spent in the soil can be allowed to be significant, as this could cause negative residues.

Therefore, the active ingredient of the product is nanoencapsulated, as research and tests have shown that this technique of packaging solid, liquid and gaseous materials confers specific properties such as: resistance to ultraviolet (UV) rays and greater solubility, offering a more efficient and effective release of active ingredients, resulting in the reduction of the indiscriminate use of pesticides (Del Puerto *et al*.,2014).

Note: This Risk Analysis and Assessment has been elaborated taking into consideration Mexican regulation and legislation. All the risks, previsions and recommendations stipulated herein shall be considered interpreted and applied in an enunciative, but not limited to, basis.

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