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**CITREX, INC.**

**MTT Cell Viability Assay**
***HepG2 Human Liver and RKO Human Colon Cells***
**Test Article: Citrex B**
**Study Number: CIT-0900b**
**September, 2000**

## SUMMARY OF RESULTS

The test article, Citrex B, was analyzed for cytoxicity at Xenometrix in September 2000. Data are shown on the following pages in Figures 1 and 2. The following is summary of the data obtained:

- The $LC_{50}$ for Citrex B in HepG2 human liver cells was 0.0026%.
- The $LC_{50}$ for Citrex B in RKO human colon cells was 0.001%.

The $LC_{50}$ is the concentration of test article estimated to cause 50% mortality in the exposed population. HepG2 cells and RKO cells were grown in separate wells of a 96-well collagen-treated (for better cell adherence) tissue culture plate and exposed to one of several concentrations of Citrex B for 48 hours. Culture viability was measured at each dose by the application of 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT). MTT is a hydrogen acceptor, which can be taken up by viable cells and reduced by mitochondria to yield a purple formazan salt. By solubilizing these formazan salt crystals in DMSO, this turnover can be measured spectrophotometrically. Only viable cells can carry out this reaction. $LC_{50}$ values represent the concentrations that reduce the MTT value to 50% of the negative control.

Study Director: Elaine Shen, Ph.D.
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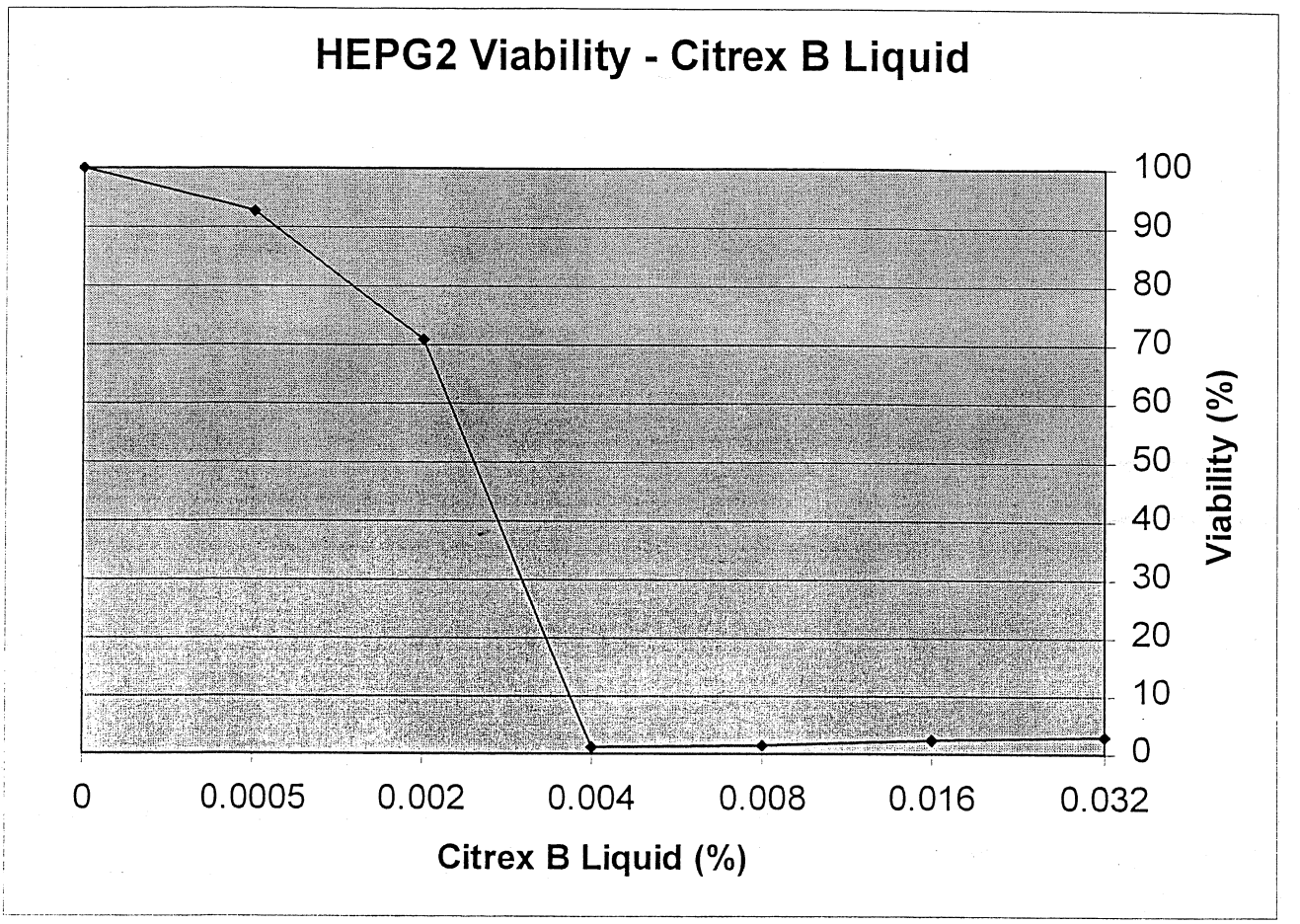
Signed: Elaine Shen

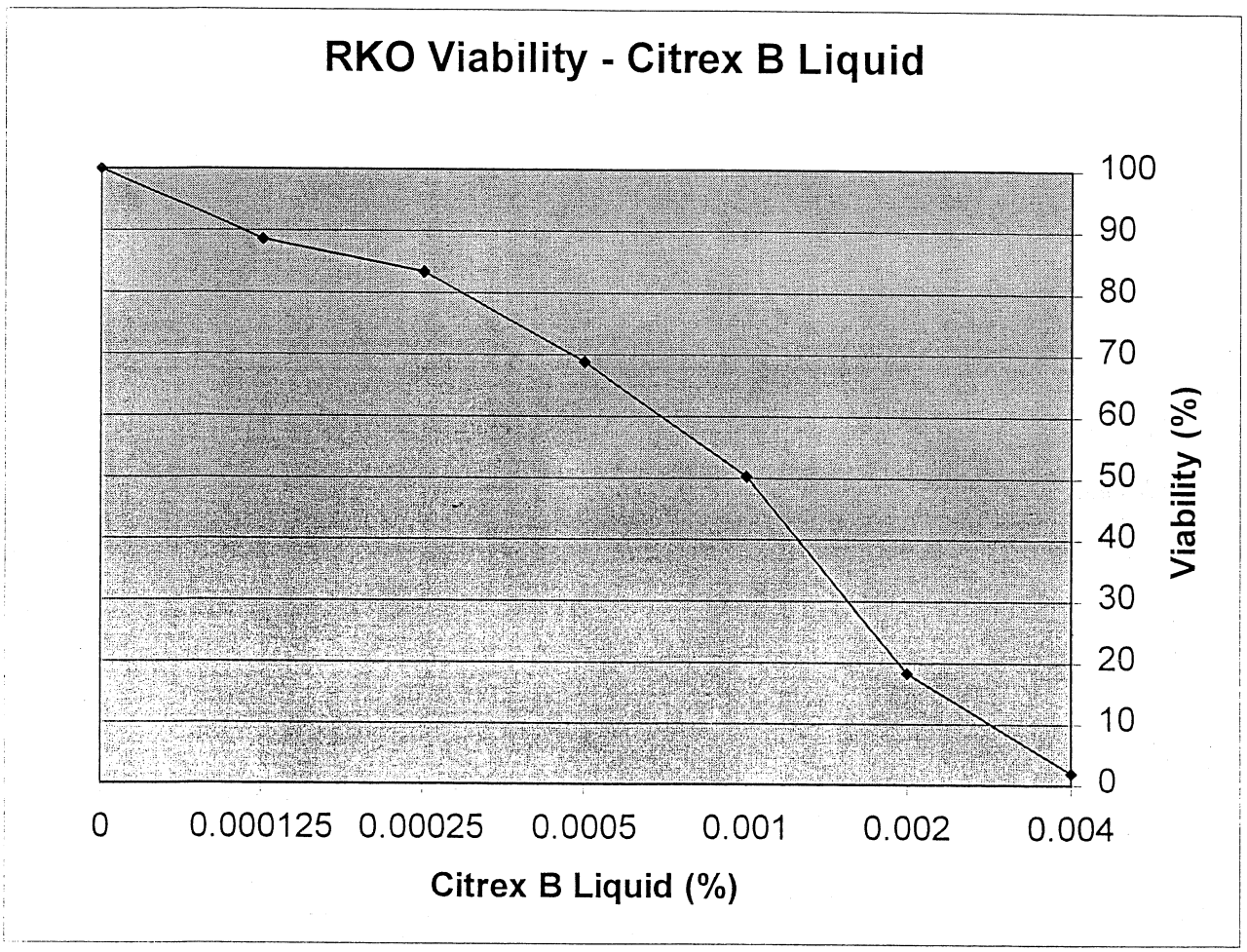
Date: September 16, 2000

## Figure 1. Effects of Citrex B on Human Liver Cell (HepG2) Viability




| % Citrex Liquid | % Viability |
|---|---|
| 0 | 100 |
| 0.0005 | 92.64 |
| 0.002 | 70.81 |
| 0.004 | 1.16 |
| 0.008 | 1.64 |
| 0.016 | 2.39 |
| 0.032 | 2.94 |

## Figure 2. Effects of Citrex B on Human Colon Cell (RKO) Viability




| % Citrex Liquid | % Viability |
|---|---|
| 0 | 100 |
| 0.000125 | 88.43 |
| 0.00025 | 83.55 |
| 0.0005 | 68.94 |
| 0.001 | 50.22 |
| 0.002 | 18.34 |
| 0.004 | 1.92 |