ACUTE ORAL 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 ORAL TOXICITY STUDY**
1.2 Analysis Number:	**22K0344-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:	2022-12-04

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 ORAL TOXICITY TEST ON **CHICHEX BED BUGS INSECTICIDE.**

3. SUMMARY:

CHICHEX BED BUGS INSECTICIDE was the product analyzed in our laboratory.

This study was conducted in three doses (2000, 4000 and 6000 mg/kg body weight). These doses were administered orally to the group of rats (3 females) and (3 males) after a fasting period of 12 hours.

After the first dosage, the animals were observed permanently during the day and then they were observed twice a day in order to take evidence of any alteration (physiological and organic) or death, as well as to determine the time of death and the duration and reversibility of the effects

After completing the study (14 days), the animals were sacrificed to carried out an autopsy and determine any macroscopic abnormality of the organs by comparing them to the control animals.

After each necropsy, histopathology tests were done in order to establish any microscopic abnormality of the organs.

With the above, it was established that, under the test conditions, the LD50 of the product **CHINCHEX BED BUGS INSECTICIDE** is higher than 4000 mg/kg body weight.

4. GUIDELINES:

OECD (2001) Guideline For Testing Of Chemicals. Guideline TG 423. Acute Oral Toxicity- Acute Toxic Class Method. Adopted: 17 December 2001.

5. INTRODUCTION:

5.1 Objective:

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

The experimental design was based on the guidelines of OECD.

5.2 Definitions:

5.2.1 Acute Oral Toxicity:

Are those effects observed in a short period of time (14 days) after the administration of a single dose or multiple doses of the product in a period nor exceeding 24 hours.

5.2.2 DL_{2000} - Median Lethal Dose:

Is a dose that contains statistically the probability of generating death in 50% of individuals exposed 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.

5.3 Specie:

Wistar Albino rat is the specie recommended by the guideline because it is the 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.

6.2 Sample preparation:

The sample was used in the same presentation of the product. As recommended in the OECD guidelines.

6.3 Experimental procedure:

The study was conducted in a group of 6 Wistar Albino rats (3 females and 3 males) per each dose level which was administered orally.

These animals were observed constantly after the first dosage during 6 hours and then they were observed daily during 14 days straight. In the end of the procedure, all the rats were euthanized and necropsied.

6.4 Animal Model - Animal Preparation:

6.4.1 Specie:

The specie of experimentation for this study was Wistar Albino Rat.

6.4.2 Previous Tests:

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

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

6.4.3 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)

The test was also done in a group of negative control animals, by applying salt solution USP (0.9%) orally in order to evaluate the animals' external aspects while handling.

6.4.4 Methodology of Adaptation - Quarantine:

The animals of the same sex were grouped randomly in each cage in such a way that the individual observation of the study was 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 groups of rats had time for adaptation and quarantine under the bioterium'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 test.

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.

The environmental conditions in the bioterium 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 Period of Exposure:

Before 12 hours of the study, the animal food was interrupted. After 6 hours of the product's administration, the food (Purina – Rodentina®) was given to the animals.

After providing the substance to the rats, 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 Oral DL_{2000}:

Three dosage levels were taken for this study (2000, 4000 and 6000 mg/kg) in order to determine observable, lethal and non-lethal effects as well as to identify the dose-response curve and the determination DL_{2000} (Median Lethal Dose). Guideline OECD 423.

6.7 Exposure:

The animals were isolated 12 hours (without food, only with water) before the single was dose administered orally.

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 when the toxicity and intensity signs appeared.

Each observation was written down. The aspects included for this analysis were: posture, movements, activity, reactivity during handling, skin, eyes, fur, membranes of the eyes, respiratory and circulatory effects, and changes of body weight (key parameter of this toxicity test).

6.9 Clinical Macroscopic Test:

The clinical tests were done once a day.

The observations were written in a detailed manner taking into account: changes of posture, movements, activity, reactivity during handling, skin, eyes, fur, membranes of the eyes, respiratory and circulatory effects, and central nervous system effects.

Body weight was checked before the administration of the substance, during the testing and after the sacrifice of the animals with the purpose to determine any alteration of body weight due to the fact that it was a key parameter of this toxicity test.

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 Guide TG-423 of the Organization for Economic Cooperation and Development" Acute Toxic Class Method", whose principle is to perform a first test, with a group of experimental animals, to which the product sample should be administered at a single dose (limit test), and if necessary, subsequent sequential tests should be performed, one test at a time, at dosages that are presumed to approach the value of the mean lethal dose of the product to facilitate the evaluation of toxicity within a range.

Consequently, for the development of the present study, three (3) consecutive tests were proposed, bearing in mind to carry out sequentially one test at a time, with ascending dosages of 2000 mg/kg, 4000 mg/kg and 6000 mg/kg respectively.

The first test proposed (2000 mg/Kg) corresponded to the limit test, in which no deaths were caused by dosing with the product sample.

The second test proposed (4000 mg/Kg) corresponded to the next higher dosage, in which one (1) specimen subjected to the study died.

The third test proposed (6000 mg/Kg), would correspond to the following ascending dosage, which was not developed taking into account that in the previous test (4000 mg/Kg), one (1) specimen died, which allowed suspending the development of additional tests, according to the recommendation of the "OECD Guide TG-423 of the Organization for Economic Cooperation and Development. "This is: to terminate the study in the test that presents one or more deaths, taking the dosage value as the Oral Median Lethal Dose of the product under test.

Taking into account that the specimens submitted to the study did not register severe or lasting signs of distress or pain, it was not necessary to withdraw them from the tests to be humanely sacrificed, thus complying with the record of observations, up to the fourteen (14) days recommended in the guide.

PROPOSED TESTS

First test at a dose of	2000 mg/kg	There were no deaths
Second test at a dose of	4000 mg/kg	**Deceased 1 Male**
Third test at a dose of	6000 mg/kg	Test not developed

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TESTS DEVELOPED

7.1 First test at a dose of 2000 mg/kg.

- Six rat-animals of both sexes (3 females and 3 males) previously selected and prepared for the development of the test were submitted to the test, to which the sample of the product **CHICHEX BED BUGS INSECTICIDE** was administered orally, and the observations were carried out according to the recommendations of the guide.

- After the oral administrations with the product sample (first day), all the specimens registered nausea, in the afternoon of the same day (first day), they showed salivation, retching, weakness and decreased activities.

- All specimens persisted with the registered signs, with similar characteristics and intensity, during the first day, the second day, the retching and salivation disappeared, from the third day the nausea and weakness disappeared and they resumed activities.

- During the fourteen (14) days of the observation period, all specimens registered normal responses to external stimuli; urinary and fecal performances in normal amounts and frequencies; water and food consumption in adequate portions, in the first and second week they increased body weight in normal proportions. In general, all individuals showed poor health conditions during the first two days and showed good health conditions from the third day of the study.

DEATHS. No specimens died in this test.

NECROPSIES. On the last day of the study, all specimens were sacrificed and necropsies were performed in which the following was observed: **"Mucosa covering the abdomen with mild inflammation".**

HISTOPATHOLOGY. Histopathological examinations were performed and were negative for tissue changes.

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7.2 Second test at a dose of 4000 mg/kg.

- Six rat-animals of both sexes (3 females and 3 males) previously selected and prepared for the development of the test were submitted to the test, to which the sample of the product **CHICHEX SED BUGS INSECTICIDE** was administered orally, and the observations were carried out according to the recommendations of the guide.

- After oral administration of the product sample (first day), all specimens showed hypersalivation, retching, nausea, decreased food consumption and abdominal swelling; in the afternoon of the same day (first day), they showed depressive attitude, grouping and decreased activities; **at the end of the same day (first day), one male also showed abdominal cramps, no response to external stimuli, lack of appetite, numbness, dehydration and died.**

- All the surviving specimens persisted with the recorded signs, with similar characteristics and intensity, during the first day, the second day the retching disappeared and the saliva production decreased, the third day, the nausea disappeared, the salivation and the abdominal swelling decreased, from the fourth day the food consumption was restored, the depression disappeared, the grouping and the activities were resumed.

- During the fourteen (14) days of the observation period, all surviving specimens registered positive responses to external stimuli; urinary and fecal performances in normal amounts and frequencies, water consumption in adequate portions, during the first three days showed a decrease in food consumption; from the fourth day they consumed food in adequate portions, in the first week they all increased body weight in low proportions; in the second week they increased weight in normal proportions.

- In general, all surviving individuals showed poor health conditions during the first three days and showed good health conditions from the fourth day of the study.

DEATHS. In this test, 1 male died (16.66%).

NECROPSIES. On the last day of the study, the surviving specimens were sacrificed and necropsies were performed, in which the following was observed: **"Slightly distended abdominal muscles."**

HISTOPATHOLOGIES. Histopathological examinations were performed in which it was observed: **"Enlarged abdominal cavity with undigested food residues".**

7.3 Third test at a dose of 6000 mg/kg.

Test not developed, bearing in mind that in the previous dosage (4000 mg/kg) 16.66% of the specimens subjected to the test died, which allowed suspending the development of additional tests, in accordance with the recommendation of the "OECD Guide TG-423 of the Organization for Economic Cooperation and Development", that is: to terminate the study in the test that presents one or more deaths, taking the dosage value as the Oral Median Lethal Dose of the product.

8. CONCLUSIONS:

At the end of the study, having used the sample of the product in the first two proposed dosages (2000 mg/kg and 4000 mg/kg), it was found that in the first test (2000 mg/kg) there was no death of specimens.

However, in the second test (4000 mg/Kg) 16.66% of the specimens submitted died.

Therefore, a value of Oral Mean Lethal Dose could be estimated for the sample of the product **CHICHEX BED BUGS INSECTICIDE**, property of the company **FRUIT TREE Ltd.** greater than 4000 mg/Kg.

LD50 Oral > 4000 mg/Kg

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9. BIBLIOGRAPHY:

9.1 OECD (2001) Guideline For Testing Of Chemicals. Guideline TG 423. Acute Oral Toxicity- Acute Toxic Class Method. Adopted: 17 December 2001.

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QUALITY CONTROL DEPARTMENT
BIOLOGICAL CONTROL AREA - ACUTE ORAL TOXICITY TEST
ANNEX 1 – BODY WEIGHT TABLE

SAMPLE: **CHICHEX BED BUGS INSECTICIDE**
START DATE: 2022-12-04
FINISH DATE: 2022-12-18

BOX No:	51V1-1	SEX:	MALES	DOSE:	2000 mg/kg

Rat No:	Identi/	Day 1	Day 3	Day 7	Day 10	Day 14	Gained Weight	Observations
1	Leg	240	245	250	260	270	30 g	No Reactivity
2	Head	230	235	239	249	258	28 g	No Reactivity
3	Tail	235	240	244	254	264	29 g	No Reactivity
4	**Control**	**250**	**256**	**261**	**263**	**265**	**15 g**	**No Reactivity**

BOX No:	51V1-2	SEX:	FEMALE	DOSE:	2000 mg/kg

Rat No:	Identi/	Day 1	Day 3	Day 7	Day 10	Day 14	Gained Weight	Observations
1	Leg	225	230	234	243	253	28 g	No Reactivity
2	Head	235	240	244	254	264	29 g	No Reactivity
3	Tail	220	224	229	238	247	27 g	No Reactivity
4	**Control**	**265**	**267**	**270**	**272**	**275**	**10 g**	**No Reactivity**

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ANNEX 1 – BODY WEIGHT TABLE

SAMPLE: **CHICHEX BED BUGS INSECTICIDE**
START DATE: 2022-12-04
FINISH DATE: 2022-12-18

BOX No:	51V1-3	SEX:	MALES	DOSE:	4000 mg/kg

Rata No:	Identi/	Day 1	Day 3	Day 7	Day 10	Day 14	Gained Weight	Observations
1	Leg	**ad**	**ad**	**ad**	**ad**	**ad**	**N/A**	**Reactivity**
2	Head	245	250	255	265	275	30 g	No Reactivity
3	Tail	230	235	239	249	258	28 g	No Reactivity
4	**Control**	**250**	**252**	**255**	**257**	**260**	**10 g**	**No Reactivity**

BOX No:	51V1-4	SEX:	FEMALE	DOSE:	4000 mg/kg

Rata No:	Identi/	Day 1	Day 3	Day 7	Day 10	Day 14	Gained Weight	Observations
1	Leg	245	250	255	265	275	30 g	No Reactivity
2	Head	225	230	234	243	253	28 g	No Reactivity
3	Tail	210	214	218	227	236	26 g	No Reactivity
4	**Control**	**245**	**247**	**249**	**252**	**255**	**10 g**	**No Reactivity**

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QUALITY CONTROL DEPARTMENT
BIOLOGICAL CONTROL AREA - ACUTE ORAL TOXICITY TEST
ANNEX 2 - OBSERVATION DATA

SAMPLE: **CHICHEX BED BUGS INSECTICIDE**
START DATE: 2022-12-04
FINISH DATE: 2022-12-18

ANIMALS		**OBSERVATION PERIOD – DOSE: 2000 mg/kg**													
		Day													
Box /Number	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
51V1-1 - 1	M	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51V1-1 - 2	M	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51V1-1 - 3	M	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
Control	**M**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**
51V1-2 - 1	F	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51V1-2 - 2	F	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51V1-2 - 3	F	bc	bc	bc	nb	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 change **nb:** no behavior change **ad:** animal death

Type of observations:

- 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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ANNEX 2 - OBSERVATION DATA

SAMPLE: **CHICHEX BED BUGS INSECTICIDE**
START DATE: 2022-12-04
FINISH DATE: 2022-12-18

ANIMALS		**OBSERVATION PERIOD – DOSE: 4000 mg/kg**													
		Day													
Box / Number	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
51V1-3 - 1	M	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**
51V1-3 - 2	M	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51V1-3 - 3	M	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
Control	**M**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**
51V1-4 - 1	F	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51V1-4 - 2	F	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51V1-4 - 3	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 change **nb:** no behavior change **ad:** animal death

Type of observations:

- 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

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QUALITY CONTROL DEPARTMENT
BIOLOGICAL CONTROL AREA - ACUTE ORAL TOXICITY TEST
ANNEX 3 – MACROSCOPIC OBSERVACION DATA AFTER NECROPSY

SAMPLE: **CHICHEX BED BUGS INSECTICIDE.**
START DATE: 2022-12-04
FINISH DATE: 2022-12-18

ANIMALS		MACROSCOPIC OBSERVATION							
		NECROPSY (BODY PART) DOSE: 2000 mg/kg							
Number	Sex	Lungs	Trachea	Mouth	Throat	Spleen	Abdomen	Stomach	Intestine
CAJA 51V1-1									
1	M	nmc	nmc	nmc	nmc	nmc	mc	mc	mc
2	M	nmc	nmc	nmc	nmc	nmc	mc	mc	mc
3	M	nmc	nmc	nmc	nmc	nmc	mc	mc	mc
Control	**M**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**
CAJA 51V1-2	F								
1	F	nmc	nmc	nmc	nmc	nmc	mc	mc	mc
2	F	nmc	nmc	nmc	nmc	nmc	mc	mc	mc
3	**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

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ANNEX 3 - MACROSCOPIC OBSERVACION DATA AFTER NECROPSY

SAMPLE: **CHICHEX BED BUGS INSECTICIDE.**
START DATE: 2022-12-04
FINISH DATE: 2022-12-18

ANIMALS		**MACROSCOPIC OBSERVATION**							
		NECROPSY (BODY PART) **DOSE: 4000 mg/kg**							
Number	**Sex**	**Lungs**	**Trachea**	**Esophagus**	**Mouth**	**Muscles**	**Kidney**	**Stomach**	**Intestine**
CAJA 51V1-3									
1	M	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**
2	M	nmc	nmc	nmc	nmc	mc	nmc	nmc	nmc
3	M	nmc	nmc	nmc	nmc	mc	nmc	nmc	nmc
Control	**M**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**
CAJA 51V1-4	F								
1	F	nmc	nmc	nmc	nmc	mc	nmc	nmc	nmc
2	F	nmc	nmc	nmc	nmc	mc	nmc	nmc	nmc
3	**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