Blood Chemistry findings with [redacted] 17-7465/001 and /002 administered to rats for 4 weeks. Data are from 4 week time point and indicate change from control group ➡ HD

| Parameter ➡<br>Animal group ↓ | GOT<br>U/L | GPT<br>U/L | ALK PHOS<br>U/l | Bilirubin<br>mg/ml |
|---|---|---|---|---|
| Male /001 | 32➡225* | 14➡392* | 283➡351 | 0.05➡0.12 |
| Male/002 | 32➡74 | 14➡44 | 283➡273 | 0.05➡0.19 |
| Female/001 | 35➡82 | 14➡66 | 152➡210 | 0.05➡1.10 |
| Female/002 | 35➡71 | 14➡62 | 152➡166 | 0.05➡0.22 |

*Elevation in these groups due primarily to one very high number. For GOT, the value was 1100, but most other values were in the 50's. For GPT, one value was 1500, but again, most of the rest of the values were in the 30-70 range.

URINALYSIS: No treatment-related findings.

ORGAN WEIGHTS: Spleens of HD [redacted] 17-7465/002 weighed approximately twice as much as those of other groups (absolute and relative weights in males and females). This returned to nearly normal after a 2 week period off drug.

GROSS PATHOLOGY: No apparent treatment-related effects noted.

HISTOPATHOLOGY: Slight increased cellularity of the red pulp (extramedullary hematopoiesis) and a moderately increased hemosiderin content observed in spleens of the HD rats [redacted] 17-7465/001: mild [redacted] 17-7465/002: moderate to marked). All findings were somewhat (but not completely) reversible after 2 weeks off of drug.
Liver had mild to moderate hemosiderin contents of HD [redacted] 17-7465/002 group, but no indications of hepatic cell damage.
In HD males, there was a slight increase in basophilia of the tubular epithelium of the kidney and slight increase in fat content of the kidneys
Kidney (males only) some basophilia and atrophy of tubular epithelium and dilation of tubuli with formation of hyaline cases in the renal cortex ([redacted] 17-7465/001: mild, [redacted] 17-7465/002: mild to moderate)
Inflammation around tail vein injection site were slightly more prevalent in treated animals.

## FOUR WEEK INTRAVENOUS TOXICITY STUDY IN DOGS WITH FRESH AND ARTIFICIALLY DECOMPOSED MIXED MICELLES (REFS III B2, III C5)

[redacted]

**PURPOSE:** To determine the toxicity of fresh and artificially decomposed mixed micelles in dogs after 4 weeks of daily administration IV.

**EXPERIMENTAL DESIGN:** "Swiss" beagle dogs (9-19 months) were randomly assigned 2/sex/group (LD and MD groups) or 3/sex/group (controls and HD of fresh and decomposed micelles) which received daily bolus doses of:
2.25 ml/kg physiological saline (controls 3/sex) (group I)

0.25, 0.75 and 2.25 ml/kg of undecomposed mixed micellar solvent (2/sex for MD and LD, 3/sex for HD) (groups II, III, IV)
0.5 and 2.25 ml/kg decomposed mixed micelles (2/sex LD, 3/sex HD). High dose group dosing was suspended after 10 days for a period of 6 days. Dosing resumed at 1.5 ml/kg. (groups V and VI).

The third animal of the various groups was permitted 2 week recovery period after the thirty days of dosing.

Following 4 weeks of treatment, 2 dogs/sex of the controls and both high dose groups were necropsied; the remaining animals were necropsied after a 2 week drug free observation period. All animals of the low and mid dose groups were necropsied after the initial 4 weeks.

## RESULTS

MORTALITY: None.

OBSERVED EFFECTS: Retching and vomiting were observed sporadically during the first 2 weeks of the MD fresh mixed micellar group II. This was more marked in the HD group III (all dogs over the whole study period). Sponsor notes that the original dose of 2.25 ml/kg of decomposed micelles proved too toxic, primarily to effects of anemia. Dose was decreased to 1.5 ml/kg after a 6 day recovery period after 10 days of treatment with the original high dose. Retching and vomiting following dosing occurred during the first 10 days, with gradual deterioration and a small weight loss.

LD decomposed group (V) exhibited sporadic vomiting.

BODY WEIGHT: It appears that bodyweights from both sexes were combined in the analysis. From the graphs provided, it appeared that there was a treatment-related decrease in body weights by week 1. However, it also appears that the different dose groups had different starting weights. When week 4 values were calculated as a % of control, there appeared to be variable decreases in body weights in all dose groups which ranged from 6-12% and were not clearly dose related. The differences were not evident in the recovery animals.

FOOD CONSUMPTION: No data were presented.

VITAL SIGNS: No changes noted in ECG, HR or body temperature.

OPHTHALMIC EXAMINATION: No findings.

HEMATOLOGY: No treatment-related effects noted for /001 treated animals. HD decomposed (/002) group (VI) exhibited intravascular hemolysis resulting in marked anemia during the first 10 days. (~5-6% decrease in RBC and Hb, increased reticulocytes from 12-28 by week 2. This continued through the end of the study and did not fully recover) This was attributed to the lysolecithin. The condition of the animals became so bad that the dosing had to be stopped for 6 days, then resumed at 1.5 ml/kg for the remainder of the study. This dose was better tolerated. Parameters were within normal limits by the end of the study.

COAGULATION: No data.

BONE MARROW: No data.

BLOOD CHEMISTRY: Plasma GOT (13-19 U/L), GPT (18-142 U/L) and ALK Phos (68-101) and cholesterol (140-247) increased in group IV (HD /001) which generally returned to normal values at the end of the recovery period (cholesterol was still slightly increased, but not significantly). LD decomposed group (V) exhibited moderately elevated serum cholesterol 18-266). HD decomposed group (VI) also exhibited increased plasma GPT (18-26), cholesterol (140-230) and bilirubin (0.12-0.14).

URINALYSIS: No treatment-related findings.

ORGAN WEIGHTS: Increased liver weight (~ 25% due, in part, to one very large liver) was noted in group IV and appeared to show a trend towards normalization (but did not reach normalization) at the end of the recovery period. Testes and prostate weights appeared to be very variable and a drug effect could not be ascribed. The increased liver weights in the HD decomposed group (VI) (~16%) did not fully recover by the end of the recovery period.

GROSS PATHOLOGY: No findings reported.

HISTOPATHOLOGY: Local tolerance was good in all groups. In the HD decomposed micelle group (VI) most animals had mild to moderate intracanalicular cholestasis which improved markedly after the dose was lowered. Hemosiderosis in the spleen was noted with both /001 and /002 after recovery.

## REPEATED DOSE TOXICITY IN DOGS AFTER ONE MONTH IV ADMINISTRATION OF CERNEVIT

**NOTE:** This study was performed by [redacted] and is dated June-July 1987. Study code is 003-072. QA and GLP statements were provided.

**PURPOSE:** To determine the toxicity of fresh Cernevit preparation in dogs after 4 weeks of daily administration IV.

**EXPERIMENTAL DESIGN:** 2 dogs/sex/group were dosed IV with Cernevit daily for one month as follows at a rate of 5 ml/min:
Physiological saline
0.25 ml/kg
1.0 ml/kg
4.0 ml/kg

## RESULTS

MORTALITY: None

OBSERVED EFFECTS: Vomiting in LD (1 animal, 2 occasions), 3 animals in the MD group and vomiting and "hiccups" in all HD animals.

BODY WEIGHT: No treatment-related changes.