UNIVERSIDAD DE GUAYAQUIL
DEPARTMENT OF CHEMICAL SCIENCES

Ciudadela Universitaria ¨Dr. Salvador Allende¨

Telephone: 2293680, E-mail: fcquimic@ug.edu.ec

Guayaquil, Ecuador

FINAL REPORT

TITLE:

Study of the acute oral toxicity of **Quina**, originating from NUTRAMEDIX Laboratories, LLC, Florida, USA

OBJECTIVES:

To study adverse side effects produced by the administration of **Quina** on body weight and different body systems.

BACKGROUND:

Quina will be used in humans and therefore the vital importance of carrying out these first-step tests. They will not only guarantee the quality of the product, but will also establish that there are no adverse side effects in humans who take the product.

As discussed in numerous international works, the study of acute toxicity is indispensable, and guarantees (within the margin of error associated with the technique) that the potential for toxicity from the compounds that will be ingested or that may enter into the system accidentally will be learned.

Describing oral acute toxicity in the international literature is a requirement that must be fulfilled for all products that are to be introduced in the market for the first time. (1, 2, 3, 4, 5)

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SCIENTIFIC, TECHNICAL AND SOCIOECONOMIC BENEFITS:
Demonstration of the innocuousness of this product is important in that the product could produce undesirable reactions in individuals who use it. Demonstrating that it does not produce toxic effects can lead to other tests that will allow it to be registered as a new medicine.

VARIABLES TO MEASURE:
- Toxic effects produced by oral application only of this product
- Weight of the animal's day 1, 7 and 14
- Mortality rates and time of death
- When clinical symptoms appear and disappear
- Anatomo-pathological exams (if required)

PROCEDURES TO FOLLOW:
Acute toxicity via oral introduction was determined using the procedures described in the OECD (Organization for Economic Cooperation and Development, comprised of the 24 most developed nations in the world) TG (Test Guidelines) 423.

CHANGES IN THE STUDY PLAN:
Changes did not take place in protocol proposed to the Unity of Quality Guarantee, whose number is referred to on Page 1**.**

SAMPLE DATA:
Product Name: **Quina**
Date of manufacture: 05/06/05
Date of packaging: 08/07/05
Solicited by: Nutramedix Laboratories, Florida, United States
Represented by: Ing. José Icaza

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Entity that carried out the work: University of Guayaquil, Department of Chemical Sciences
Address: Ciudadela Universitaria ¨Dr. Salvador Allende¨
Represented by: Dr. Walter Herrera Arguello
Form of product presentation: glass bottle containing 30 ml
Storage: The product was stored at room temperature, was protected from light and kept under lock and key

INFORMATION WITH RESPECT TO HANDLING:
No special handling instructions were needed

PRODUCT COMPOSITION:
Quina bark extract (*Cinchona calisaya*)
Mineral water
Ethanol (20 -25 %)

EXPERIMENTAL PROCEDURE:

INTRODUCTION:
This test was performed with the intention of determining the Acute Toxicity by oral intake of the product to be evaluated, given that this is one of the ways proposed for human intake.

DOSAGE USED IN THE TEST:
Data used indicates that:

Manufacturer's recommended dosage of 15 drops twice a day as the maximum daily dose. Taking into account that 15 drops are approximately 1 mL, each mouse was administered 20 mL/kg, or 120 times the recommended human dose, taking into account the average mouse weight of 25 grams.

Mortality rates and other clinical observations as are discussed in Table 1 were used as fundamental test parameters.

PRINCIPAL TEST PROCEDURE FOLLOWED:
Those that are described in the norms of the OECD. (5)

METHODS AND TECHNIQUES:

Study Material: **Quina**

Animal Model: The test was carried out with a species of rodent (mouse), with a minimum of 5 male animals with an average weight of 25 grams (7), belonging to the Swiss line and originating from the Chemistry Department of the University of Guayaquil. These mice were appropriate for carrying out the study of acute toxicity via oral intake.

The animals were maintained in climate-controlled and quarantine conditions according to established procedures (8, 9), during a period of at least 5 days.

Access to food and water was “ad libitum.¨(10, 11)

Animals were distributed randomly among the different groups. (12)

Food was denied to the animals 18 hours before exposure to the test substance.

The test lasted 19 days (5 days of acclimation, 14 test days)

METHOD DEVELOPMENT:
The evening before the experiment food was denied to the animals with the testing carried out after this fasting. After the fasting all animals were weighed to determine the appropriate dosage.

The substance administered was the study product, in a single dosage of 20 mL/kg of animal weight. The solution's volume remained constant given that there was no need for various administrations to complete the appropriate dosage per body weight (20 mL/kg). Two to three hours after the administration of the product the animals were allowed access to food again.

After the product's administration observations were conducted and systematically recorded for each individual animal, several times on the first day and at least once a day for the next 13 days.

Given that oral ingestion of the product could cause delayed toxic reactions, the animals were weighed on the first, seventh, and 14th days.

At the end of the experiment, the animals were euthanized in a saturated ether atmosphere. (13)

If any abnormality were detected during the examination of the organs (lungs, heart, kidneys and stomach or other organs that may have shown clinical symptoms during the clinical studies), samples were taken for pathological studies (14).

RESULTS CALCULATIONS:
The weights of the mice at different times were statistically processed to obtain the mean and the standard deviation (2).

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DESCRIPTION OF THE DOSAGE, ADMINISTRATION METHOD AND DURATION OF THE EXPERIMENT:

The experiment was conducted following the guidelines of OECD TG 423.

The method of administration was oral, using an intra-gastric canella.

The experiment lasted 19 days (5 of acclimation and 14 of testing).

It is important to realize that this experiment was carried out using a volume of 20 ml per kilogram of body weight. In comparison, a human of 60 kilogram of weight would be expected to ingest a maximum of 20 drops, or approximately 0.6 mL dissolved in 120 mL of water, this means the human will ingest 0.005 mL per 60 kg, therefore for each kilogram of weight he will be ingesting 0.0000833 mL and since the animal received 20 mL per kilogram, the animal therefore receives 240,963 times the expected human dosage, which indicates that the safety margin of the product is very high.

ANALYTICAL RESULTS:

Results of the daily observations during the 14-day experimental period are recorded in Tables #1 and #2.

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TABLE 1 - CLINICAL SYMPTOMS

PRODUCT: Quina **PRODUCT ORIGEN: NutraMedix, LLC, Florida, USA**
DOSAGE: 20 ml/kg **SEX: Male**
START DATE: 07/12/05 **END DATE: 07/26/05**

CLINICAL SYMPTOMS	DAYS													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
EYES	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MUCOUS MEMBRANES	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RESPIRATORY SYS.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CIRCULATORY SYS.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AUTONOMO	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CENTRAL NERV. SYS.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CHANGES IN HAIR	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TREMBLING	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CONVULSIONS	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SALIVATION	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SKIN	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SEDATION	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOMULENCE	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DEATH	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OTHER	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PLEASE NOTE: THE NUMBER OF ANIMALS WITH THE SYMPTOM IS NOTED ON THE CHART.

TECNICIAN RUNNING THE STUDY
RESPONSIBLE PROFESSIONAL:
Dr. Diadelis Remirez **Signature:**

Date of Dissemination: 07/26/05

As can be seen in Table # 1, there are no clinical symptoms in any of the study groups.

TABLE # 2. BODY WEIGHT (GRAMS) VARIATION AND STANDARD DEVIATION OF THE FEMALE ANIMALS IN THE EXPERIMENT OF ACUTE TOXICITY VIA ORAL INGESTION OF AMANTILLLA RELAX.			
GROUP	**TIME (Days)**		
	0	**7**	**14**
MALES	25±6	30.6 ± 8	34.0 ± 10.0

Table # 2 demonstrates the results obtained for body weight (in measured values and standard deviation) for days 1, 7, and 14 of the experiment.

BODY WEIGHT:
As can be seen from Table # 2, the animals treated with Quina showed weight gain between the weighing sessions.

HISTO-PATHOLOGY:
Samples taken from selected organs showed no affects from under the microscope, thus the pathologist did not take histo-pathological samples.

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CONCLUSIONS:

1- Autopsies revealed no affects to selected organs.

2- The product did not affect weight gain of the animals in the study.

3- No toxic effects are produced when administering Quina in an acute form to the animals.

4- Quina is a compound whose potential for toxicity is very low based on the fact that its security margin is very high. In addition, it was administered undiluted, in contrast to suggested human use, in which it is diluted in 120 ml of drinking water.

GENERAL CONCLUSIONS:

Quina did not produce toxic effects when used in accordance to the guidelines described in OECD TG 423, using a volume of 20 mL/kg. This study demonstrates the innocuousness of the mentioned product.

PERSONNEL RESPONSIBLE FOR THE STUDY:

DIRECTOR OF THE STUDY: **SIGNATURE:**
DR. WALTER HERRERA

Responsible Professional: **Signature:**
Dr. Diadelis Ramirez Figueredo

Date: 07/26/05

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