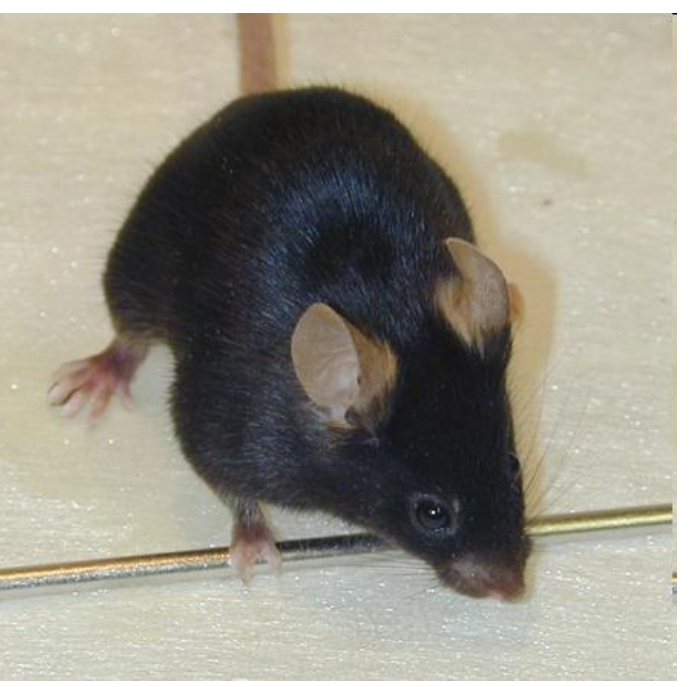




# Oral Bioavailability of a Novel *Francisella tularensis* Chemotherapeutic

L. Best, B. Cranmer, S. Knudson, J. Cummings, K. England, P. Tonge, R. Slayden

College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO

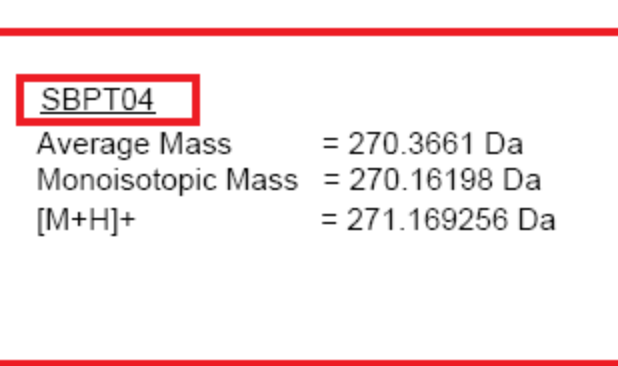
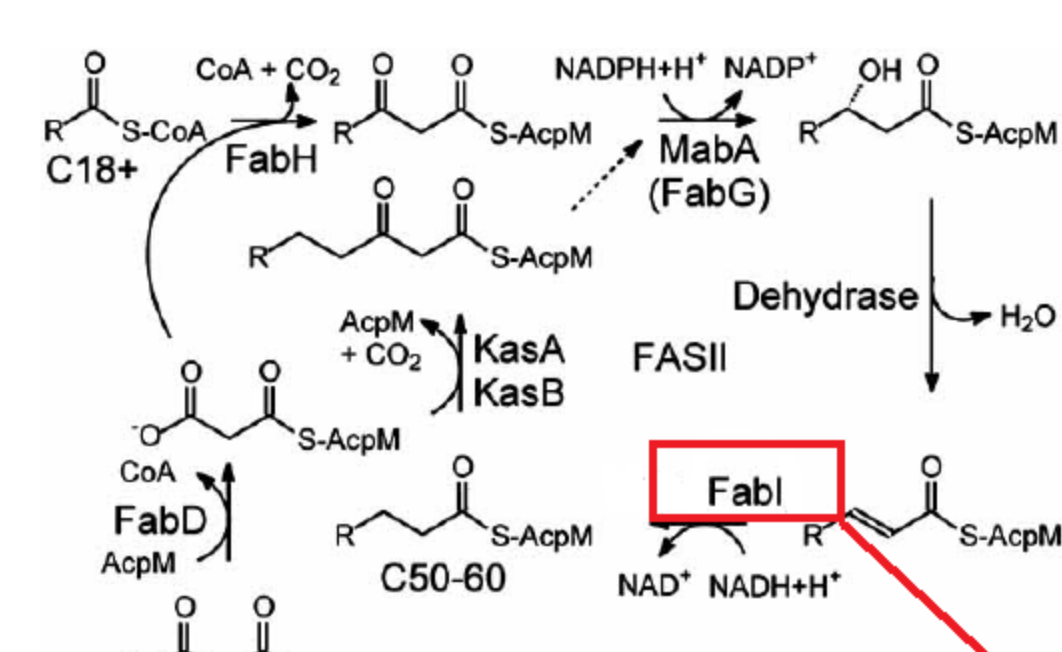


## Abstract

*Francisella tularensis* is a gram-negative bacterium that causes the disease Tularemia in mammals. *F. tularensis* is considered a potential biological weapon by the United States government due to its low infectious dose, potential for aerosol transmission, and high virulence. A lead-based alkyl-substituted diphenyl ether, SBPT04, has shown promise as an antimicrobial against *F. tularensis*. A survival study explored the efficacy of three different formulations of PT04 delivered via oral gavage against a Shu4 infection of *F. tularensis* in a murine model. Listed in ascending order of treatment success the formulations were: 5% ethanol, 10% ethanol 6% cremaphore 1% citric acid, and 10% ethanol 30% PEG 400 6% EtPGS. A second study aimed to identify the effect of different formulations on SBPT04 bioavailability and tissue distribution in C57/BL6 mice. Plasma, lung, liver, and spleen samples were analyzed using LC/MS for concentrations of SBPT04 and the glucuronidated conjugate (SBPT04g). 10% ethanol 30% PEG 400 6% EtPGS formulation was least effective in the survival study but achieved the greatest amount of SBPT04 tissue perfusion.

## Introduction

•*Francisella tularensis* is a gram-negative pathogenic bacterium that causes the disease Tularemia in mammals and is considered a potential biological weapon by the United States government.  
 •A series of lead-based diphenyl ether compounds were synthesized and one compound, SBPT04, was found to be a potent inhibitor of the *F. tularensis* enoyl reductase (FtuFabI) in the bacterial fatty acid synthesis cascade.

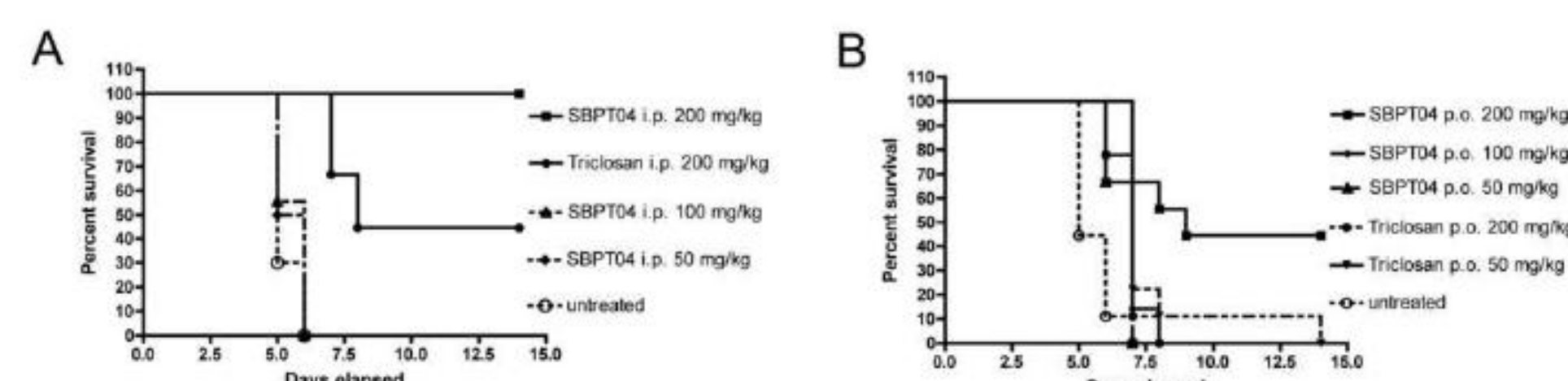


## Commonly Used *F. tularensis* Antimicrobials and SBPT04

|                     | MIC <sub>90</sub> (µg/mL) | LC <sub>50</sub> (µg/mL) | SI  |
|---------------------|---------------------------|--------------------------|-----|
| <b>SBPT04</b>       | 0.2                       | 100                      | 500 |
| <b>Streptomycin</b> | 4                         | >100                     | 25  |
| <b>Gentamycin</b>   | 2                         | >100                     | 50  |
| <b>Doxycyclin</b>   | 1                         | 3                        | 3   |

Selective index of SBPT04 and leading *F. tularensis* antimicrobials was calculated as the MIC / LC50. MICs were determined using an LVS strain of *F. tularensis* and cytotoxicity was determined using African green monkey kidney cells.

•SBPT04 has proved effective *in vivo* against *F. tularensis* strain Shu4 when administered at 200 mg/kg IP, but appears to have problems with oral bioavailability.



## Objectives

- To determine the effect of PO formulation of SBPT04 on survival rates of mice infected with a *Shu4* strain of *F. tularensis*.
- To determine the effect of PO formulations of SBPT04 on metabolism and tissue distribution of SBPT04 in a murine model.

## Experiment One: Survival Study

- Six week old ICR mice were inoculated with *F. tularensis* (Shu4) by aerosol delivery. Mice were separated into 6 groups of 15 and each group was given 5 daily 200mg/kg doses of SBPT04 formulated as described below.
- Three mice were sacrificed from each group on days 2 and 4 to determine bacterial loads in the lungs and spleen and the remaining 9 mice were monitored for survival.

| Group | Formulation                        | Mouse Survival |       |       |       |       |       |       |       |       |       |        |        |
|-------|------------------------------------|----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|--------|
|       |                                    | Day 0          | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 | Day 30 |
| 1     | Control                            | 15             | 15    | 12    | 12    | 9     | 9     | 5     | 1     | 1     | 0     | 0      | 0      |
| 2     | 5% Ethanol                         | 15             | 15    | 12    | 12    | 9     | 9     | 9     | 8     | 7     | 7     | 7      | 7      |
| 3     | 10%EtOH,6%cremaphore,1%citric acid | 15             | 15    | 12    | 12    | 9     | 9     | 2     | 2     | 2     | 2     | 2      | 2      |
| 4     | 20%cyclodextrin, 5%D5W             | 15             | 15    | 12    | 12    | 9     | 8     | 3     | 1     | 1     | 0     | 0      | 0      |
| 5     | 5% Ethanol, 8%Solutol              | 15             | 15    | 12    | 12    | 9     | 9     | 5     | 1     | 1     | 1     | 1      | 1      |
| 6     | 10%Ethanol, 30%PEG400, 6% E TPGS   | 15             | 15    | 12    | 12    | 9     | 9     | 5     | 1     | 1     | 0     | 0      | 0      |

- The most effective formulation was 5% ethanol with a survival rate of 7 / 9 mice after one month.
- In order to determine the possible cause of diminished SBPT04 efficacy when delivered PO an oral bioavailability study was performed using formulations of varying effectiveness in the survival study: 5% EtOH (7 / 9), 10% EtOH 6% cremaphore 1% citric acid (2 / 9), and 10% EtOH 30% PEG400 6% E TPGS (0 / 9).

## Experiment Two: Metabolism and Tissue Distribution

- Three groups of 10 c57/bl6 mice were dosed with 200 mg/kg SBPT04 PO. Each group received the drug in a different formulation;:

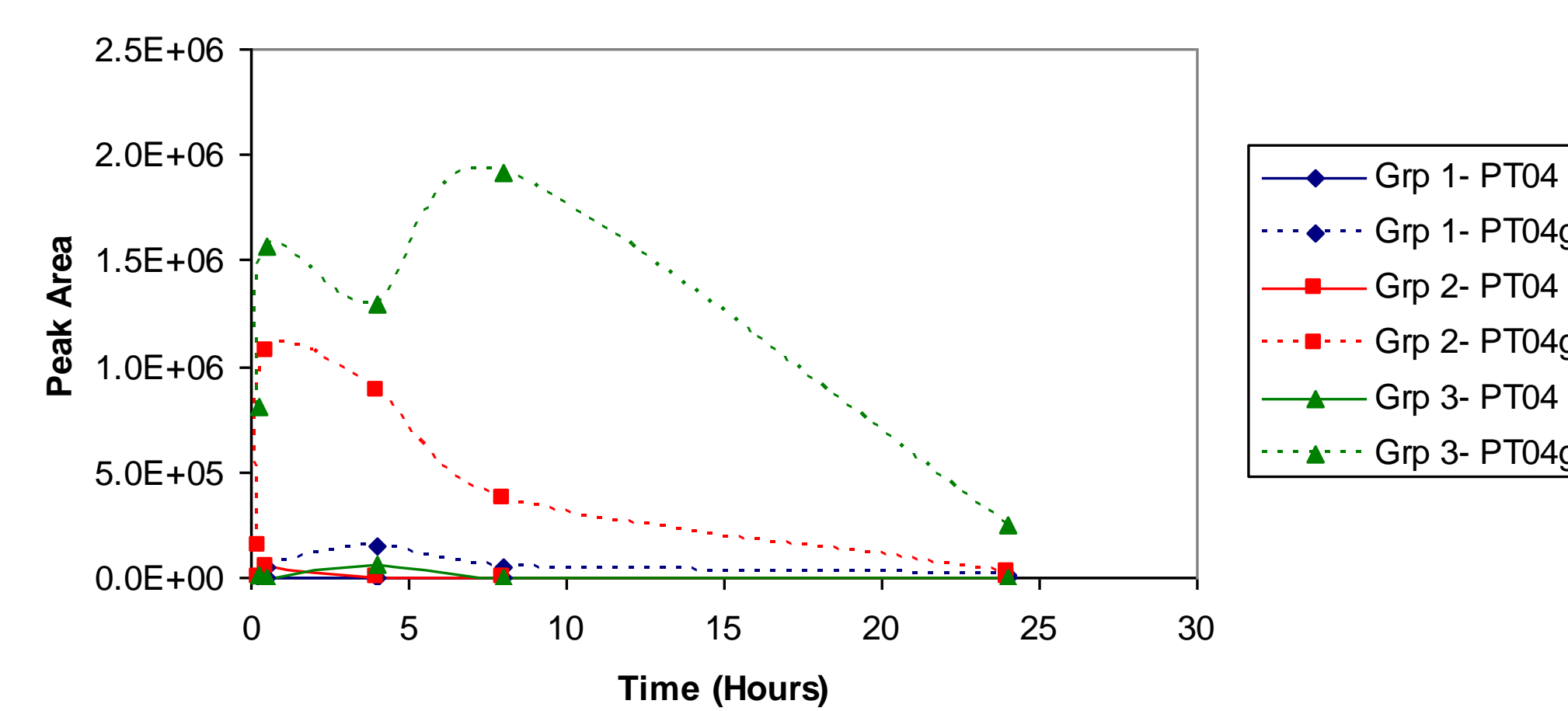
**Group 1: 5% EtOH**

**Group 2: 10% EtOH 6% cremaphore 1% citric acid**

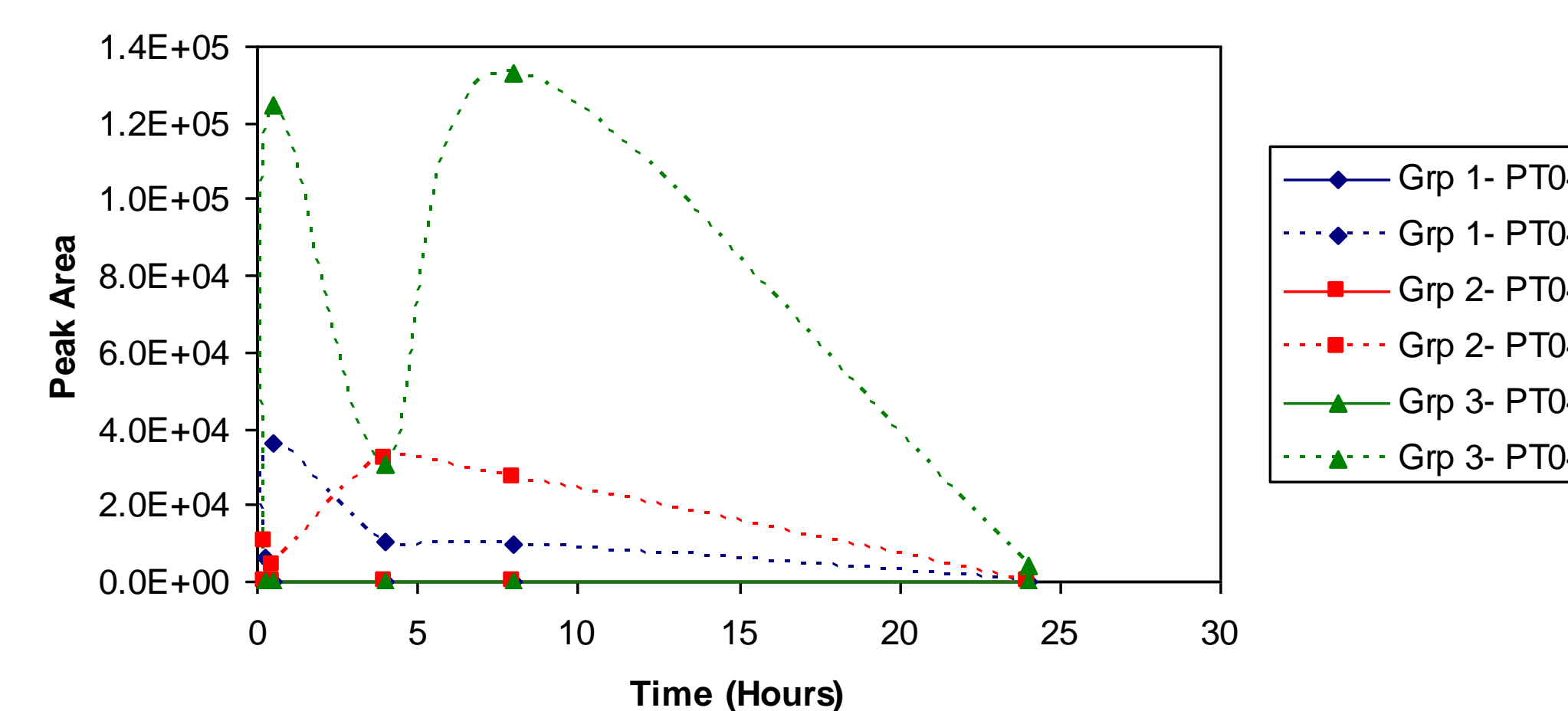
**Group 3: 10% EtOH 30% PEG400 6% ETPGS.**

- 2 mice from each group were sacrificed at 15 minutes, 30 minutes, 4 hours, 8 hours and 24 hours. Lung, spleen, liver and plasma samples were collected and analyzed using LC/MS for the parent compound and the glucuronidated conjugate (SBPT04g).

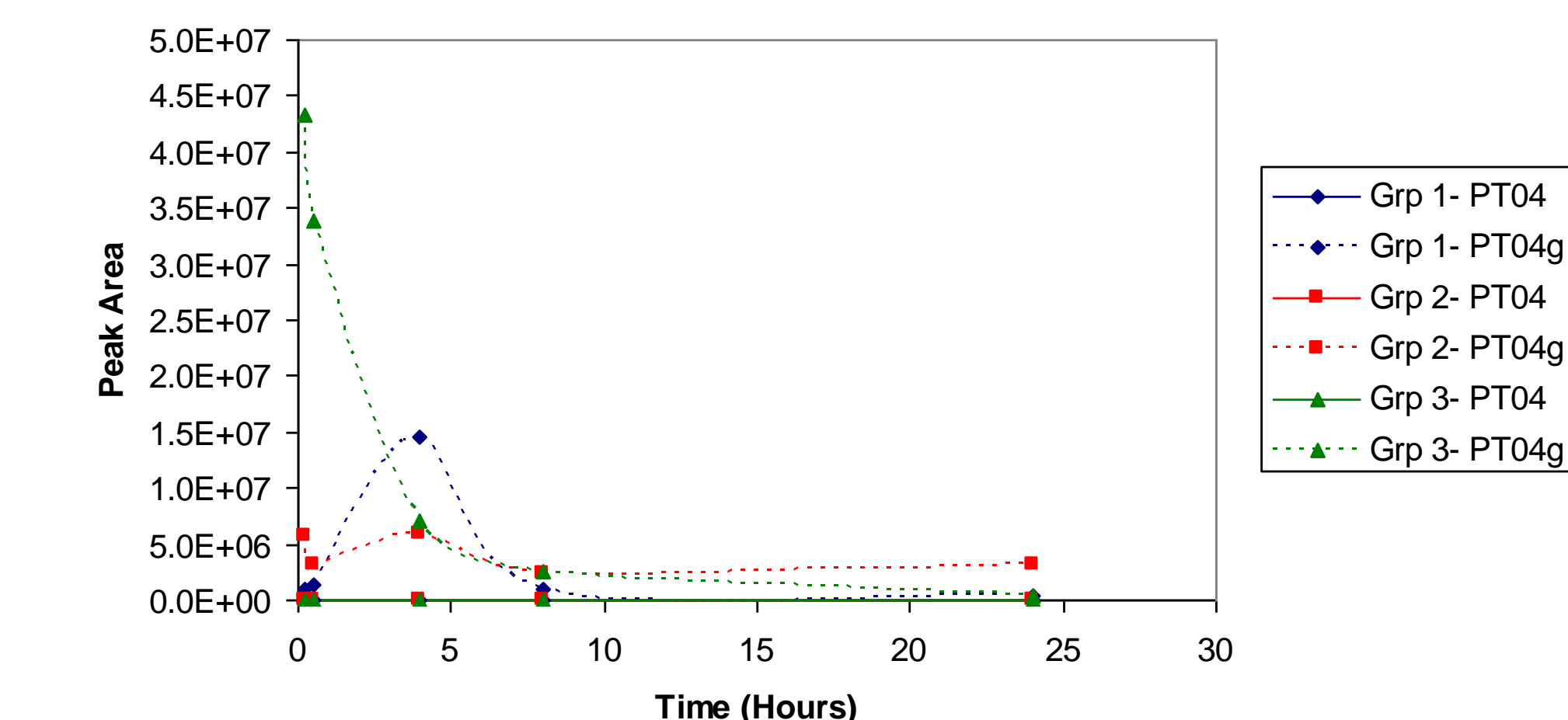
### Lung



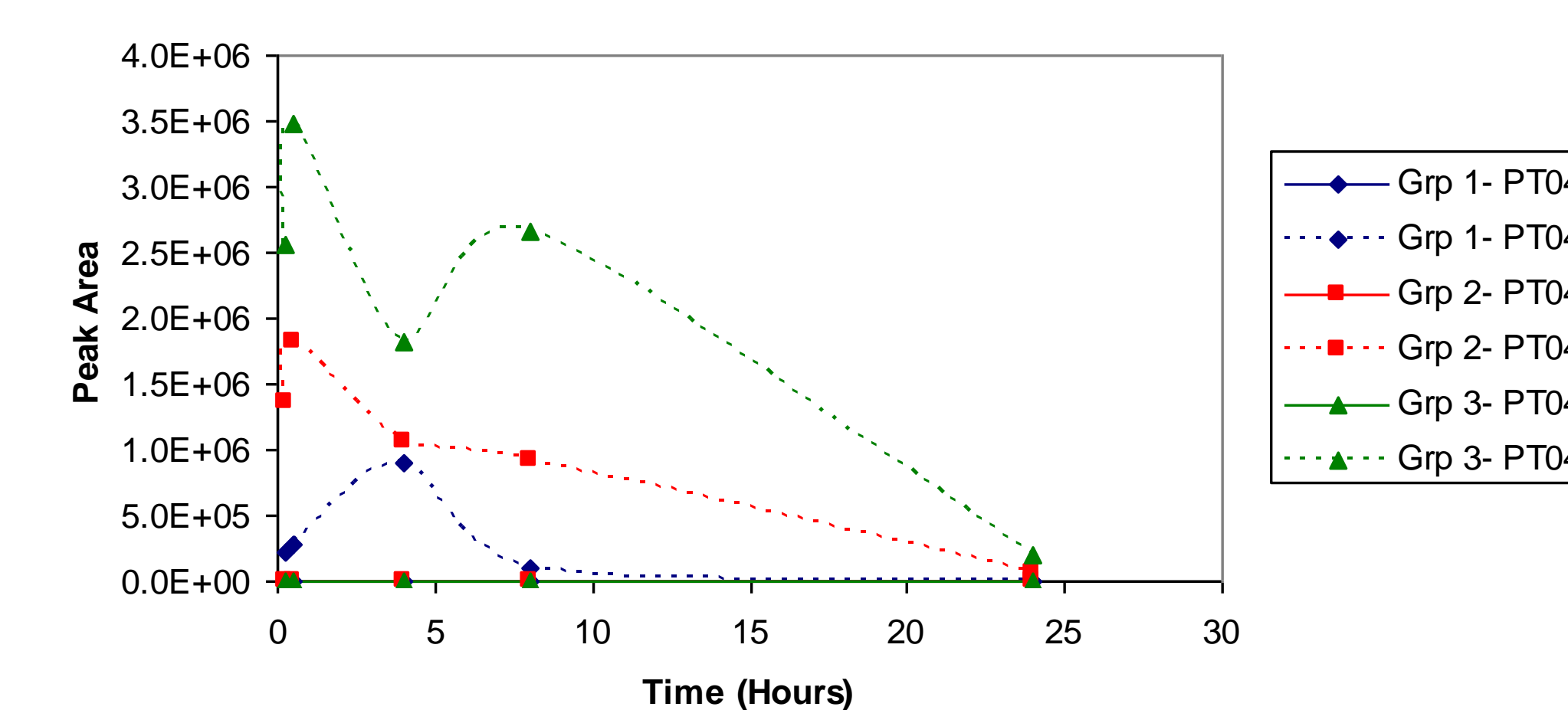
### Spleen



### Liver

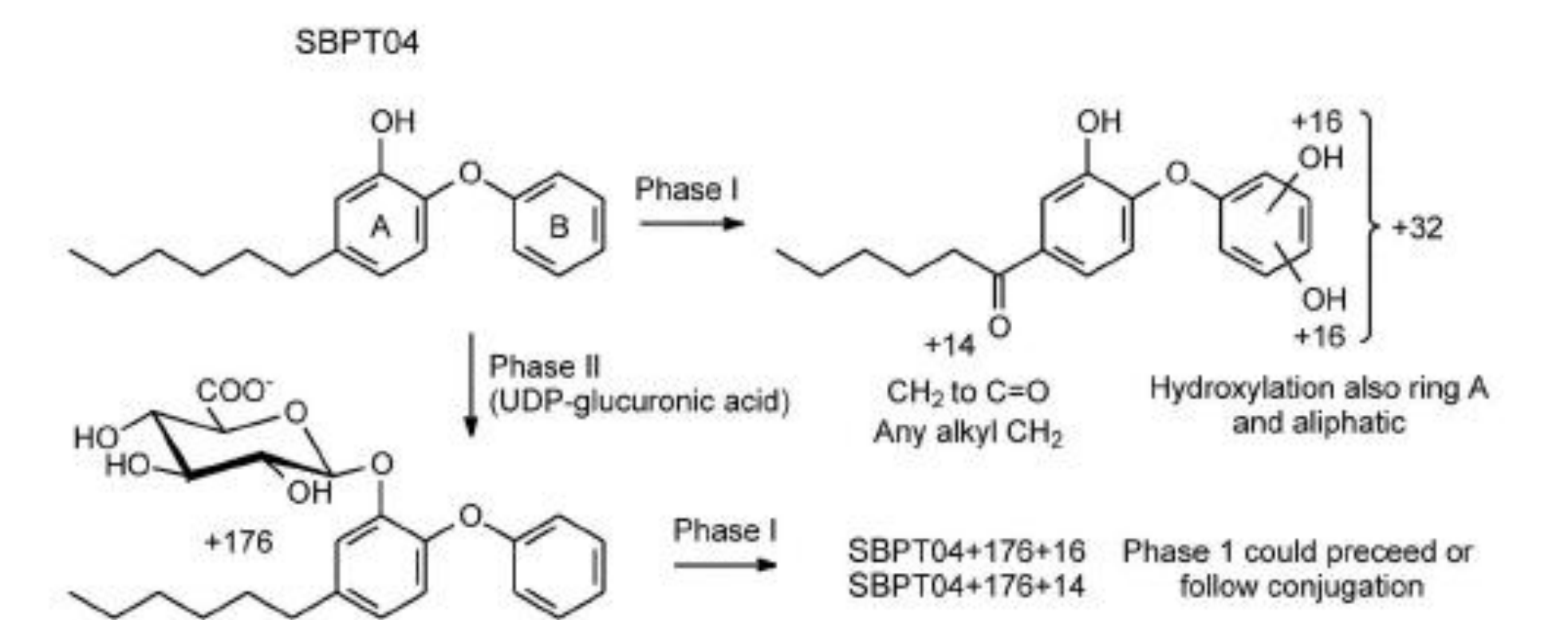


### Plasma

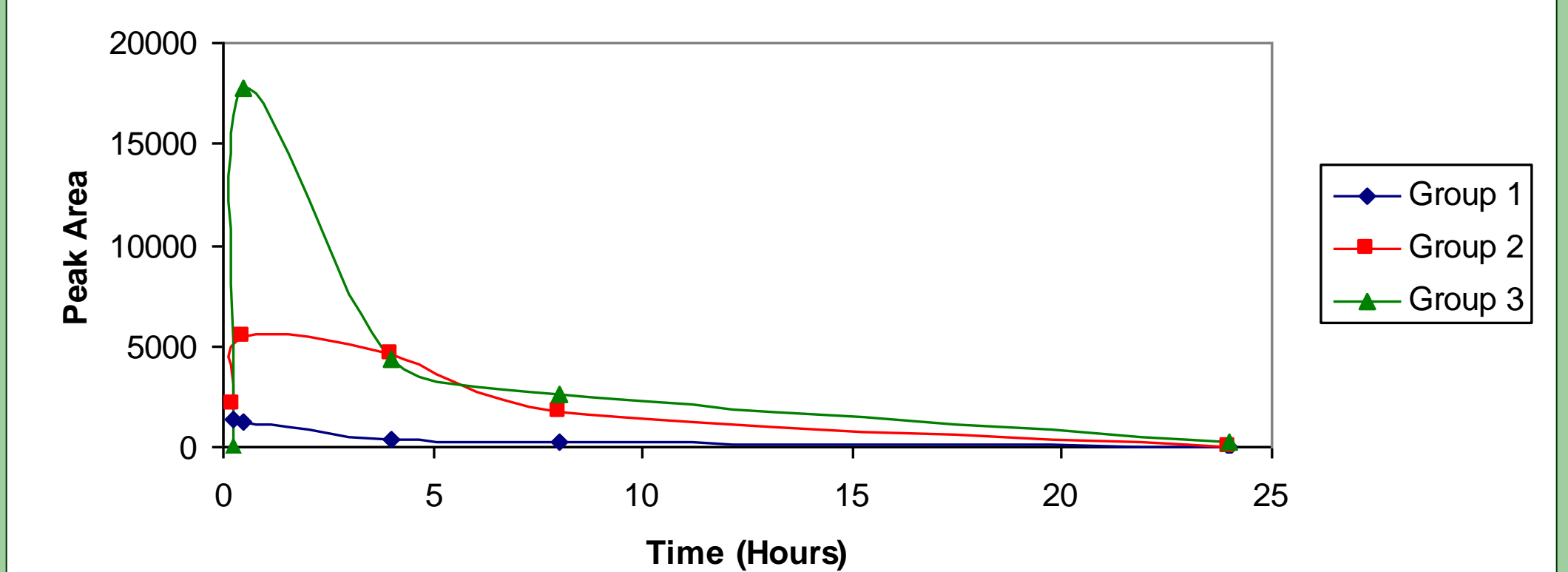


## Experiment Two: Metabolism and Tissue Distribution

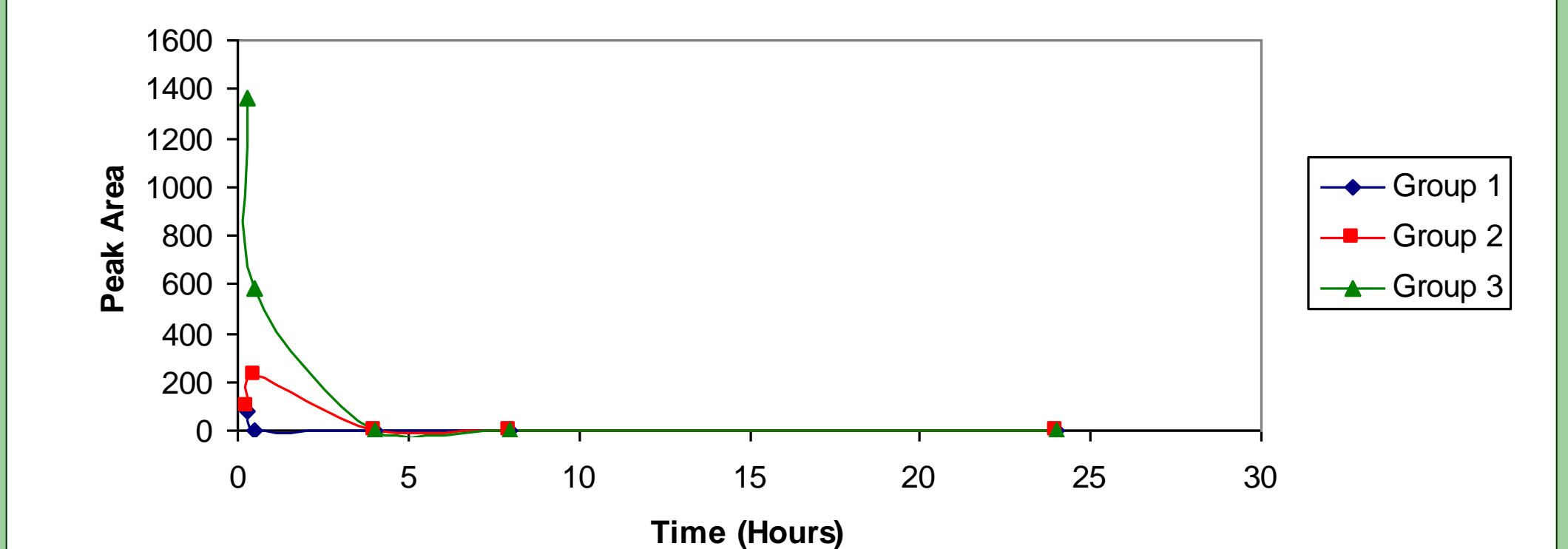
### Metabolism of SBPT04



### SBPT04 Liver Distribution



### SBPT04 Plasma Distribution



- No detectable amounts of the SBPT04 parent compound were found in the lung or spleen.

## Conclusion

•SBPT04 appears to have the greatest tissue perfusion when formulated with 10% EtOH 30% PEG400 6% ETPGS and the least perfusion when formulated with 5% ethanol.

•The formulation with the greatest tissue perfusion was also the least effective against an *F. tularensis* infection in the murine model.

•This suggests that although SBPT04 is effective *in vivo* against *F. tularensis* it may be toxic to the animal if it perfuses the tissues at higher concentrations.

•Further *in vivo* cytotoxicity studies using the different formulations need to be performed to address this issue.

## Acknowledgements

Funding for this project was generously provided by the Merck-Merial summer fellowship program.

I would also like to thank Terry Nett, Collin Clay, Richard Slayden, Brian Cranmer, Jason Cummings, and Susan Knudson for their guidance and assistance throughout the project.

