

Segment II study, the HD produced maternal toxicity. 2 does died and weight gain of remaining does was less than 1/2 the control value during the treatment period. This appeared to catch up in the period of days 20-30 of gestation after treatment. There were no findings in the LD group. In the HD group, there was no effect on the resorption rate, body weight or survival rate of fetuses, but the number of abortions was markedly increased in this group between days 23 and 28. The sponsor attributed the abortions to maternal toxicity.

**BODY WEIGHT:** Treated animals had a decrease in mean weight gain by 1/3 of controls. Body weight gain increased after dosing was stopped. By day 30, the HD animals had nearly restored their body weights to control levels.

**FETAL PARAMETERS:** No change in resorption rate. However the number of fetuses aborted before cesarean section was markedly higher in the micelles group (0 in control and LD, 43 in HD group). This resulted in a smaller number of live fetuses at term. The sponsor concluded that the increased abortions reflected a secondary effect due to maternal toxicity. Neither body weight nor survival rate of live fetuses was affected in the treatment group.

There were no external, skeletal or soft tissue abnormalities in either treatment group.

#### SEGMENT III PERI AND POSTANATAL DEVELOPMENTAL STUDIES IN RATS WITH ARTIFICIALLY DECOMPOSED MIXED MICELLES SOLUTION (REFS III C4)

**NOTE:** Sponsor's title: "Peri- and postnatal study in rats with intravenous administration of the mixed-micelles solution [REDACTED] 17-7465/002 (artificially decomposed). Segment III study". Study performed by [REDACTED] Dated August 27, 1985. GLP statement was provided 10/10/85. QA statement was provided 10/7/85. [REDACTED] report number: B-105'595.

**PURPOSE:** To evaluate the developmental toxicity of the partially degraded mixed micelle preparations in rats.

**EXPERIMENTAL DESIGN:** Mated female Fu-Albino rats (20/group) were randomly assigned to treatment groups which were administered daily IV doses as follows:

Group I (controls) 2.0 ml/kg physiological saline

Group II (LD) 1.0 ml/kg stored mixed micelles [REDACTED] 17-7465/002

Group III (HD) 2.0 ml/kg stored mixed micelles [REDACTED] 17-7465/002

Dosing occurred between day 16 of gestation through lactation day 11. Rats were allowed to litter spontaneously and to rear the offspring up to weaning age. The observation period ended on lactation day 23. 8 litters/dosage group underwent physical and functional evaluation (hair growth, eruption of upper incisors, opening of external auditory canal, opening of palpebral fissure, auditory startle, pupillary contraction). At the same time period, 8 litters/group were sacrificed, dissected and wet weight of liver, heart and kidneys determined. 20 pups/sex/group were randomly chosen for learning and memory ability in a water-filled E-maze approximately 2 weeks after weaning. Does were also sacrificed and necropsied on lactation day 23 and examined for staining of the uteri for implantation sites.

## RESULTS

MORTALITY: No compound related maternal death or toxicity were noted.

BODY WEIGHT: No effects in treated dams.

FETAL PARAMETERS: No change in resorption rate, litter size or body weight of fetuses compared to control group. The duration of gestation was slightly prolonged in the high dose group. There was a nonsignificant reduction in survival rate of the pups during lactation in both treated groups. The sponsor attributed this to stress of the dams during the IV infusions during lactation. The weight of pups was not affected during lactation.

Physical and functional developmental parameters were not significantly altered in treated groups except for a slight delay in incisor eruption and opening of the eye in the high dose group. Learning and memory tests were not affected by treatment.

There were no external, skeletal or soft tissue abnormalities in either treatment group. Organ weights were not altered in weanlings.

## MUTAGENICITY AND CARCINOGENICITY

No carcinogenicity assessment was performed. The sponsor claimed that the risk of parenterally administered mixed micelles was negligible or non-existent. This reviewer believes that this has not been assessed and no statement should be made regarding expected results of studies that were not performed.

The sponsor concluded that the Ames assay alone was a sufficient evaluation of mutagenic capacity.

### AMES SALMONELLA ASSAY WITH ARTIFICIALLY DECOMPOSED MIXED MICELLES SOLUTION (III D1)

Note: Study performed by [REDACTED] Dated July 7, 1982. GLP statement was provided (date could not be read). No QA statement was provided. [REDACTED] report number: 77 722. GLP statement was provided dated 7/16/82.

Purpose: *In Vitro* assessment of mutagenic potential of partially decomposed mixed micelles (Ro 17-7465/002) using a bacterial *his*<sup>+</sup> reversion assay ("Ames Test"). Study completed:

Experimental Design: Standard plate incorporation assay.

Salmonella Strains: TA 1535, TA 1537, TA 1538 TA100 and TA 98

Metabolic Activation System: Phenobarbital-induced S9 rat liver homogenate.

Test article: [REDACTED] 17-7465/002 which contains approximately 20% of lecithin hydrolyzed into lysolecithin and free fatty acids. (Study lists test compound as [REDACTED] 17-7465/763, but the composition appears to be the same as /002) Doses up to 500 microliters/plate were tested.

Vehicle control: Water

Positive control: Cyclophosphamide. 200 µl/plate ± S9 activation mix.

Criteria for Positive Result: Not specified.

Results: According to the sponsor, there was a slight, reproducible increase in the number of revertants was found in TA 100 in the presence and absence of metabolic activation. The data indicate that while there was a potentially weak response in the TA100, but it does not reach the level of doubling the control value which would indicate a positive finding. This assay had negative results.

Evaluation: Sponsor considered the response too weak to classify the mixed micelles as mutagenic. The data indicate that while there was a potentially weak response in the TA100, it does not reach the level of doubling the control value which would indicate a positive finding. This assay had negative results. This assay did not take into account the T:A-specific bacterial strains TA97 or *E. coli* WP2uvrA. Although this test article is considered more toxic than the native product, this test does not really address the mutagenicity of [REDACTED] P(7465/001). Therefore, it is not appropriate to include this finding in the label.

## HEMOLYSIS AND IRRITATION STUDIES

### IN VIVO HEMOLYSIS TESTING IN DOGS (REF. III Q1)

NOTE: Study performed by [REDACTED] Dated June 6, 1984. GLP statement was provided (6/84). QA statement was provided (5/25/84). Sponsor title: "Venous Irritation and Hemolysis Testing with [REDACTED] 15-1788/014 (Benzodiazepam antagonist) Parenteral Formulation".

PURPOSE: To determine the hemolytic activity of mixed micelles formulations in dogs *in vivo*.

EXPERIMENTAL DESIGN: 6 dogs/sex/group were treated IV with a total volume of 1 ml in the cephalic vein at a rate of 3-4 ml/min as follows:

1. [REDACTED] 15-1788/014 (1 mg dissolved in mixed micelles)
2. Mixed micelles solution
3. Normal saline (control)

The single IV dose was administered after a 19 h fast.

### RESULTS

No significant hemolysis occurred following a single injection IV. There was a slight to well-defined erythema and occasional thrombus noted, but this did not appear to be dose related. There was one incidence of visible hemolysis in the undiluted plasma of a dog in the group 1 and group 2.