## Composition of Cernevit™-12 IV Multivitamins

| COMPONENT | QUANTITY per 5 ml (vial contents) | NUTRITIONAL REQUIREMENTS | TOXIC LEVEL |
|---|---|---|---|
| ACTIVE COMPONENTS | | | |
| Retinol palmitate (Vitamin A) | 3500 U | 700-1000 retinol equivalents* | Acute Tox detected at 1-30X$10^6$ in adults Chronic tox at 50-1000x $10^3$ IU |
| Cholecalciferol (Vitamin $D_3$) | 200 U | 400 IU* (considered arbitrary, 100 IU is sufficient) | MLD Est. 50,000 IU for adult humans (1000-2000 IU for infants or adults with certain infections and metabolic diseases. |
| DL-α-Tocopherol (Vitamin E) | 10.2mg<br>11.2 IU | 8-10 mg/dl<br>12-15 IU*<br>(may be lower for some disease states) | $LD_{50}$ rats>2000 mg/kg<br>In humans, doses of 3200 IU/day are relatively well tolerated. |
| Ascorbic acid (Vitamin C) | 125 mg | 60 mg* (increase to 80 and 100 during pregnancy and lactation | Relatively non-toxic: 10 g/day does not constitute a serious health risk. |
| Nicotinamide (Vitamin $B_3$) | 46 mg | 13-20* mg (niacin equiv.) | No data |
| Dexpanthenol (corresponding to pantothenic acid) | 16.15 mg<br>(17.25 mg) | 4-7 mg/day* | No data |
| Pyridoxine HCl (corresponding to pyridoxine) (Vitamin $B_6$) | 5.5 mg<br>4.53 mg | 1.4-2.0* mg | No data |
| Riboflavin sodium phosphate (corresponding to riboflavin/Vitamin $B_2$) | 5.67<br>4.14 | 1.3-1.8*mg/day for adult females and males | Oral doses of 10g/kg in rat and 2 g/kg produced no toxic effects. $LD_{50}$ IP=560 mg/kg in rats. |
| Cocarboxylase tetrahydrate corresponding to thiamine (Vitamin $B_1$) | 5.8 mg<br>3.51 mg | 1-1.5* mg/day for adult females and males | No data |
| Folic acid | 414 | 200 mcg/day* | 10 mg/day for 5 years was not toxic to humans |
| D-Biotin | 60 mcg | 100-200mcg/day** | IV LD50 in rabbits = 0.41 mcmol/day in 30 days<br>In dogs = 6.15 mcmol/day in 10 days |
| Cyanocobalamin (Vitamin $B_{12}$) | 5.5 mcg | 2 mcg/day** | GRAS |
| OTHER INGREDIENTS | | | |
| Glycine | 250 mg | | |
| Glycocholic acid | 140 mg | | |
| Soybean lecithin | 112.5 mg | | |
| NaOH and/or HCl | To adjust pH to 5.9 | | |

*Recommended Daily Allowance set by the Nutritional Research Council-US (range given for males and females aged 10-70 + years) data taken from Handbook of Vitamins, 2nd edition. Ed. L. J. Machlin 1991.

** Recommended by Food and Nutrition Board of the U.S. National Academy of Sciences

The proposed formulation of vitamin contents reflects, with the exception of Biotin, low multiples of the RDA. Vitamin E is close to the range specified by the RDA. Literature indicates that the toxic levels of vitamins listed above are considerably higher than the RDA recommendations, so it is not expected that the vitamin content would approach the toxic range for these compounds. The key toxicology issue for this NDA is the safety of the micelle component of the formulation. This has been addressed by the following studies provided by the sponsor.

# RESEARCH REPORT NO. B-119'757: MIXED MICELLES MONOGRAPH, [redacted] 17-7465

Monograph dated 12/12/85

SUMMARY:

The glycocholic acid-lecithin mixed micelles colloid is based on a naturally occurring system. The mixed micelles formed by this system are thermodynamically stable and non-cytolytic. This specific mixed micelle system [redacted] 17-7465) appears to be limited by the chemical stability of lecithin which and be partly hydrolyzed into lysolecithin and fatty acid with prolonged storage at high temperatures. It is the lysolecithin that appears to contribute to the toxicity of this mixture and has been studied as part of this monograph. The lysolecithin is an endogenous substance, which is usually removed by one of several enzymatic reactions to prevent accumulation to toxic levels.

**Human data of glycocholic acid indicate that the amounts of glycocholic acid should not be toxic when administered as a bolus injection to patients with normally functioning livers. However, the author suggests that if administered to patients with severe hepatopathy or to neonates, this should be considered a potential hazard until a systematic investigation in humans proves otherwise.**

LD50 of glycocholic acid in rats, mice and rabbits with fresh [redacted] 17-7465 resulted in similar values (3.5-7.1 ml/kg, which corresponds to 300-600 mg/kg glycocholic acid). The contents of a single vial include 140 mg of glycocholic acid. This is ~ 2.8 mg/kg (~ 100 mg/m$^2$) for human exposure. Based on surface area comparisons, the LD50's are <u>at least</u> 9 times the proposed daily human exposure (using 300 mg/kg and converting to surface area with the lowest species multiplier: mice = 900 mg/m$^2$). For partly degraded Ro17-7465, the NOEL in rats and dogs was 0.75 ml/kg and 20 mg/kg lysolecithin. This represents a multiple of approximately 4 times (mg/m$^2$ basis) the human exposure if a similar mixture would be administered to humans. There were no reproductive or teratogenic toxicities detected with the partially degraded product. The Ames assay was negative. No allergenic potential was detected in the guinea pig sensitization assay. There was no local irritation detected and likelihood for hemolysis appears to be low.

There was no change noted in behavior of dogs at i.v. doses of 0.1-1 ml/kg [redacted] 17-7465 and there was no change in chemically or electrically induced convulsions in mice with i.v. dose up to 3 ml/kg. Doses up to 2.2 ml/kg did not affect the blood pressure or heart rate of conscious spontaneous hypertensive rats although 0.66 ml/kg did produce a moderate short duration increase in heart rate of conscious normotensive rabbits. Normotensive dogs had a slight decrease in blood pressure and heart rate increase after 0.3 ml/kg. Partly degraded mixed micelles were several times more potent that undegraded formulation at decreasing blood pressure. It was estimated that approximately 15% of the hypotensive effect of degraded Ro17-7465 could be due to stimulation of muscarinic receptors, but histamine receptor activity did not appear to be involved. 1.0 ml/kg increased diuresis in rats and dogs.

Pharmacokinetics:
In dogs, an IV administration as mixed micelles or aqueous formulation resulted in similar plasma concentration-time curves. The sponsor suggests that this provided evidence that the mixed micelles breakdown into the component parts early in the distribution phase. In vitro studies designed to examine the interaction of the mixed micelles with protein binding of various drugs demonstrated that there was no displacement from the serum albumin binding of the two test compounds (diazepam and warfarin).

In healthy volunteers administered 50 ml of solubilizer for 6 days (approximately 10 times the proposed human dose), the tolerance of mixed micelles in animals and humans was similar.

Considerations for development of a mixed micellar delivery system include:
- Toxicity
- Stability
- Hemolytic/cytolytyic effects
- Anaphylactic reactions
- Local tolerance
- Size of micelle
- Viscosity (upper limit of concentration can be determined by the ability to withdraw into syringe.

Structural characteristics of bile salt-lecithin micelles:
- May vary in structure and solubilizing capabilities depending upon relative concentration of components
- Dilution of mixed micelles may be associated with conversion into liposomes with various diameters depending upon composition. This still allows for solubilization of the apolar agents; dilution of simple micelles may result in precipitation of the agent.
- In a thermodynamically stable system, shelf life is limited by the chemical stability of the components. In this system, lecithin may be hydrolyzed into lysolecithin and fatty acids.

Biological characteristics of bile salt-lecithin micelles:
- Cytolytic properties: *In vitro* hemolysis tests indicated that continued addition of lecithin to a fixed sodium glycocholate solution reduced the hemolytic activity from 100% to 0. In stored samples, the amount of protective lecithin decreases and the resultant lysolecithin contributes to an increased hemolytic activity of the mixed micelle preparation.
- Lecithin saturated mixed micelles were demonstrated to have good local tolerance when administered IV to the ear veins of rabbits and when administered IM to rats.
- The sponsor proposes that if liposomes were formed upon administration of the mixed micelle preparation, they would only be transiently present (no evidence was presented for this). It was demonstrated that lipid-soluble diazepam was rapidly freed from its association with mixed micelles as measured by timing of pharmacological activity which was equivalent to the administration of diazepam in an organic solvent:aqueous solution. When administered in dogs, PK studies indicate that labeled glycocholic acid was rapidly separated from the mixed micelle into its components after injection.
- Guinea pig sensitization tests were negative by ID or IM routes with the mixed micelle formulation, but was positive for the polyethoxylated castor oil positive control.

Review of main components of mixed micelles:
The sponsor presumes that the mixed micelles rapidly dissociate into the individual components. Thus the biological significance (toxicity) was pursued with the individual components.

## Glycocholic acid

Total pool size of endogenous cholic and chenodeoxycholic acid synthesized by the liver is approximately 500-800 mg of primary bile acids/day. The total pool size of cholic acid is approximately 0.9-1.5 g/day and chenodeoxycholic acid is 0.6-1.4 g/day with smaller amounts of deoxycholic and lithocholic acids. The pool undergoes enterohepatic circulation 6-10 times/day. The liver extracts the bile acids from the portal and general blood circulation and secretes them into the bile (along with newly synthesized bile acids). These are ultimately stored in the gall bladder until released into the small intestine. 96% of the bile acids are absorbed into the terminal small intestine and returned to the liver via the portal venous system. Daily loss of bile acids through the feces is approximately 0.2-0.8 g. Fewer than 5% of the bile salts are excreted in the urine, mostly as sulfate esters. First pass extraction of cholyglycine is approximately 85% with a plasma disappearance half-life of 2.6 minutes indicating that the healthy liver has a considerable capacity to extract bile acids. The majority of the bile acid molecules in the body occur in micellar solution. The bile constituents (lecithin and cholesterol) are incorporated into the bile acid micelles to form mixed micelles.

In infants, the enterohepatic bile circulation is immature and the daily bile salt synthesis and total bile acid pool size are very small. Also, in patients with cholestasis and acute hepatitis, serum bile acid levels are elevated up to 60 and 100 times normal, respectively. In liver cirrhosis, systemic and portal bile acid concentrations are substantially elevated. Thus, the safety of the mixed micellar preparation remains to be demonstrated in these populations.

In mice, LD 50 for glycocholic acid was 320 mg/kg by the IV route. Oral LD50 for small rodents is over 2000 mg/kg. In human volunteers, a single IV injection of up to 1 g of sodium cholate produced no systemic or local toxic effects, but the sponsor states that "care must be taken to avoid hemolysis". It is not clear how this was demonstrated or guarded against.

## Lecithin

Lecithin is the most widely distributed phospholipid and is found in mammalian species in all cellular membranes, in plasma lipoproteins and bile. Several grams of lecithin are ingested in the normal human diet daily. Liver and intestine are the most active sites of synthesis. The catabolism of lecithin is regulated by hydrolytic enzymes with the product being lysolecithin. In the blood, lecithin is transported in the form of plasma lipoproetins. The biological half life of lecithin ranges from several hours in some tissues to almost 2 months in the myelin fraction of the brain. Lecithins have been used as oral therapeutic agents at doses up to 80 g. The sponsor did not provide information on the levels given in IV use of lecithins.

Lysolecithin

Lysolecithin is formed when fatty acid is split from the parent lecithin. This is found in several tissues (plasma, bile and intestinal contents) at lower levels than lecithin. Nevertheless, up to 3 g/day of lysolecithin may be formed by the human body. It is continuously produced by the activity of enzymes, which are involved in the remodeling of cellular membranes and the catalysis of the transfer of fatty acid from lecithin to cholesterol. Enzymatic reactions prevent accumulation of lysolecithin to toxic levels. The most important toxic effect of lysolecithin is cytolysis, which is important in regards to this product as it raises the possibility of hemolytic activity.

## SPONSOR SUMMARY SAFETY EVALUATION

Sponsor indicates that the proposed formulation containing glycocholic acid should be safe based upon the following considerations:

1. Good tolerance of up to 1 g sodium cholate administered as an IV bolus in humans.
2. Size of bile acid pool in adults.
3. Capacity of the normal liver to extract bile acids from the blood and secrete them into the bile with a very short plasma half-life.
4. Repeated daily infusions of >4g/day have been well tolerated by normal volunteers

Sponsor indicates that the proposed formulation containing lecithin should be safe based upon the following considerations:

1. Normally functioning mammals can handle endogenous and exogenous lysolecithin and prevent its accumulation. This, however, does not take into account what might happen to the handling of lysolecithin in a patient population where some of these mechanisms might be impaired.
2. Toxicity studies in animals with lysolecithin administered up to 20 mg/kg exogenous lysolecithin was considered to be well-tolerated and produced no hemolytic responses.
3. Calculations by the sponsor suggest that a daily dose of 25 ml of decomposed mixed micelles, containing approximately 1 g of lysolecithin should not be hemolytic.

## PRECLINICAL DATA

Preclinical studies utilized both fresh, undecomposed mixed micelles and artificially aged and decomposed mixed micelles as well as mixed micelles stored 4 months at ambient temperature. The composition of test articles/ml was as follows:

[redacted] 17-7465.001:

88.5 mg glycocholic acid
19.0 µl NaOH 40%
169.3 mg Phospholipon 100 (lecithin)
15 µl Benzyl alcohol
1.0mg Sodium metabisulfite pure
q.s. 1 N HCl to pH 6.0
Water for injection to 1 ml