UNIVERSIDAD DE ANTIOQUIA
1803

Programa Ofidismo Escorpionismo

Medellin

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POE-046-12

Mr. Sergio Urrego
Executive Director
PROGAL BT
Medellín

Mr. Urrego:

As the Coordinator of the Ophidism / Scorpionism Program, I should inform you that during 2010 and 2011 several assessments on Acute Toxicity, Sub-Chronic Toxicity, and Cytotoxicity were made to a sample (beige powder, Lot No. 100909) provided by you. Results obtained were as follows:

Acute Toxicity (assessment conducted in compliance with Standard 423 of the Organization for Economic Co-Operation and Development - OECD):

• The product provided by you did not cause death to any experimentation animal (mice) administered with 5, 50, 300, and 2000 mg/kg doses during 14 days.
• No signs of toxicity were observed in the following organic systems analyzed:

– Central Nervous System.
– Muscular System.
– Integumentary System (skin) and mucosae.

Conclusion: No adverse effects were observed after oral administration of the sample in experimentation animals.

Sub-Chronic Toxicity (assessment conducted in compliance with Standard 408 of the Organization for Economic Co-Operation and Development – OECD):

- Administration of the sample at repeated doses during 90 days did not cause directly the death of three of the experimentation animals (in both control group and tested groups). This fact was verified in a partially repeated assay where no mice died despite their exposure to the same experimental environmental conditions and doses.
- Some animals from both control group and tested groups (with no incidence on the dose) exhibited some effects such as brain hypereosinophlia, liver vacuolar changes, lung interstitial pneumonia, and kidney glomerulonephritis. These effect were caused by factors associated to food provided and/or environmental conditions of the vivarium and/or associated genetic factors.
- Effects observed after using the highest doses of the tested substance show a potential protective effect of the substance on organs such as lungs and kidneys. This fact should be verified in future trials intended to verify the protective effect initially observed in this trial.

Conclusion:

Conclusion: No meaningful adverse effects were observed after repeated oral administration of the sample in experimentation animals.

Reference: Organization for Economic Co-Operation and Development - OCDE (Rome, 1995). Report of the Consultation Meeting on Sub-chronic and Chronic Toxicity/Carcinogenicity Testing. OECD Guideline for Testing of Chemicals. [Internet Site]: http://www.oecd.org. Date consulted: May 2010.

Cytotoxicity:

Normal Human Cells:
Assessments conducted with mononuclear cells of human peripheral blood using the Mosmann methodology in 1983, and amended in 1976 by *Francois Denizot* y *Rita Lang,* based on the metabolic decrease of Bromide 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium (MTT) through the mitochondrial enzyme succinate dehydrogenase in a compound dyed with blue color (formazan), allow concluding that no toxic effects on cells occur at a 4 mg/ml concentration during 48 hours.

Reference: Mosmann T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assay. J. Inmunol. Methods 1983; 65: 55–63.

Denizot F., Lang R. Rapid Colorimetric Assay for Cell Growth and Survival, Modifications to the Tetrazolium Dye Procedure Giving Improved Sensitivity and Reliability. J. Immunol. Methods 1986; 89: 271–277.

Murine Cells:

Assessments conducted in C_2C_{12} cells, following the method described by Lomonte et al. 1999, showed that no toxicity occurs in these cells at any dose evaluated.

Reference: Lomonte, B., Angulo, Y., Rufini, S., Cho, W., Giglio, J.R., Ohno, M., Daniele, J.J., Geoghegan, P., Gutiérrez, J.M., 1999. Comparative Study of the Cytolytic Activity of Myotoxic Phospholipases A2 on Mouse Endothelial (tEnd) and Skeletal Muscle (C2C12). Cells *in vitro*. Toxicon 37, 145-158.

Sincerely yours,

Juan Carlos Alarcón P.
Coordinator
Ophidism and Scorpionism Program
serpentario@udea.edu.co
Phone: 219 65 35 - 263 19 14