



UNIVERSIDAD DE GUAYAQUIL
FACULTAD DE CIENCIAS QUIMICAS.

Ciudadela Universitaria "Dr. Salvador Allende"
Telephone 2293680, Email fcquimic@ug.edu.ec
Guayaquil, Ecuador
FINAL REPORT

CODE: BIO-IP-013-01

TITLE:

Study of the acute oral toxicity of ENULA, originating from NUTRAMEDIX Laboratories, LLC, Florida, USA.

OBJECTIVES:

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

BACKGROUND:

ENULA will be used in humans because of 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).



UNIVERSIDAD DE GUAYAQUIL

FACULTAD DE CIENCIAS QUIMICAS.

Ciudadela Universitaria "Dr. Salvador Allende"

Telephone 2293680, Email fcquimic@ug.edu.ec

Guayaquil, Ecuador

FINAL REPORT

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 animals day 1, 7 and 14.
- Mortality rates and time of death.
- When clinical symptoms appear and disappear.
- Anatomico-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: ENULA.

Represented by: Ing. José Icaza.

Entity that carried out the work: University of Guayaquil, Department of Chemical Sciences.



UNIVERSIDAD DE GUAYAQUIL

FACULTAD DE CIENCIAS QUIMICAS.

Ciudadela Universitaria "Dr. Salvador Allende"
Telephone 2293680, Email fcquimic@ug.edu.ec
Guayaquil, Ecuador

FINAL REPORT

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:

Elecampane roots extract
Jalapa roots extract
Blood Wism stems extract
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:

Suggested use is 30 drops dissolved in a 120 mL glass of water twice a day. With this data in mind, each mouse with body weight of 200 g received 42.5 drops of undissolved product which is the same as 1.1 mL / 200g of body weight and without dissolving ENULA in water. Dosage used for the purpose of this study is 100 times more than the therapeutic suggested dosage of this product.



UNIVERSIDAD DE GUAYAQUIL
FACULTAD DE CIENCIAS QUIMICAS.

Ciudadela Universitaria "Dr. Salvador Allende"
Telephone 2293680, Email fcquimic@ug.edu.ec
Guayaquil, Ecuador

FINAL REPORT

Mortality rates and other clinical observations are disclosed in Table 1 and data is 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: ENULA.

Animal Model: The test was carried out with a species of rodent (mouse), with a minimum of 6 animals per test of the same sex. That is, 6 females were used in accordance with recommendations with a mean weight of $238 \text{ g} \pm 20\%$ (7), and belonging to the Wistar 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 the mice 18 hours before exposure to the test substance.

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



UNIVERSIDAD DE GUAYAQUIL

FACULTAD DE CIENCIAS QUIMICAS.

Ciudadela Universitaria "Dr. Salvador Allende"
Telephone 2293680, Email fcquimic@ug.edu.ec
Guayaquil, Ecuador
FINAL REPORT

METHOD DEVELOPMENT.

Two experimental groups were created. Only females were used, as is in accordance with OECD guidelines 423 of 2001, asserting that the female is more sensitive than the male, making it a more appropriate choice for acute toxicity studies.

The evening before the experiment food was denied 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 1.1 mL / 200 g of body weight. 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).



UNIVERSIDAD DE GUAYAQUIL

FACULTAD DE CIENCIAS QUIMICAS.

Ciudadela Universitaria "Dr. Salvador Allende"

Telephone 2293680, Email fcquimic@ug.edu.ec

Guayaquil, Ecuador

FINAL REPORT

FINAL REPORT RESULTS CALCULATIONS:

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

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 cannula.

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

It is important to realize that this experiment was carried out using a volume of 1.1 mL per 200 g of body weight. In comparison, a human would be expected to ingest 15 drops twice a day (approximately 0.75 mL dissolved in 120 mL of water per day). The mouse therefore receives 500 times more dosage than the expected human dosage per day.

ANALYTICAL RESULTS.

Results of the daily observations during the 14-day experimental period are recorded in Table 1.



UNIVERSIDAD DE GUAYAQUIL
DEPARTMENT OF CHEMICAL SCIENCES

TABLE 1. CLINICAL SYMPTOMS

PRODUCT: ENULA

PRODUCT ORIGEN: NutraMedix, LLC, Florida, USA.

DOSAGE: 1.1 mL/ 200 g

SEX: Female

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

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

PROFESSIONAL RESPONSIBLE:

Q F. Glenda Sarmiento

SIGNATURE:



UNIVERSIDAD DE GUAYAQUIL
FACULTAD DE CIENCIAS QUIMICAS.

Ciudadela Universitaria "Dr. Salvador Allende"
Telephone 2293680, Email fcquimic@ug.edu.ec
Guayaquil, Ecuador

FINAL REPORT

As recorded on table 1, no clinical symptoms were noted in the study group.

Table 2 demonstrates the body weights of the animals on day 1, 7, and 14 of the experiment.

TABLE # 2. BODY WEIGHT (GRAMS) VARIATION AND STANDARD DEVIATION OF THE FEMALE ANIMALS IN THE EXPERIMENT OF ACUTE TOXICITY VIA ORAL INGESTION OF ENULA.			
GROUP	TIME (Days)		
	0	7	14
FEMALES I	181.87 ± 9.41	204.23 ± 2.39	208.65 ± 1.62
FEMALES II	189.93 ± 4.07	213.28 ± 5.53	217.90 ± 3.75

As can be seen from Table 2, the females treated with ENULA showed weight gain between the weighing sessions.

The following bar graphs were created with the values from Table 2.

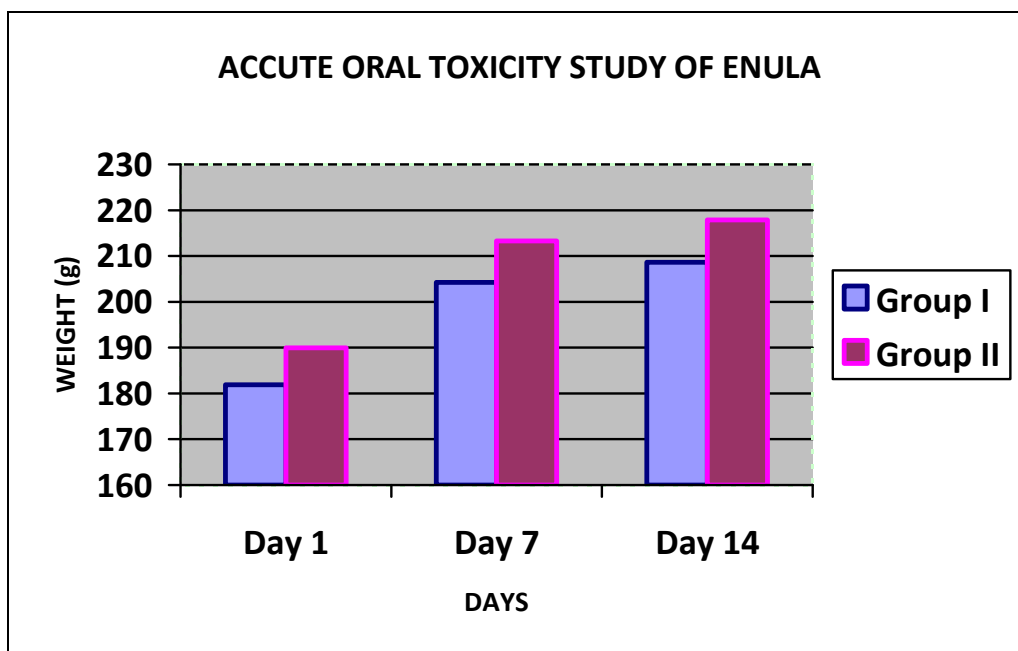


UNIVERSIDAD DE GUAYAQUIL
FACULTAD DE CIENCIAS QUIMICAS.

Ciudadela Universitaria "Dr. Salvador Allende"
Telephone 2293680, Email fcquimic@ug.edu.ec

Guayaquil, Ecuador

FINAL REPORT



HISTO-PATHOLOGY

Samples taken from selected organs showed no affects from the macroscopic point of view, thus the pathologist did not take histo-pathological samples.

CONCLUSIONS.

- 1- Clinical symptoms were not observed in the animals.
- 2- Autopsies revealed no affects to selected organs.



UNIVERSIDAD DE GUAYAQUIL
FACULTAD DE CIENCIAS QUIMICAS.

Ciudadela Universitaria "Dr. Salvador Allende"
Telephone 2293680, Email fcquimic@ug.edu.ec
Guayaquil, Ecuador

FINAL REPORT

- 3- The product did not affect weight gain of the animals in the study.
- 4- No toxic effects are produced when administering ENULA in an acute form to the animals.

GENERAL CONCLUSIONS

ENULA did not produce toxic effects when used in accordance to the guidelines described in OECD TG 423, thus the product is considered practically innocuous for humans when administered in the acute form. Therefore studies of acute toxicity at higher doses in humans are not necessary.

PERSONEL RESPONSABLE FOR THE STUDY:

DIRECTOR OF THE STUDY:

NELSON MONTOYA, M. SC:

SIGNATURE:

Analyst:

Q. F. Glenda Sarmiento

SIGNATURE:

DEAN OF THE C.C.Q.Q. FACULTY

Dr. Carlos Silva Huilcapi, M. Sc

SIGNATURE:



UNIVERSIDAD DE GUAYAQUIL
FACULTAD DE CIENCIAS QUIMICAS.

Ciudadela Universitaria "Dr. Salvador Allende"
Telephone 2293680, Email fcquimic@ug.edu.ec

Guayaquil, Ecuador

FINAL REPORT

BIBLIOGRAPHY.

1. Hukeri VI, Kalyani GA, Kakarni (1988). Hypoglycemic activity of flavonoids of *Phyllanthus fraternus* in rat *Fitoterapia* 68-70.
2. Copra RN., Nayar SI, Copra LC (1956). Glossary of Indian medicinal plants. Editorial CSIR, Nueva Delhi pp 191.
3. Roig JT (1974) Plantas medicinales aromáticas o venenosas de Cuba. Ciencia y Técnica, la Habana, pp 404, 569, 808.
4. Hayes W., Principles and Methods of Toxicology. Principles and Methods for Acute Toxicity and Eye Irritancy Ed. Raven Press, Ltd. N.Y., 169-220, 1989
- 4A. Ibid, Statistic for toxicologist. 435-483, 1984
5. Organización para la Cooperación Económica y el Desarrollo. (OECD) Guidelines for testing of chemical. Paris. 423, 2001.
6. García G. Los estudios toxicológicos de primera barrera y la toxicología Alternativa. Tesis para optar por el grado académico de Maestro en Ciencias. La Habana, junio del 2000.
7. Procedure. Peso Corporal de las ratas.
8. Procedure. Guía para el cuidado de los animales de Laboratorio.
9. Procedure. Cuarentena .
10. Procedure. Suministro de Agua manual de rutina.
11. Procedure. Asignación aleatoria de las especies roedoras.
12. Procedure. Eutanasia.
13. Procedure. Técnicas de Anatomía Patológica.