ZYCHROME® SAFETY OVERVIEW

Zychrome® is a unique, patent-pending chromium (Cr) complex consisting of chromium, niacin and L-cysteine (chromium dinicocysteinate)*In vitro* and *in vivo* preclinical research demonstrate that Zychrome is more efficacious than other forms of chromium in decreasing fasting glucose levels, glycated hemoglobin levels (HbA1c), insulin levels and vascular inflammation as assessed by CRP, MCP-1, ICAM-1 and oxidative stress levels.[1] Zychrome exerts these effects through the regulation of cell signal transduction pathways associated with insulin function, glucose control and vascular inflammation.[1] A randomized, double-blind, placebo-controlled clinical study demonstrated that Zychrome significantly modulated insulin levels and insulin resistance as well as the inflammatory cytokine, TNF-□ and the oxidative stress marker, protein carbonyl.[2] An array of toxicological studies on Zychrome demonstrate a wide margin of safety suggesting that it is safe for human consumption.[2,3]

Summary of Zychrome® Safety Profile

Clinical and Animal Tolerance[2,3]

- Well tolerated by humans and animals with no reported adverse effects
- Does not affect liver, kidney, or heart function
- No allergic reactions

Toxicological Studies Conducted in GLP-Approved Labs[2,3]

- 90-day Sub-Chronic Toxicity: Non-toxic up to 1,000-fold the human equivalency dose (> 5.7 mg/kg)
- Acute Oral Toxicity: LD_{50} > 2,000 mg/kg
- Acute Dermal Toxicity: LD_{50} > 2,000 mg/kg
- Primary Skin Irritation: Slightly Irritating
- Primary Eye Irritation: Severely Irritating
- Ames' Bacterial Reverse Mutation Assay: Non-mutagenic
- Mammalian Erythrocyte Micronucleus Test: Non-mutagenic

GRAS[4]

Human Studies

- In a randomized, double-blind, placebo-controlled clinical study Zychrome supplementation (400 mcg Cr daily) for 90 days was shown to be safe and well tolerated with no significant adverse events reported in study participants. Zychrome did not adversely affect liver, kidney, or heart function as demonstrated by normal serum levels of ALT, BUN and CK.[2]

Animal Studies

Subchronic Toxicity Study

- *Repeated-dose 90-Day Oral Toxicity Study* - Blood, clinical chemistry and microscopic tissue evaluations did not show any adverse effects in organs after a 90-day sub-chronic toxicity study of Zychrome administered in increasing doses in male and female Sprague-Dawley (SD) rats. No changes were observed in liver or kidney function as demonstrated by ALT and BUN levels after 90 days of supplementation with 0.23, 2.3 or 5.7 mg/kg of Zychrome a day. The no-observed-adverse-effect-level (NOAEL) for Zychrome was determined to be > 5.7 mg/kg/day.[5]

Toxicity Studies

- *Acute Oral Toxicity* - Administration of Zychrome at single dose of 2,000 mg/kg in female SD rats did not reveal any significant changes in all tissues examined. Based on these results, the oral LD_{50} of Zychrome was determined to be > 2,000 mg/kg.[3]
- *Acute Dermal Toxicity* – Administration of a single 2,000 mg/kg dose of Zychrome applied directly to the skin of male and female rats for 24 hours revealed no dermal irritation, adverse effects, or abnormal behavior. Based on these results, the acute dermal LD_{50} of Zychrome was > 2,000 mg/kg.[3]
- *Primary Dermal Irritation* – A single 560 mg dose of Zychrome applied directly to the skin of rabbits caused a slight erythema one hour after topical application. The overall incidence and severity of irritation decreased with time. All animals were free from irritation by 72 hours. Thus, Zychrome was classified as slightly irritating to the skin.[3]
- *Primary Eye Irritation* – Ocular irritation was tested in rabbits using a single ocular instillation of 70 mg of Zychrome. Within 48 hours of instillation, all treated eyes exhibited corneal opacity, iritis and positive conjunctivitis. The overall incidence and severity of irritation decreased gradually thereafter. Apart from the eye irritation noted, all animals appeared active and healthy. There were no other signs of gross toxicity, adverse effects, or abnormal behavior. Under the conditions of this study, Zychrome was classified as severely irritating to the eye.[3]

Genotoxicity Study

- *Ames' Bacterial Reverse Mutation Assay* – Four strains of *Salmonella typhimurium* and one strain of *Escherichia coli* were used to evaluate the mutagenic potential of Zychrome in the presence and absence of metabolic activation. Zychrome was determined to be non-mutagenic.[3]
- *Mammalian Erythrocyte Micronucleus Test* – The mutagenic potential of Zychrome to induce micronuclei (clastogenicity or aneugenicity) was assessed in polychromatic erythrocytes in murine bone marrow. Zychrome did not induce structural or numerical chromosomal damage in the immature erythrocytes of the mouse.[3]

GRAS

- Determined GRAS by the Burdock Group, an independent leading toxicology group.[4]

Conclusions

- No adverse effects were observed in 90-day repeated oral dose toxicity study or in the clinical study done in 83 type 2 diabetic subjects.
- GRAS determined.
- Overall, the above studies demonstrate the broad spectrum safety of Zychrome suggesting it to be safe for human consumption.

1. Jain SK, Croad JL, Velusamy T, Rains JL, Bull R. Chromium dinicocysteinate supplementation can lower blood glucose, CRP, MCP-1, ICAM-1, creatinine, apparently mediated by elevated blood vitamin C and adiponectin and inhibition of NF-κB, Akt, and Glut -2 in livers of zucker diabetic fatty rats. *Mol Nutr Food Res*. 2010;54:1-10.
2. Jain SK, Kanlon G, Moorehead L, et al. The effect of chromium dinicocysteinate supplementation on circulating levels of insulin TNF-α, oxidative stress and insulin resistance in type 2 diabetic patients: Randomized, double-blind, placebo-controlled clinical study. *Mol Nutr Res*. 2012;56:1333-1341.
3. Sreejayan N, Marone PA, Lau FC, Yasmin T, Bagchi M, Bagchi D. Safety and toxicological evaluation of a novel chromium(III) dinicocysteinate complex. *Toxicol Mech Meth*. 2010;20:321-333.
4. Burdock Group. Dossier in Support of the Generally Recognized as Safe (GRAS) Status of Chromium(III) Dinicocysteinate Complex (Zychrome®) As A Food Ingredient. Internal data, 2012.