ACUTE DERMAL TOXICITY TEST
FOR THE PRODUCT CHICHEX BED BUGS INSECTICIDE
OF THE ENTERPRISE FRUIT TREE Ltd.

QUALITY CONTROL DEPARTMENT

BIOLOGICAL ANALYSIS

TOXICITY TEST

1. COMPOSITION CERTIFICATE:

1.1 Type of study:	**ACUTE DERMAL TOXICITY STUDY**
1.2 Analysis Number:	**22K0345-T**
1.3 Client:	**FRUIT TREE Ltd.**
1.4 Trade name:	**CHICHEX BED BUGS INSECTICIDE**
1.5 Composition:	**Active Ingredient:** • Silicon Dioxide 550 gr/kg • Amorphous Silica 450 gr/kg
1.6 Date of received samples:	2022-11-24
1.7 Date of analysis:	2022-12-05

NOTE: Result according to sample sent and sampled by FRUIT TREE Ltd.

* The composition certificate is a true transcription of the one sent and authorized by FRUIT TREE Ltd.

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2. TITLE:

ACUTE DERMAL TOXICITY TEST ON **CHICHEX BED BUGS INSECTICIDE.**

3. SUMMARY:

The product was to assess **CHICHEX BED BUGS INSECTICIDE** white New Zealand rabbits were used.

This study was conducted in one doses (4000 mg/kg body weight). These doses were administered once in the epidermis of the rabbits (5 females) after a fasting period of 12 hours.

The administration of the doses was done through 5 patches.

After the first dosage, the animals were observed permanently during the day and then they were observed twice a day in order to have evidence of edema and erythema. This study was done in 14 days.

After completing the study (14 days), the animals were sacrificed and necropsied to establish macroscopic abnormalities in organs and compare them to the control animals.

The results showed that under the conditions of this study, Dermal Dose DL_{50} of the product **CHICHEX BED BUGS INSECTICIDE** was greater than 4000 mg/kg body weight.

4. GUIDELINES:

OECD (2017) Guideline For Testing Of Chemicals. Guideline TG 402. Acute Dermal Toxicity: Fixed Procedure. Adopted: 09 October 2017.

5. INTRODUCTION:

5.1 Objective:

The objective of this study is to evaluate the Acute Dermal Toxicity of the sample taken from the product **CHICHEX BED BUGS INSECTICIDE** when it is administered once in the epidermis.

The experimental design was based on the guidelines of EPA and OPPTS.

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5.2 Definitions:

5.2.1 Acute Toxicity:

Is the adverse effect observed in a short time periods (14 days) after the administration of a single or multiple dose of the product, for a period of time not exceeding 24 hours.

5.2.2 DL_{50} - Median Lethal Dermal Dose:

Is the dose that has statistically the probability of generating 50% of death in individuals, under very well-defined experimental conditions.

5.2.3 Dosage:

Is the term that determines the dose, frequency and duration.

5.2.4 Dose:

Is the quantity of substance administered in order to be analyzed. The dose given is expressed in terms of milligrams (mg) or grams (g) per unit of weight of each animal.

5.2.5 Dose – Response relationship

Is the relationship between the dose and the proportion of a biological effect in individuals or a population.

5.2.6 Dose - Answer:

Is the relationship between the dose and the proportion of a population that shows a well-defined effect.

5.2.7 Dermal Irritation:

Is a reaction located in skin as a result of one or more exposures of a chemical or physical agent.

5.2.8 Dermal Corrosion:

Is the effect caused by the tissues' damage after the direct contact of a substance, generally producing cellular death in the affected area.

5.3 **Specie:**
New Zealand While rabbit is the specie recommended by the guideline because this animal that more accurately reflects the toxic effects of a substance. These effects can be extrapolated to the effects on human health.

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6. MATERIALS AND METHODS:

6.1 Received Sample:

CHICHEX BED BUGS INSECTICIDE was given by **FRUIT TREE Ltd.** on November 24, 2022, in form of finished product.

6.2 Sample Preparation:

The substance is applied directly without dissolving according to OECD protocol.

6.3 Experimental Procedure:

The study was conducted in a group of 5 rabbits (5 females) per each dose level.

CHICHEX BED BUGS INSECTICIDE was administered in the animals' epidermis.

These animals were observed constantly after the first dosage during 6 hours, then after 24 and 72 hours. The observation was done daily during 14 days straight. In the end of the procedure, all the rabbits were euthanized and necropsied.

6.4 Animal Model:

The specie of experimentation was the New Zealand White rabbit . All animals were analyzed clinically.

6.4.1 Previous Test:

Young and healthy animals were used in this study. Their physical condition and behavior were analyzed before entering the vivarium. The analyzed aspects were:

- Mobility (active and dynamic)
- Appetite
- Posture
- Physical appearance

6.4.2 Health Examinations:

Before the test, the animals' health condition was analyzed clinically by making the following medical examinations:

- Fur (softness, brightness and appearance)
- Mucosa (Eyes, nose, external genitalia, and nearby areas which have to be free from any accumulation of extraneous material, as well as the inside of the external ear canal)
- Color (any change of body appearance, iris, skin and the internal face of the ears)
- Touch (palpation of any irregularity, flaking, and scabs)
- Deformities (dilatation of abdomen, protuberances and asymmetries)

6.4.3 Methodology of Adaptation - Quarantine:

The animals were placed individually in stainless steel cages cage in such a way that the individual observation of the study and the animals' normal performance were not interfered.

Each animal was marked and each group was identified with registration cards which included the number of the animal, gender, date of treatment, weight and observations.

The animals were moved to a respective area of accommodation in the laboratory, under environmental conditions of brightness of 12 X 12, average temperature of 20° C and relative humidity between 30 - 70 %.

During the time of adaptation and quarantine, food of composition known as ad-libitum and water were administered to the animals.

The rabbits had time for adaptation and quarantine under the vivarium's environmental conditions during a period of 15 days.

After providing the substance, the animals were moved to an observation place where were evaluated individually during the whole period of testing.

The animals were kept in an individual and isolated area under the environmental conditions required for a period of observation of 14 days after the administration of the substance.

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The environmental conditions in the vivarium for this study were: Temperature of 20 ±2°C, relative humidity between 30 – 70% and a period of light of 12 hours and a period of darkness of 12 hours in order to keep the animals' cardiac cycle.

6.5 Exposure Period:

Before 24 hours of the study, animal fur was removed of an area of the dorsal region, which was sufficiently large to administer the dose (10% of the body surface). Before 12 hours of the study, the animal food was interrupted. After 6 hours of the product's administration, the food (Purina – Conejina ®) was given to the animals.

Water and food (whose composition is known as ad-libitum) were administered during 14 days, length of time for this study.

6.6 Dosage Levels – Determination of Dermal Dose DL_{50}:

One dosage level were taken for this study. Their limit value was 4000 mg/kg body weight (4000 mg/kg) in order to determine observable, lethal and non-lethal effects as well as to identify the dose-response curve and the determination Dermal Dose DL_{50} (Median Lethal Dose). Guideline OECD 402.

6.7 Exposure:

The animals were isolated 12 hours (without food, only with water) before the single dose was administered in the epidermis. The substance was poured homogenously with a spatula on the shaved area which was covered by a gauze bandage during 24 hours.

After 6 hours of the administration of the substance, food (ad-libitum) was given to the animals.

6.8 Animal Observation:

The animals were observed during a period of 14 days. The first day (day of the administration of the substance) needed greater attention during the observation. There was special attention if the toxicity and intensity signs appeared or any case of death.

The following observations were written: erythema, edema, changes of posture, movements, activity, reactivity during handling, grip strength, gestures, stereotype (walk backward), skin, eyes, fur, membranes of the eyes, respiratory and circulatory effects, and changes of body weight (key parameter of this toxicity test).

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6.9 Clinical Macroscopic Test:

The clinical tests were done once a day.

The observation was written in a detailed manner taking into account: erythema, edema, changes of posture, movements, weight loss, seizures, prostration, reactivity during handling, skin, eyes, fur, eyes' membranes, respiratory system's alteration, and nervous system's alterations, salivation and death.

Body weight was checked before the administration of the substance and euthanasia. Body weight is registered within 3, 7, 10 and 14 days. The weight variability was a key parameter as a toxicity marker.

After 14 days, the animals were weighed, sacrificed and necropsied to observe any pathological and macroscopic change.

The necropsy report is annexed.

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7. RESULTS:

The present study was developed in accordance with the guidelines adopted in the "OECD TG-402 Guide of the Organization for Economic Cooperation and Development", using the "fixed dose" method, which provides information on the risks and hazardous health properties likely to arise from short-term exposure to a chemical by the dermal route and allows classification according to the value or range of the acute dermal median lethal dose found. However, the method states that the test chemicals should not be administered in doses known to cause severe pain and distress to the animals subjected, due to possible corrosive or severely irritating actions.

The principle of the test indicates that a group of animals of one sex should be exposed via the dermal route with a sample of the test product in a staged procedure using an appropriate "fixed dose" initially. The initial dose level is selected at the concentration expected to produce clear signs of toxicity without causing serious toxic effects or mortality.

In addition to the above, the "fixed dose" method allows that other groups of animals may be tested at higher or lower fixed doses, depending on the presence or absence of signs of toxicity or mortality. It also indicates that, for justified reasons, the study should be continued with additional applications (one at a time) until the dose that causes toxicity is identified, or no more than one death is identified, or when no effects are observed with the highest dose, or when deaths occur with the lowest dose.

Likewise, observations of effects and deaths should be made, if possible during the first fourteen (14) days after applications. Animals that die during the trial should be autopsied and at the end of the trial, surviving animals should be sacrificed and autopsied.

According to the principle of the method described above, for the present study, a "fixed dose" level of 4000 mg/kg of the sample of the product **CHICHEX BED BUGS INSECTICIDE** was proposed and applied topically on 5 animals - Albino rabbits, of one sex only (5 females) previously selected and prepared for the study. Subsequently, the observations were recorded during the time recommended in the guide, and at the end of the study all the specimens were sacrificed and necropsies were performed in order to identify possible changes in their organs.

In the present case, it was not considered necessary to continue the study with additional, higher or lower dosages, since, in the initial "fixed dose" test carried out, no deaths or moribund states were recorded in the specimens submitted.

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Observations

After the dermal applications with the product sample (first day), none of the specimens showed dermal changes; during the posture of the patches, no signs of irritation were observed; during the time of contact with the product sample (with the patches semioccluded), no topical responses were registered around the patches or in the adjacent areas; when the patches were removed, a transparent rash was observed in the treated area of all the specimens, which was tiny, tiny in size and tiny in thickness.
When the patches were removed, a transparent rash of minute size and thickness was observed in the treated area of all the specimens.

All the specimens of the test persisted with the registered sign, with similar characteristics, until the second day; on the third day, the rash showed a decrease in size, from the fourth day the rash healed and in the afternoon of the same day (fourth) it disappeared completely, on the fifth day the specimens registered hair growth and on the tenth day of the study all the specimens completed their fur.

During the fourteen (14) days of the observation period, the test specimens registered positive responses to external stimuli; water and food consumption in adequate portions; urinary and fecal performances in normal amounts and frequencies; in the first and second week, they increased body weight in normal proportions in the first and second week of the study increased body weight in normal proportions and all showed good health conditions. No specimens died during the study.

On the last day of the test, all individuals were sacrificed and necropsies were performed, which were negative for morphological changes.

8. CONCLUSIONS:

At the end of the study, having used the sample of the product at the fixed dose proposed (4000 mg/kg), it was found that there were no systemic toxic effects, there was no irreversible damage in the treated skin areas and no specimen died during the observation period.

Therefore, it could be concluded that the sample of the product **CHICHEX BED BUGS INSECTICIDE,** property of the company **FRUIT TREE Ltd.** has a value of Dermal Mean Lethal Dose higher than 4000 mg/Kg.

Dermal LD_{50} > 4000 mg/Kg

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9. BIBLIOGRAPHY

9.1 OECD (2017) Guideline For Testing Of Chemicals. Guideline TG 402. Acute Dermal Toxicity: Fixed Procedure. Adopted: 09 October 2017.

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BIOLOGICAL CONTROL AREA - ACUTE DERMAL TOXICITY TEST
ANNEX 1 – BODY WEIGHT TABLE

SAMPLE: **CHICHEX BED BUGS INSECTICIDE**
START DATE: 2022-12-05
FINISH DATE: 2022-12-19

Animal	Sex	Identifi/ Cage No	DOSE	Day 0	Day 3	Day 7	Day 10	Day 14	Gained Weight
1	F	51W1-1	4000 mg/kg	2330	2377	2423	2520	2617	287 g
2	F	51W1-1	4000 mg/kg	2380	2428	2475	2574	2673	293 g
3	F	51W1-1	4000 mg/kg	2395	2443	2491	2590	2690	295 g
4	F	51W1-1	4000 mg/kg	2335	2382	2428	2526	2623	288 g
5	F	51W1-1	4000 mg/kg	2385	2433	2480	2580	2679	294 g
6	**F**	**Control**	**N/A**	**2730**	**2811**	**2891**	**2918**	**2945**	**215 g**

Note: Body weight is given in Gram – All animals showed weight gain.

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ANNEX 2 - OBSERVATION DATA

SAMPLE: **CHICHEX BED BUGS INSECTICIDE**
START DATE: 2022-12-05
FINISH DATE: 2022-12-19

ANIMALS		**OBSERVATION PERIOD – DOSE: 4000 mg/kg**													
		Day													
Cage/Number	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
51W1-1/1	F	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51W1-1/2	F	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51W1-1/3	F	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51W1-1/4	F	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51W1-1/5	F	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
Control	**F**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**

M: Male **F:** Female **bc:** behavior changes **nb:** no behavior changes **ad:** animal death

Type of observations:

- There were no erythema or edema
- Changes in the level of activity
- Movements and posture
- Reactivity during handling
- Reactivity towards sensory stimulus
- Stereotyped behavior
- Skin evaluation
- Fur evaluation
- Respiratory effects
- Circulatory effects
- Effects on the central nervous system

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BIOLOGICAL CONTROL AREA - ACUTE DERMAL TOXICITY TEST
ANNEX 3 – MACROSCOPIC OBSERVACION DATA AFTER NECROPSY

SAMPLE: **CHICHEX BED BUGS INSECTICIDE**
START DATE: 2022-12-05
FINISH DATE: 2022-12-19

ANIMALS		MACROSCOPIC OBSERVATION - DOSE: 4000 mg/kg							
		NECROPSY (BODY PART)							
Cage / Number	Sex	Lungs	Trachea	Heart	Liver	Spleen	Kidney	Stomach	Intestine
51W1-1/1	F	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
51W1-1/2	F	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
51W1-1/3	F	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
51W1-1/4	F	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
51W1-1/5	F	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
Control	**F**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**

M: Male **F:** Female **mc:** macroscopic changes **nmc:** no macroscopic changes **ad:** animal death

Type of observations:

- Inflammation
- Necropsy
- Hemorrhages
- Ulcerations
- Changes in morphology
- Changes in coloration