ACUTE INHALATION TOXICITY TEST
FOR THE PRODUCT CHINCHEX BED BUGS INSECTICIDE
OF THE ENTERPRISE CHINCHEX LIMITED

QUALITY CONTROL DEPARTMENT

BIOLOGICAL ANALYSIS

TOXICITY TEST

1. COMPOSICION CERTIFICATE:

1.1 Type of study:	**ACUTE INHALATION TOXICITY STUDY**
1.2 Analysis Number:	**23B0076-T**
1.3 Client:	**CHINCHEX LIMITED**
1.4 Trade name:	**CHINCHEX BED BUGS INSECTICIDE**
1.5 Composition:	**Active Ingredient:** • Silicon Dioxide 550 gr/kg • Amorphous Silica 450 gr/kg
1.6 Date of received samples:	2023-02-24
1.7 Date of analysis:	2023-03-09

NOTE: Result according to sample sent and sampled by CHINCHEX LIMITED

* The composition certificate is a true transcription of the one sent and authorized by CHINCHEX LIMITED

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2. TITLE:

ACUTE INHALATION TOXICITY TEST ON **CHINCHEX BED BUGS INSECTICIDE.**

3. SUMMARY:

The product to be evaluated was **CHINCHEX BED BUGS INSECTICIDE**. Wistar rats were used.

The study was carried out at four doses (0.5 mg/L, 2.0 mg/L, 10.0 mg/L and 20.0 mg/L) which were dosed by inhalation to groups of 5 males per dose, after a fasting period of 12 hours.

The observation of the animals after dosing was carried out permanently during the first day of administration and then twice a day to control and evidence behavioral, physiological and organic alterations, death, in order to determine the duration and reversibility of the effects and time of death.

At the end of the study (14 days) all animals were sacrificed and necropsied to establish macroscopic abnormalities of the organs, compared with the control animals.

After the necropsy, histopathological examinations were performed to establish the microscopic abnormalities of the organs.

With the above, the conditions of average lethal dose of the product **CHINCHEX BED BUGS INSECTICIDE** was established to be greater than 20.0 mg/L. Which corresponds to a GHS classification 5

4. GUIDELINES:

OECD (2018) Guideline For Testing Of Chemicals. Guideline TG 433. Acute inhalation toxicity: Fixed concentration procedure. Adopted: 25 June 2018.

5. INTRODUCTION:

5.1 Objective:

The objective of this study is to evaluate the Acute Inhalation Toxicity of the sample taken from the product **CHINCHEX 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:

CHINCHEX BED BUGS INSECTICIDE was given by **CHINCHEX LIMITED** on February 24, 2023.

6.2 Sample Preparation:

The sample to be evaluated was dissolved in a specific solvent and then vehicleized with sterile water, added together with the sample to the vaporizer in the inhalation chamber according to OECD 433 protocol.

6.3 Experimental Procedure:

The study was conducted in a group of 5 Wistar rats (5 males) per dose level. The product **CHINCHEX BED BUGS INSECTICIDE** was administered by inhalation.

The animals were permanently observed for the first 6 hours after dosing and then daily observations were made for 14 consecutive days. At the end, all 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:

Groups of 5 animals of the same sex per cage were randomly housed so as not to interfere with individual observation.

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.

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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}:

Four dosage levels were taken: 0.5 mg/L, 2.0 mg/L, 10.0 mg/L and 20.0 mg/L for 4 hours, to determine observable, lethal and non-observable effects and thus determine the dose-response curve and the determination of the LC50 (Lethal Mean Concentration). OECD Guide 433.

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.

7. RESULTS:

The present study was developed according to the guidelines adopted in the "OECD TG-433 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 product sample, 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, in concentrations that are presumed to be close to 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 0.5 mg/L, 2.0 mg/L, 10.0 mg/L and 20.0 mg/L mg/liter, during four (4) hours of exposure, using in each test, four (4) groups of experimental animals, to be exposed to inhalation with the sample of the product **CHINCHEX BED BUGS INSECTICIDE.**

The first proposed concentration (0.5 mg/liter), in which no deaths were caused by inhalation with the product sample.

The second proposed inhalation (2.0 mg/liter) corresponded to the next consecutive test, in which no animals died.

The third proposed concentration (10.0 mg/liter) corresponded to the next consecutive inhalation, in which there were no deaths.

The fourth proposed concentration (20.0 mg/liter) would correspond to the next consecutive inhalation, in which one animal died.

During the inhalations and the following fourteen (14) days, which 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 histopathological observation was carried out.

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	0,5 mg/liter/air	No Death
Second test in concentration of	2,0 mg/liter/air	No Death
Third test in concentration of	10,0 mg/liter/air	No Death
Fourth concentration test of	20,0 mg/liter/air	**Died 1 male**

TESTS DEVELOPED

7.1 First test in concentration of 0,5 mg/liter/air.

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

- Inside the inhalation chamber they showed no abnormal behavioral signs and respiration was normal.

- 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 efficient health conditions during the whole study from the first 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: **"Normal behavior".**

HISTOPATHOLOGY. Histopathological examinations were performed, in which the following was not observed: **"No histopathological damage".**

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7.2 Second test at a concentration of 2.0 mg/litre.

- For the development of the present test, 5 rat-animals (5 males) previously selected and prepared for the study were submitted to exposure with the sample of the product **CHINCHEX 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 behaved normally.

- During the fourteen (14) days of the observation period, all surviving 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 the survivors increased body weight in low proportions, in the second week they increased weight in normal proportions.

- In general, all individuals showed efficient health conditions during the whole study from the first day of the study.

DEATHS. No specimens 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 was observed: **"Normal behavior".**

HISTOPATHOLOGY. Histopathological examinations were performed, in which the following was not observed: **"No histopathological damage".**

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

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

- Inside the inhalation chamber they showed no abnormal behavioral signs and respiration was normal.

- During the first two days they presented sweating and nasal irritation.

- 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 efficient health conditions during the whole study from the first day of the study.

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7.4 Third test at a concentration of 20.0 mg/liter.

- For the development of the present test, 5 rat-animals (5 males) previously selected and prepared for the study were submitted to exposure with the sample of the product **CHINCHEX 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, hyperlagrimation and sweating of their bodies; these signs persisted during the four (4) hours of inhalation. On leaving the chamber, the specimens additionally showed fever, depressive attitude, drowsiness, bluish skin and decreased activities. In the afternoon of the same day (first), **one male, increased 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 amounts 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. Deceased 1 Male 20%.

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 diagnosis of Acute Silicosis".**

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8. CONCLUSIONS:

At the end of the study, having used the sample of the product, in the first three concentrations proposed **(0.5 mg/L, 2.0 mg/L, 10.0 mg/L),** it did not cause death in any of the specimens. However, in the fourth test **(20.0 mg/liter), 20%** of the specimens submitted died.

Therefore, for the sample of the product **CHINCHEX BED BUGS INSECTICIDE**, property of the company **CHINCHEX LIMITED.,** a value of Lethal Mean Concentration greater than 20.0 mg/liter could be estimated, which corresponds to a GHS classification in category 5.

CL_{50} Inhalation > 20.0 mg/liter.

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9. BIBLIOGRAPHY:

9.1 OECD OECD (2018) Guideline For Testing Of Chemicals. Guideline TG 433. Acute Inhalation Toxicity: Fixed Concentration Procedure. Adopted: 25 June 2018.

Responsible of this study : **CARLOS MANUEL BUSTOS BOLIVAR**
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QUALITY CONTROL DEPARTMENT
BIOLOGICAL CONTROL AREA - ACUTE INHALATION TOXICITY TEST
ANNEX 1 – BODY WEIGHT TABLE

SAMPLE: **CHINCHEX BED BUGS INSECTICIDE**
STAR DATE: 2023-03-09
FINISH DATE: 2023-03-23

Box/Animal	Sex	Identifi/	DOSES	Day 1	Day 3	Day 7	Day 10	Day 14	Gained Weight
58H1-1/1	M	Leg	0,5 mg/L de Air	250	255	260	270	281	31 g
58H1-1/2	M	Head	0,5 mg/L de Air	245	250	255	265	275	30 g
58H1-1/3	M	Tail	0,5 mg/L de Air	260	265	270	281	292	32 g
58H1-1/4	M	Back	0,5 mg/L de Air	240	245	250	260	270	30 g
58H1-1/5	M	Hand	0,5 mg/L de Air	230	235	239	249	258	28 g
58H1-1/6	**M**	**Control**	**N/A**	**290**	**301**	**312**	**315**	**324**	**34 g**

Note: Weight of each animal is in grams - -- All animals showed weight gain.

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FINISH DATE: 2023-03-23

Box/Animal	Sex	Identifi/	DOSES	Day 1	Day 3	Day 7	Day 10	Day 14	Gained Weight
58H1-2/1	M	Leg	2,0 mg/L de Air	265	270	276	287	298	33 g
58H1-2/2	M	Head	2,0 mg/L de Air	275	281	286	297	309	34 g
58H1-2/3	M	Tail	2,0 mg/L de Air	280	286	291	303	314	34 g
58H1-2/4	M	Back	2,0 mg/L de Air	275	281	286	297	309	34 g
58H1-2/5	M	Hand	2,0 mg/L de Air	260	265	270	281	292	32 g
58H1-2/6	**M**	**Control**	**N/A**	**315**	**328**	**340**	**347**	**354**	**39 g**

Note: Weight of each animal is in grams - -- All animals showed weight gain.

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Box/Animal	Sex	Identifi/	DOSES	Day 1	Day 3	Day 7	Day 10	Day 14	Gained Weight
58H1-3/1	M	Leg	10,0 mg/L de Air	240	245	250	260	270	30 g
58H1-3/2	M	Head	10,0 mg/L de Air	245	250	255	265	275	30 g
58H1-3/3	M	Tail	10,0 mg/L de Air	250	255	260	270	281	31 g
58H1-3/4	M	Back	10,0 mg/L de Air	255	260	265	276	286	31 g
58H1-3/5	M	Hand	10,0 mg/L de Air	260	265	270	281	292	32 g
58H1-3/6	**M**	**Control**	**N/A**	**330**	**343**	**355**	**362**	**369**	**39 g**

Note: Weight of each animal is in grams - -- All animals showed weight gain.

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SAMPLE: **CHINCHEX BED BUGS INSECTICIDE**
STAR DATE: 2023-03-09
FINISH DATE: 2023-03-23

Box/Animal	Sex	Identifi/	DOSES	Day 1	Day 2	Day 5	Day 10	Day 14	Gained Weight
58H1-4/1	M	Leg	20,0 mg/L de Air	**m**	**m**	**m**	**m**	**m**	**N/A**
58H1-4/2	M	Head	20,0 mg/L de Air	275	281	286	297	309	34 g
58H1-4/3	M	Tail	20,0 mg/L de Air	285	291	296	308	320	35 g
58H1-4/4	M	Back	20,0 mg/L de Air	260	265	270	281	292	32 g
58H1-4/5	M	Hand	20,0 mg/L de Air	265	270	276	287	298	33 g
58H1-4/6	**M**	**Control**	**N/A**	**295**	**308**	**320**	**327**	**334**	**39 g**

Note: Weight of each animal is in grams - -- All animals showed weight gain.

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

SAMPLE: **CHINCHEX BED BUGS INSECTICIDE**
STAR DATE: 2023-03-09
FINISH DATE: 2023-03-23

ANIMALS		OBSERVATION PERIOD– DOSE 0,5 mg/L													
		Day													
Box /Number	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
58H1-1/1	M	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-1/2	M	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-1/3	M	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-1/4	M	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-1/5	M	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-1/6	**M (Control)**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**ni**	**nb**	**nb**	**nb**	**nb**

M: Male **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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SAMPLE: **CHINCHEX BED BUGS INSECTICIDE**
STAR DATE: 2023-03-09
FINISH DATE: 2023-03-23

ANIMALS		OBSERVATION PERIOD – DOSE 2,0 mg/L													
		Day													
Number	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
58H1-2/1	M	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-2/2	M	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-2/3	M	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-2/4	M	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-2/5	M	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-2/6	**M (Control)**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**

M: Male **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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SAMPLE: **CHINCHEX BED BUGS INSECTICIDE**
STAR DATE: 2023-03-09
FINISH DATE: 2023-03-23

ANIMALS		OBSERVATION PERIOD – DOSE 10,0 mg/L													
		Day													
Number	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
58H1-3/1	M	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-3/2	M	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-3/3	M	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-3/4	M	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-3/5	M	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-3/6	**M (Control)**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**

M: Male **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

ACUTE INHALATION TOXICITY TEST
FOR THE PRODUCT CHINCHEX BED BUGS INSECTICIDE
OF THE ENTERPRISE CHINCHEX LIMITED

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

SAMPLE: **CHINCHEX BED BUGS INSECTICIDE**
STAR DATE: 2023-03-09
FINISH DATE: 2023-03-23

ANIMALS		OBSERVATION PERIOD – DOSE 20,0 mg/L													
		Day													
Number	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
58H1-4/1	M	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**
58H1-4/2	M	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-4/3	M	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-4/4	M	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-4/5	M	bc	bc	bc	bc	nb	nb	nb	nb	nb	nb	nb	nb	nb	nb
58H1-4/6	**M (Control)**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**	**nb**

M: Male **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
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ACUTE INHALATION TOXICITY TEST
FOR THE PRODUCT CHINCHEX BED BUGS INSECTICIDE
OF THE ENTERPRISE CHINCHEX LIMITED

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

SAMPLE: **CHINCHEX BED BUGS INSECTICIDE**
STAR DATE: 2023-03-09
FINISH DATE: 2023-03-23

ANIMALS			MACROSCOPIC OBSERVATION - DOSE: 0,5 mg/L						
			NECROPSY (BODY PART)						
Box / Number	Sex	Lungs	Trachea	Heart	Liver	Mouth	Nose	Stomach	Intestine
58H1-1/1	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-1/2	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-1/3	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-1/4	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-1/5	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-1/6	**M(Control)**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**

M: Male **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 CHINCHEX BED BUGS INSECTICIDE
OF THE ENTERPRISE CHINCHEX LIMITED

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

SAMPLE: **CHINCHEX BED BUGS INSECTICIDE**
STAR DATE: 2023-03-09
FINISH DATE: 2023-03-23

ANIMALS		MACROSCOPIC OBSERVATION - DOSE: 2,0 mg/L							
		NECROPSY (BODY PART)							
Box / Number	Sex	Lungs	Trachea	Heart	Skin	Mouth	Nose	Stomach	Intestine
58H1-2/1	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-2/2	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-2/3	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-2/4	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-2/5	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-2/6	**M(Control)**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**

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

Type of observations:

- Inflammation
- Necropsy
- Hemorrhages
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ACUTE INHALATION TOXICITY TEST
FOR THE PRODUCT CHINCHEX BED BUGS INSECTICIDE
OF THE ENTERPRISE CHINCHEX LIMITED

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

SAMPLE: **CHINCHEX BED BUGS INSECTICIDE**
STAR DATE: 2023-03-09
FINISH DATE: 2023-03-23

ANIMALS		MACROSCOPIC OBSERVATION - DOSE: 10,0 mg/L							
		NECROPSY (BODY PART)							
Box / Number	**Sex**	**Lungs**	**Trachea**	**Heart**	**Skin**	**Mouth**	**Nose**	**Stomach**	**Intestine**
58H1-3/1	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-3/2	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-3/3	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-3/4	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-3/5	M	nmc	nmc	nmc	nmc	nmc	nmc	nmc	nmc
58H1-3/6	**M(Control)**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**

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

Type of observations:

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

SAMPLE: **CHINCHEX BED BUGS INSECTICIDE**
STAR DATE: 2023-03-09
FINISH DATE: 2023-03-23

ANIMALS		MACROSCOPIC OBSERVATION - DOSE: 20,0 mg/L							
		NECROPSY (BODY PART)							
Box / Number	**Sex**	**Lungs**	**Trachea**	**Heart**	**Skin**	**Mouth**	**Nose**	**Stomach**	**Intestine**
58H1-4/1	M	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**	**ad**
58H1-4/2	M	mc	mc	nmc	mc	mc	mc	nmc	nmc
58H1-4/3	M	mc	mc	nmc	mc	mc	mc	nmc	nmc
58H1-4/4	M	mc	mc	nmc	mc	mc	mc	nmc	nmc
58H1-4/5	M	mc	mc	nmc	mc	mc	mc	nmc	nmc
58H1-4/6	**M(Control)**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**	**nmc**

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

Type of observations:

- Inflammation
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