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

QUALITY CONTROL DEPARTMENT

BIOLOGICAL ANALYSIS

TOXICITY TEST

1. COMPOSICION CERTIFICATE:

1.1 Type of study:	**ACUTE INHALATION TOXICITY STUDY**
1.2 Analysis Number:	**22K0346-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:	2022-12-06

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

3. SUMMARY:

CHICHEX BED BUGS INSECTICIDE was the product analyzed in our laboratory. Wistar Albino rats were used for this analysis.

This study was conducted in three doses (2,5 mg/L, 5,0 mg/L y 10,0 mg/L air x 4 hours). These doses were administered to the group of rats (5 females and 5 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 and compare 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 CL_{50} of the product **CHICHEX BED BUGS INSECTICIDE** is higher than 5,0 mg/L of air.

4. GUIDELINES:

OECD (2009) Guideline For Testing Of Chemicals. Guideline TG 403. Acute Inhalation Toxicity: Fixed Procedure. Adopted: 07 September 2009.

5. INTRODUCTION:

5.1 Objective:

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

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

5.2 Definitions:

5.2.1 Acute Inhalation Toxicity:

Is the adverse effect caused by a substance after a simple and interrupted exposure by inhalation within a short time period (24 hours or less)

5.2.2 DL_{50} - 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. A vaporizer was used for this study.

6.3 Experimental Procedure:

The study was conducted in a group of 10 Wistar Albino rats (5 females and 5 males) per each dose level which was administered by the inhalation route.

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 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)

This test is also done in a group of negative control animals by applying vapor to evaluate the external aspects during 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 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.

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.

The animals of the same sex were grouped and placed in a hermetically-sealed inhalator chamber. Then, they were given oxygen (19%) with a dynamic flow of 10 air changes per hour.

The chamber has a control system that guaranties a temperature between 20°C+- 2°C and relative humidity between 30 - 70 %.

The inhalation chamber is for body use (whole body to develop the test in a type of habitat). The volume of the group of the animals does not exceed 5% of the volume of the chamber which allows to guaranty the total exposure of the substance.

The chamber has a hermetically-sealed system that emanates vapors of the substance by inducing it into the chamber, which permits a balance in the concentrations.

When the balance is achieved in the chamber, automatically the four-hour process is activated.

After 4 hours, the equipment automatically makes the vent.

After administering the substance, the animals were moved and placed in their cages. After 6 hours, water and food (whose composition is known as ad-libitum) were provided during 14 days, period of time for this study.

6.6 Dosage Levels - Determination CL_{50}:

Three dosage levels were taken for this study. Their limit value was 5,0 mg/L Air x 4 hours (2,5 mg/L, 5,0 mg/L y 10,0 mg/L Air during 4 hours), in order to determine observable, lethal and non-lethal effects as well as to identify the dose-response curve and the determination CL_{50} (Median Lethal Concentration).Guideline OECD 403.

6.7 Exposure:

The animals were isolated 12 hours (without food, only with water) before the single dose was 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 or any case of death.

Each observation was written down. The aspects included for this analysis were: 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).

6.9 Clinical Macroscopic Test:

The clinical tests were done once a day.

The observation was written in a detailed manner taking into account: changes of posture, movements, activity, reactivity during handling, grip strength, gestures, stereotype (walk backward), skin, eyes, fur, eyes' membranes, 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.

After each necropsy, histopathology reports were done.

The histopathology reports were done in intestines, kidneys, lungs, central nervous system, pancreas, skin, heart and spleen.

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7. RESULTS:

The present study was developed according to the guidelines adopted in the "OECD TG-403 Guide of the Organization for Economic Cooperation and Development", using the methodology corresponding to the "traditional protocol", in which initially, a first group of animals should be taken to be subjected to inhalation with the sample of the product, at a concentration limit (limit test), and if necessary, other tests should be carried out with other experimental animals, through consecutive inhalations step by step, at concentrations that are presumed to approach the value of the mean lethal concentration of the product.

In the test that one or more deaths occur, the study should be terminated and the concentration should be taken as the mean lethal concentration value of the product to facilitate the evaluation of toxicity within a range.

In accordance with the above, for the development of the present study, it was planned to perform three (3) consecutive tests, step by step, at the concentrations of 2.5 mg/liter/air, 5.0 mg/liter/air and 10.0 mg/liter/air, during four (4) hours of exposure, using in each test, three (3) groups of experimental animals, to be exposed to inhalation with the sample of the product **CHICHEX BED BUGS INSECTICIDE.**

The first concentration proposed (2.5 mg/liter/air), corresponded to the limit test, in which no deaths were caused by inhalation with the product sample.

The second proposed inhalation (5.0 mg/liter/air) corresponded to the next consecutive test, in which two (2) specimens died by inhalation with the product sample.

The third concentration proposed (10.0 mg/liter/air), corresponded to the following consecutive inhalation, which was not developed, bearing in mind that in the previous test (5.0 mg/liter/air), two (2) specimens died, which is considered a reason to terminate the study, according to the recommendation of the "OECD TG-403 Guide of the Organization for Economic Cooperation and Development. "This is: to terminate the study in the test that one or more deaths occur, taking the concentration value as the mean lethal concentration of the product.

During the inhalations and the following fourteen (14) days that corresponded to the theoretical period of the study, observations were carried out and toxic effects and deaths were recorded; at the end of each test, both the specimens that died and the survivors were sacrificed and necropsied and later underwent histopathological observation.

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

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PROPOSED TESTS

First test at a concentration of	2,5 mg/liter/air	No fatalities
Second test in concentration of	5,0 mg/liter/air	**2 Males died**
Third test in concentration of	10,0 mg/liter/air	**Test not developed**

TESTS DEVELOPED

7.1 First test in concentration of 2,5 mg/liter/air.

- For the development of the present test, 10 rat-animals of both sexes (5 females and 5 males) previously selected and prepared for the study were submitted to exposure with the sample of the product **CHICHEX BED BUGS INSECTICIDE**, in an inhalation chamber for four (4) hours. These specimens were observed during the time indicated in the guide.

- Inside the chamber, all specimens exhibited coughing, difficulty breathing, nasal irritation, tearing, sweating in their bodies, these signs persisted during the four (4) hours of inhalation. When leaving the chamber, the specimens also showed general weakness, depressive attitude and drowsiness.

- All the specimens of the test persisted with the registered signs, with similar characteristics and intensity, during the whole first day, in the morning of the second day, the sweating in their bodies disappeared, the frequency and intensity of coughing decreased and they showed slight improvement in the quality of breathing; On the afternoon of the same day (second day), the production of tears, nasal irritation and general weakness disappeared; on the third day, nasal irritation and coughing disappeared and they showed normal breathing; from the fourth day, the production of tears, depressive attitude and somnolence disappeared.

- During the fourteen (14) days of the observation period, all specimens registered positive responses to sound stimuli; urinary and fecal performances in normal amounts and frequencies, water and food consumption in adequate portions, in the first week they increased body weight in low proportions and in the second week they increased weight in normal proportions.

- In general, all individuals showed poor health conditions during the first three days and showed good health conditions from the fourth 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**: "Respiratory Canal with mild inflammation".**

HISTOPATHOLOGY. Histopathological examinations were performed, in which it was observed: **"Respiratory Canal with moderate narrowing and bluish skin of the extremities".**

7.2 Second test at a concentration of 5,0 mg/liter/air.

- For the development of the present test, 10 rat-animals of both sexes (5 females and 5 males) previously selected and prepared for the study were submitted to exposure with the sample of the product **CHICHEX BED BUGS INSECTICIDE**, in an inhalation chamber for four (4) hours. These specimens were observed for the time indicated in the guide.

- Inside the chamber, all specimens exhibited coughing, difficulty breathing, nasal irritation, hyperlagrimation and sweating of their bodies; these signs persisted during the four (4) hours of inhalation. Upon leaving the chamber, the specimens additionally showed fever, depressive attitude, drowsiness, bluish skin and decreased activities. **In the afternoon of the same day (first), two males increased their body temperature, additionally registered delayed responses to sound stimuli, pectoral noises, mouth and nose stupor, asphyxia, severe respiratory distress and died.**

- All the surviving specimens persisted with the recorded signs, with similar characteristics and intensity until the morning of the second day; in the afternoon of the same day (second), sweating in their bodies and fever disappeared; On the third day, the production of tears and the depressive attitude disappeared; in the afternoon of the same day (third), the frequency and intensity of coughing decreased and showed slight improvement in the quality of breathing; on the fourth day, nasal irritation, blue color of the skin and drowsiness disappeared; from the fifth day, coughing disappeared, they showed normal breathing and resumed activities.

- During the fourteen (14) days of the observation period, all the surviving specimens registered positive responses to sound stimuli, urinary and fecal performances in normal quantities and frequencies; water and food consumption in adequate portions; in the first week the survivors increased body weight in low proportions; in the second week they increased weight in normal proportions.

- In general, all survivors showed poor health conditions during the first four days and showed good health conditions from the fifth day of the study.

DEATHS. Two males (20%) died in this test.

NECROPSIES. On the last day of the study, all surviving specimens were sacrificed and necropsies were performed, in which the following were observed: **"Bluish skin" and "Edematous respiratory canal".**

HISTOPATHOLOGY. Histopathological examinations were performed, in which it was observed: **"Inflamed and irritated lungs, with fluid deposits inside, with a diagnosis of Acute Silicosis".**

7.3 Third test in concentration of 10,0 mg/liter/air

Test not developed, considering that the previous inhalation (5.0 mg/liter/air) caused the death of two (2) specimens, which is considered a reason to suspend the study, according to the recommendation of the "OECD Guide TG-403 of the Organization for Economic Cooperation and Development", i.e.: terminate the study in the test that presents one or more deaths, taking the concentration value as the average lethal concentration of the product.

8. CONCLUSIONS:

At the end of the study, having used the product sample in the first two concentrations proposed (2.5 mg/liter/air and 5.0 mg/liter/air), it was found that in the first test (2.5 mg/liter/air) none of the specimens died. However, in the second test (5.0 mg/liter/air), death was recorded in 20% of the specimens submitted.

Therefore, for the sample of the product **CHICHEX BED BUGS INSECTICIDE,** property of the company **FRUIT TREE Ltd.** a value of Lethal Mean Concentration greater than 5.0 mg/liter/air could be estimated.

Inhalation LC50 > 5,0 mg/liter/air

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9. BIBLIOGRAPHY:

9.1. OECD (2009) Guideline For Testing Of Chemicals. Guideline TG 403. Acute Inhalation Toxicity: Fixed Procedure. Adopted: 07 September 2009.

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Laboratory registered in ICA (Colombian Agricultural Institute). Resolution 3173 on Nov 25, 2002

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

SAMPLE: **CHICHEX BED BUGS INSECTICIDE**
STAR DATE: 2022-12-06
FINISH DATE: 2022-12-20

Box/Animal	Sex	Identifi/	DOSES	Day 0	Day 3	Day 7	Day 10	Day 14	Gained Weight
51X1-1/1	M	Leg	2,5 mg/L de Air	255	260	265	276	286	31 g
51X1-1/2	M	Head	2,5 mg/L de Air	250	255	260	270	281	31 g
51X1-1/3	M	Tail	2,5 mg/L de Air	260	265	270	281	292	32 g
51X1-1/4	M	Back	2,5 mg/L de Air	240	245	250	260	270	30 g
51X1-1/5	M	Hand	2,5 mg/L de Air	230	235	239	249	258	28 g
51X1-1/6	**M**	**Control**	**N/A**	**270**	**281**	**292**	**295**	**304**	**34 g**
Animal	**Sex**	**Identifi/**	**DOSES**	**Day 0**	**Day 3**	**Day 7**	**Day 10**	**Day 14**	**Gained Weight**
51X1-2/1	F	Leg	2,5 mg/L de Air	245	250	255	265	275	30 g
51X1-2/2	F	Head	2,5 mg/L de Air	255	260	265	276	286	31 g
51X1-2/3	F	Tail	2,5 mg/L de Air	250	255	260	270	281	31 g
51X1-2/4	F	Back	2,5 mg/L de Air	240	245	250	260	270	30 g
51X1-2/5	F	Hand	2,5 mg/L de Air	250	255	260	270	281	31 g
51X1-2/6	**F**	**Control**	**N/A**	**275**	**286**	**298**	**304**	**310**	**35 g**

M: Male **F:** Female

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

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SAMPLE: **CHICHEX BED BUGS INSECTICIDE**
STAR DATE: 2022-12-06
FINISH DATE: 2022-12-20

Box/Animal	Sex	Identifi/	DOSES	Day 1	Day 2	Day 5	Day 10	Day 14	Gained Weight
51X1-3/1	M	Leg	5,0 mg/L de Air	260	**ad**	**ad**	**ad**	**ad**	**N/A**
51X1-3/2	M	Head	5,0 mg/L de Air	270	**ad**	**ad**	**ad**	**ad**	**N/A**
51X1-3/3	M	Tail	5,0 mg/L de Air	285	291	296	308	320	35 g
51X1-3/4	M	Back	5,0 mg/L de Air	270	275	281	292	303	33 g
51X1-3/5	M	Hand	5,0 mg/L de Air	265	270	276	287	298	33 g
51X1-3/6	**M**	**Control**	**N/A**	**300**	**313**	**325**	**332**	**339**	**39 g**
Box/Animal	**Sex**	**Identifi/**	**DOSES**	**Day 1**	**Day 2**	**Day 5**	**Day 10**	**Day 14**	**Gained Weight**
51X1-4/1	F	Leg	5,0 mg/L de Air	245	250	255	265	275	30 g
51X1-4/2	F	Head	5,0 mg/L de Air	235	240	244	254	264	29 g
51X1-4/3	F	Tail	5,0 mg/L de Air	225	230	234	243	253	28 g
51X1-4/4	F	Back	5,0 mg/L de Air	240	245	250	260	270	30 g
51X1-4/5	F	Hand	5,0 mg/L de Air	255	260	265	276	286	31 g
51X1-4/6	**F**	**Control**	**N/A**	**270**	**281**	**292**	**295**	**304**	**34 g**

M: Male **F:** Female **ad:** animal death

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

ACUTE INHALATION TOXICITY TEST
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OF THE ENTERPRISE FRUIT TREE Ltd

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

SAMPLE: **CHICHEX BED BUGS INSECTICIDE**
STAR DATE: 2022-12-06
FINISH DATE: 2022-12-20

ANIMALS		OBSERVATION PERIOD– DOSE 2,5 mg/L													
		Day													
Box /Number	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
51X1-1/1	M	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-1/2	M	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-1/3	M	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-1/4	M	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-1/5	M	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-1/6	**M (Control)**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**
51X1-2/1	F	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-2/2	F	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-2/3	F	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-2/4	F	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-2/5	F	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-2/6	**F (Control)**	**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:

- 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**
STAR DATE: 2022-12-06
FINISH DATE: 2022-12-20

ANIMALS		OBSERVATION PERIOD – DOSE 5,0 mg/L													
		Day													
Number	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
51X1-3/1	M	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**
51X1-3/2	M	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**
51X1-3/3	M	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-3/4	M	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-3/5	M	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-3/6	**M (Control)**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**
51X1-4/1	F	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-4/2	F	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-4/3	F	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-4/4	F	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-4/5	F	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
51X1-4/6	**F (Control)**	**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:

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

SAMPLE: **CHICHEX BED BUGS INSECTICIDE**
STAR DATE: 2022-12-06
FINISH DATE: 2022-12-20

ANIMALS			MACROSCOPIC OBSERVATION - DOSE: 2,5 mg/L						
			NECROPSY (BODY PART)						
Box / Number	Sex	Lungs	Trachea	Heart	Liver	Mouth	Nose	Stomach	Intestine
51X1-1/1	M	mc	mc	nmc	nmc	mc	mc	nmc	nmc
51X1-1/2	M	mc	mc	nmc	nmc	mc	mc	nmc	nmc
51X1-1/3	M	mc	mc	nmc	nmc	mc	mc	nmc	nmc
51X1-1/4	M	mc	mc	nmc	nmc	mc	mc	nmc	nmc
51X1-1/5	M	mc	mc	nmc	nmc	mc	mc	nmc	nmc
51X1-1/6	**M(Control)**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**
51X1-2/1	F	mc	mc	nmc	nmc	mc	mc	nmc	nmc
51X1-2/2	F	mc	mc	nmc	nmc	mc	mc	nmc	nmc
51X1-2/3	F	mc	mc	nmc	nmc	mc	mc	nmc	nmc
51X1-2/4	F	mc	mc	nmc	nmc	mc	mc	nmc	nmc
51X1-2/5	F	mc	mc	nmc	nmc	mc	mc	nmc	nmc
51X1-2/6	**F(Control)**	**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

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

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

SAMPLE: **CHICHEX BED BUGS INSECTICIDE**
STAR DATE: 2022-12-06
FINISH DATE: 2022-12-20

ANIMALS		MACROSCOPIC OBSERVATION - DOSE: 5,0 mg/L							
		NECROPSY (BODY PART)							
Box / Number	**Sex**	**Lungs**	**Trachea**	**Heart**	**Skin**	**Mouth**	**Nose**	**Stomach**	**Intestine**
51X1-3/1	M	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**
51X1-3/2	M	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**
51X1-3/3	M	mc	mc	nmc	mc	mc	mc	nmc	nmc
51X1-3/4	M	mc	mc	nmc	mc	mc	mc	nmc	nmc
51X1-3/5	M	mc	mc	nmc	mc	mc	mc	nmc	nmc
51X1-3/6	**M(Control)**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**
51X1-4/1	F	mc	mc	nmc	mc	mc	mc	nmc	nmc
51X1-4/2	F	mc	mc	nmc	mc	mc	mc	nmc	nmc
51X1-4/3	F	mc	mc	nmc	mc	mc	mc	nmc	nmc
51X1-4/4	F	mc	mc	nmc	mc	mc	mc	nmc	nmc
51X1-4/5	F	mc	mc	nmc	mc	mc	mc	nmc	nmc
51X1-4/6	**F(Control)**	**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
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