**Experiment Number:** K06898 **Toxicokinetics Data Summary Request Date:** 7/11/2023

**Route:** Gavage, IV **Compound:** alpha/beta-Thujone mixture/ **Analyte:** alpha-Thujone **Request Time:** 10:03:16

**Species/Strain:** Rats/Fischer 344 **CAS Number:** 76231-76-0 **Lab:** Battelle Columbus

**Male**

**Treatment Group (mg/kg)**

 **3.0 IV**  **Plasmac,e**  **25 Gavage Plasmab,d  50 Gavage Plasmab,d**

|  |  |  |  |
| --- | --- | --- | --- |
| C\_0min\_pred (ng/mL) | 1110 ± 200 |  |  |
| Cmax\_pred (ng/mL) |  | 255 ± 29 | 462 ± 55  |
| Tmax\_pred (minute) |  | 5.1 ± 3.7 | 12.9 ± 3.7 |
| Cmax\_obs (ng/g) |  | 286 ± 125 | 666 ± 100 |
| Tmax\_obs (minute) |  | 9.75 ± 0.50 | 10.0 ± 0.0 |
| Alpha Half-life (minute) | 6.48 ± 1.10 |  |  |
| Beta Half-life (minute) | 165 ± 9 |  |  |
| k01 (minute-1)  |  | 0.281 ± 0.093 | 0.344 ± 0.129 |
| k01 Half-life (minute) |  | 2.47 ± 0.81 | 2.01 ± 0.75 |
| k10 (minute-1) | 0.0349 ± 0.0057 | 0.00435 ± 0.00042 | 0.00427 ± 0.00039 |
| k10 Half-life (minute) | 19.8 ± 3.2 | 160 ± 16 | 162 ± 15 |
| k12 (minute-1) | 0.0633 ± 0.0127 |  |  |
| k21 (minute-1) | 0.0129 ± 0.0014 |  |  |
| Cl1 (mL/min/kg) | 94.2 ± 4.2 |  |  |
| Cl2 (mL/min/kg) | 171 ± 21 |  |  |
| Cl1\_F (mL/min/kg) |  | 400 ± 37 | 437 ± 41 |
| V1 (mL/kg) | 2700 ± 490 |  |  |
| V2 (mL/kg) | 13200 ± 1000 |  |  |
| V1\_F (mL/kg) |  | 92000 ± 12000 | 102000 ± 13000 |
| MRT (minute) | 169 ± 9 |  |  |
| AUC\_0-T (ng mL-1 min) |  | 57500 | 96400 |
| AUCinf\_pred (ng\*mL-1\*min) | 31800 ± 1400 | 62600 ± 5800 | 114000 ± 11000 |
|  |  |  |  |

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**Experiment Number:** K06898 **Toxicokinetics Data Summary Request Date:** 7/11/2023

**Route:** Gavage, IV **Compound:** alpha/beta-Thujone mixture/ **Analyte:** alpha-Thujone **Request Time:** 10:03:16

**Species/Strain:** Rats/Fischer 344 **CAS Number:** 76231-76-0 **Lab:** Battelle Columbus

**Female**

**Treatment Group (mg/kg)**

 **3.0 IV**  **Plasmac**  **25 Gavage Plasmab,d  50 Gavage Plasmab,d**

|  |  |  |  |
| --- | --- | --- | --- |
| C\_0min\_pred (ng/mL) | 885 ± 185 |  |  |
| Cmax\_pred (ng/mL) |  | 383 ± 88 | 483 ± 87 |
| Tmax\_pred (minute) |  | 16.0 ± 7.0 | 16.0 ± 6.5 |
| Cmax\_obs (ng/g) |  | 731 ± 127 | 752 ± 112 |
| Tmax\_obs (minute) |  | 10.0 ± 0.0 | 10.0 ± 0.0 |
| Alpha Half-life (minute) | 6.79 ± 1.17 |  |  |
| Beta Half-life (minute) | 53.2 ± 10.8 |  |  |
| k01 (minute-1)  |  | 0.213 ± 0.135 | 0.259 ± 0.142 |
| k01 Half-life (minute) |  | 3.25 ± 2.07 | 2.68 ± 1.46 |
| k10 (minute-1) | 0.00807 ± 0.00142 | 0.00446 ± 0.00067 | 0.0746 ± 0.0113 |
| k10 Half-life (minute) | 9.29 ± 1.41 | 85.9 ± 15.1 | 156 ± 23 |
| k12 (minute-1) | 0.0227 ± 0.0061 |  |  |
| k21 (minute-1) | 0.0178 ± 0.0042 |  |  |
| Cl1 (mL/min/kg) | 253 ± 22 |  |  |
| Cl2 (mL/min/kg) | 76.8 ± 17.2 |  |  |
| Cl1\_F (mL/min/kg) |  | 464 ± 85 | 430 ± 63 |
| V1 (mL/kg) | 3390 ± 710 |  |  |
| V2 (mL/kg) | 4310 ± 970 |  |  |
| V1\_F (mL/kg) |  | 57400 ± 16200 | 96400 ± 19600 |
| MRT (minute) | 30.5 ± 4.2 |  |  |
| AUC\_0-T (ng mL-1 min) |  | 44300 | 106000 |
| AUCinf\_pred (ng\*mL-1\*min) | 11900 ± 11000 | 53900 ± 19800 | 116000 ± 117000 |

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**Experiment Number:** K06898 **Toxicokinetics Data Summary Request Date:** 7/11/2023

**Route:** Gavage, IV **Compound:** alpha/beta-Thujone mixture/ **Analyte:** alpha-Thujone **Request Time:** 10:03:16

**Species/Strain:** Rats/Fischer 344 **CAS Number:** 76231-76-0 **Lab:** Battelle Columbus

**Male**

**Treatment Group (mg/kg)**

 **6.0 IV**  **Braina**  **40 Gavage Braina  80 Gavage Braina**

|  |  |  |  |
| --- | --- | --- | --- |
| Cmax\_obs (ng/g) | 2560 ± 590 | 508 ± 75 | 1400 ± 210 |
| Tmax\_obs (minute) | 9.00 | 42.0 | 17.0 |
| Half-life (minute) | 54.9 | 80.7 | 106 |
| AUC\_0-T (ng\*g-1\*min) | 82700 |  |  |
| AUC\_0-T (ng/g\*min) |  | 101000 | 192000 |
| AUCinf\_pred (ng\*g-1\*min) |  | 107000 | 218000 |
| AUCinf\_pred (ng\*mL-1 min) | 83500 |  |  |
|  |  |  |  |

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**Experiment Number:** K06898 **Toxicokinetics Data Summary Request Date:** 7/11/2023

**Route:** Gavage, IV **Compound:** alpha/beta-Thujone mixture/ **Analyte:** alpha-Thujone **Request Time:** 10:03:16

**Species/Strain:** Rats/Fischer 344 **CAS Number:** 76231-76-0 **Lab:** Battelle Columbus

**Female**

**Treatment Group (mg/kg)**

 **6.0 IV**  **Braina**  **40 Gavage Braina  80 Gavage Braina**

|  |  |  |  |
| --- | --- | --- | --- |
| Cmax\_obs (ng/g) | 3090 ± 200 | 2180 ± 260 | 2900 ± 500 |
| Tmax\_obs (minute) | 10.7 | 16.3 | 16.0 |
| Half-life (minute) | 61.5 | 121 | 141 |
| AUC\_0-T (ng\*g-1\*min) | 95600 |  |  |
| AUC\_0-T (ng/g\*min) |  | 188000 | 396000 |
| AUCinf\_pred (ng\*g\*min) |  | 191000 | 407000 |
| AUCinf\_pred (ng\*g-1\* min) | 96600 |  |  |
|  |  |  |  |

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**Experiment Number:** K06898 **Toxicokinetics Data Summary Request Date:** 7/11/2023

**Route:** Gavage, IV **Compound:** alpha/beta-Thujone mixture/ **Analyte:** alpha-Thujone **Request Time:** 10:03:16

**Species/Strain:** Rats/Fischer 344 **CAS Number:** 76231-76-0 **Lab:** Battelle Columbus

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LEGEND

MODELING SOFTWARE

 WinNonlin Version 5.0.1

MODELING METHOD & BEST FIT MODEL

 aWinNonlin, Version 5.0.1, Pharsight Corporation, Mountain View, CA, Noncompartmental Analysis (NCA)

bWinNonlin, Version 5.0.1, Pharsight Corporation, Mountain View, CA, one-compartment with first order absorption and elimination with 1/Yhat2 weighting (Model No. 3)

cWinNonlin, Version 5.0.1, Pharsight Corporation, Mountain View, CA, Two compartment model with bolus input and first order elimination with 1/Yhat2 weighting (Model No. 8)

EXCEPTION

 dAUC 0-T standard error of the mean, SE, was ND, not detected.

 eThe 37.53 ng/mL concentration at 90 minutes was not used in modeling.

ANALYTE

 Alpha-Thujone

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**Experiment Number:** K06898 **Toxicokinetics Data Summary Request Date:** 7/11/2023

**Route:** Gavage, IV **Compound:** alpha/beta-Thujone mixture/ **Analyte:** alpha-Thujone **Request Time:** 10:03:16

**Species/Strain:** Rats/Fischer 344 **CAS Number:** 76231-76-0 **Lab:** Battelle Columbus

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TK PARAMETERS

 C\_0min\_pred = Fitted plasma concentration at time zero (IV only)

 Cmax\_pred = Observed or Predicted Maximum plasma (or tissue) concentration

 Tmax\_pred = Time at which Cmax predicted or observed occurs

 Cmax\_obs = Observed or Predicted Maximum plasma (or tissue) concentration

 Tmax\_obs = Time at which Cmax predicted or observed occurs

 Half-life = Lambda z Half life, t 1/2, the terminal elimination half-life based on non-compartmental analysis

 Alpha Half-Life = Half-life for the alpha phase

 Beta Half-Life = Half-life for the beta phase

 k01 = Absorption rate constant, ka

 k01 Half-life = Half-life of the absorption process to the central compartment

 k10 = Elimination rate constant from the central compartment also ke or kelim

 k10 Half-life = Half-life for the elimination process from the central compartment

 k12 = Distribution rate constant from first to second compartment

 k21 = Distribution rate constant from second to first compartment

 Cl1 = Clearance of central compartment, Clapp or apparent clearance for intravenous groups

 Cl2 = Clearance of the secondary compartment

 CL1\_F = Apparent clearance of the central compartment, also Cl\_F for gavage groups in non-compartmental model

V1 = Volume of distribution of the central compartment, includes Vd and V volume of distribution, Vz apparent volume of distribution NCA,

Vapp apparent volume of distribution for intravenous studies

 V2 = Volume of distribution for the peripheral compartment

 V1\_F = Apparent volume of distribution for the central compartment includes Vd\_F, V\_F for oral groups, and Vc\_F

 MRT = Mean Residence Time

 AUC\_0-T = Area under the plasma concentration versus time curve, AUC, from time ti (initial) to tf (final), AUClast

 AUCinf\_pred = Area under the plasma concentration versus time curve, AUC, extrapolated to time equals infinity

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**Experiment Number:** K06898 **Toxicokinetics Data Summary Request Date:** 7/11/2023

**Route:** Gavage, IV **Compound:** alpha/beta-Thujone mixture/ **Analyte:** alpha-Thujone **Request Time:** 10:03:16

**Species/Strain:** Rats/Fischer 344 **CAS Number:** 76231-76-0 **Lab:** Battelle Columbus

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TK PARAMETERS PROTOCOL

 ANALYSIS METHOD

Target times for blood and brain collection for the intravenous phase of the study were - male rats at 5, 10, 20, and 45 minutes, and 1, 2, 4, 6, 8, and 12 hours; female rats at 5, 10, 15, 30, and 45 minutes, and 1, 1.5, 2, 2.5, and 3 hours; and male and female mice at 2, 5, 7, 10, 15, 20, 30, and 45 minutes, and 1 and 1.5 hours. Target times for blood and brain collection for the gavage phase of the study were: male and female rats at 2, 5, 10, and 30 minutes, and 1.5, 3, 6, and 12 hours; and male and female mice at 2, 5, 10, 20, and 40 minutes, and 1.5 hours, 2 hours (40 mg/kg female mice only), 3 hours, 4 hours (80 mg/kg female mice only), 5 hours (40 mg/kg male mice only), and 6 hours (80 mg/kg male mice only).

 TK\_INTRAVENOUS PLASMA

 6.0 mg/kg Male and Female

The test article had a purity of 70 percent alpha-Thujone and 11 percent beta-Thujone. Thirty animals/species/sex/compound/dosage group (excluding replacements) were given a single IV injection of a,b-thujone in Cremophor-ethanol-water (1,1,8) using a catheter surgically implanted by the animal supplier into the jugular vein. Dosages were administered at a volume of 2 mL/kg (rats) and 4 mL/kg (mice). Animals were weighed the morning of dosing for calculation of the dosing volume. The dosing volume was administered as a bolus push. Dosed 7/1-3/02.

 TK\_GAVAGE PLASMA

 40 mg/kg, 80 mg/kg Male and Female

Twenty-four animals/species/sex/compound/dosage group (excluding replacements) were given a single oral gavage administration of a,b-thujone in 0.5 percent aqueous methylcellulose. Doses were administered at a volume of 5 mL/kg (rats) and 10 mL/kg (mice). Non-fasted animals were given a single gavage administration. Dosed 12/17-20/02.

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**Experiment Number:** K06898 **Toxicokinetics Data Summary Request Date:** 7/11/2023

**Route:** Gavage, IV **Compound:** alpha/beta-Thujone mixture/ **Analyte:** alpha-Thujone **Request Time:** 10:03:16

**Species/Strain:** Mice/Fischer 344 **CAS Number:** 76231-76-0 **Lab:** Battelle Columbus

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TK PARAMETERS PROTOCOL (cont’d)

 TK\_INTRAVENOUS BRAIN

 6.0 mg/kg Male and Female

The test article had a purity of 70 percent alpha-Thujone and 11 percent beta-Thujone. Thirty animals/species/sex/compound/dosage group (excluding replacements) were given a single IV injection of a,b-thujone in Cremophor-ethanol-water (1,1,8) using a catheter surgically implanted by the animal supplier into the jugular vein. Dosages were administered at a volume of 2 mL/kg (rats) and 4 mL/kg (mice). Animals were weighed the morning of dosing for calculation of the dosing volume. The dosing volume was administered as a bolus push. Dosed 7/1-3/02.

 TK\_GAVAGE BRAIN

 40 mg/kg, 80 mg/kg Male and Female

Twenty-four animals/species/sex/compound/dosage group (excluding replacements) were given a single oral gavage administration of a,b-thujone in 0.5 percent aqueous methylcellulose. Doses were administered at a volume of 5 mL/kg (rats) and 10 mL/kg (mice). Non-fasted animals were given a single gavage administration. Dosed 12/17-20/02.

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