Experiment Number: K18071 | **Toxicokinetics Data Summary** | **Request Date:** 11/27/2019

Route: Gavage , Fetal transfer | **Compound/Analyte:** Hydroxyurea/Hydroxyurea | **Request Time:** 2:30:16

Species/Strain: Rat/Harlan Sprague-Dawley | **CAS Number:** 127-07-1 | **Lab:** RTI

Female (Dam)

Treatment Group (mg/kg/day)

	18.8 Gav[a] Plasma	18.8 Gav[b] Amniotic Fluid	18.8 Gav[a] Fetus, Whole
Cmax_pred (ng/mL)	295	.	
Cmax_pred (ng/g)			101
Tmax_pred (hour)	0.333	.	0.333
Lambda_z (hour^-1)	0.644	.	1.405
Half-life (hour)	1.08	.	0.49
AUC_0-T (ng•h/mL)	487	.	
AUC_0-T (ng•h/g)			470
AUCinf_pred (ng•h/mL)	509	.	
AUCinf_pred (ng•h/g)			497

Experiment Number: K18071 | **Toxicokinetics Data Summary** | **Request Date:** 11/27/2019

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Species/Strain: Rat/Harlan Sprague-Dawley | **CAS Number:** 127-07-1 | **Lab:** RTI

Female (Dam)

Treatment Group (mg/kg/day)

	75 Gav[c] Plasma	75 Gav[d] Amniotic Fluid	75 Gav[c] Fetus, Whole
Cmax_pred (ng/mL)	3840	433	
Cmax_pred (ng/g)			4080
Tmax_pred (hour)	0.5	0.5	0.5
Lambda_z (hour^-1)	0.376	.	0.644
Half-life (hour)	1.84	.	1.04
AUC_0-T (ng•h/mL)	5580	2975	
AUC_0-T (ng•h/g)			6269
AUCinf_pred (ng•h/mL)	5689	.	
AUCinf_pred (ng•h/g)			6321

Experiment Number: K18071 | **Toxicokinetics Data Summary** | **Request Date:** 11/27/2019

Route: Gavage , Juvenile and Adolescent | **Compound/Analyte:** Hydroxyurea/Hydroxyurea | **Request Time:** 2:30:16

Species/Strain: Rat/Harlan Sprague-Dawley | **CAS Number:** 127-07-1 | **Lab:** RTI

Dams (for Juveniles) and Pups

Treatment Group (mg/kg/day)

	18.8 Gav[a] Dam Plasma GD17-PND21	18.8 Gav[a] Female Pup Plasma Juvenile PND 10-21	18.8 Gav[a] Male Pup Plasma Juvenile PND 10-21	18.8 Gav[a] Female Pup Plasma Adolescent PND 10-34	18.8 Gav[a] Male Pup Plasma Adolescent PND 10-34
Cmax_pred (ng/mL)	1390	3930	3930	2390	1980
Tmax_pred (hour)	0.166	0.667	0.667	0.5	0.667
Lambda_z (hour^-1)	3.556	0.570	0.997	0.483	0.454
Half-life (hour)	0.19	1.22	0.70	1.44	1.53
AUC_0-T (ng•h/mL)	1000	13346	14351	3298	5257
AUCinf_pred (ng•h/mL)	1003	13641	14385	3340	5442

Experiment Number: K18071
Route: Gavage , Juvenile and Adolescent
Species/Strain: Rat/Harlan Sprague-Dawley

Toxicokinetics Data Summary
Compound/Analyte: Hydroxyurea/Hydroxyurea
CAS Number: 127-07-1

Request Date: 11/27/2019
Request Time: 2:30:16
Lab: RTI

Dams (for Juveniles) and Pups

Treatment Group (mg/kg/day)

	75 Gav[c] Dam Plasma GD17-PND21	75 Gav[c] Female Pup Plasma Juvenile PND 10-21	75 Gav[c] Male Pup Plasma Juvenile PND 10-21	75 Gav[c] Female Pup Plasma Adolescent PND 10-34	75 Gav[c] Male Pup Plasma Adolescent PND 10-34
Cmax_pred (ng/mL)	4490	18900	20800	17900	13400
Tmax_pred (hour)	0.333	0.333	0.333	0.5	0.333
Lambda_z (hour^-1)	0.889	0.471	0.616	0.304	0.524
Half-life (hour)	0.78	1.47	1.13	2.28	1.32
AUC_0-T (ng•h/mL)	8662	65392	84995	40140	40113
AUCinf_pred (ng•h/mL)	8680	67537	86477	46070	41661

Experiment Number: K18071 | **Toxicokinetics Data Summary** | **Request Date:** 11/27/2019

Route: Gavage , Lactational transfer | **Compound/Analyte:** Hydroxyurea/Hydroxyurea | **Request Time:** 2:30:16

Species/Strain: Rat/Harlan Sprague-Dawley | **CAS Number:** 127-07-1 | **Lab:** RTI

Female (Dam)

Treatment Group (mg/kg/day)

	18.8 Gav[a] Dam Plasma GD17-PND14	18.8 Lactational Transfer[e] Female Pup Plasma PND14	18.8 Lactational Transfer[e] Male Pup Plasma PND14
Cmax_pred (ng/mL)	1060	23.2	17.9
Tmax_pred (hour)	0.333	6	0.5
Lambda_z (hour^-1)	0.302	.	.
Half-life (hour)	2.29	.	.
AUC_0-T (ng•h/mL)	1792	254	230
AUCinf_pred (ng•h/mL)	1918	.	.

LEGEND

MODELING METHOD & BEST FIT MODEL

[a] Phoenix Winnonlin (Version 6.3) The half daily target dose (9.4 mg/kg) was used in modeling; non-compartmental analysis Model 200. AUCinf is the observed not the predicted value

[b] Phoenix Winnonlin (Version 6.3) The half daily target dose (9.4 mg/kg) was used in modeling; non-compartmental analysis Model 200. The concentration for all but one sample of amniotic fluid in this group was less than the LOD (11 ng/mL), so no parameters could be calculated

[c] Phoenix Winnonlin (Version 6.3) The half daily target dose (37.5 mg/kg) was used in modeling; non-compartmental analysis Model 200. AUCinf is the observed not the predicted value.

[d] Phoenix Winnonlin (Version 6.3) The half daily target dose (37.5 mg/kg) was used in modeling; non-compartmental analysis Model 200. For amniotic fluid, the r^2 was 0.005 indicating a poor curve fit so Lamda_z and Half-life were not reported. AUCinf is the observed not the predicted value.

[e] Phoenix Winnonlin (Version 6.3) The half daily target dose (9.4 mg/kg) was used in modeling. non-compartmental analysis Model 200. Pups were not gavage dosed. Plasma concentrations from the two pups per sex per time point were averaged for WinNonlin analysis. Lambda_z, Half-life and AUCinf(obs) had values that were below ELOQ or below the LOD. Curve fits were poor (r^2=0.0148 for female, no fit was possible for the male) so these parameters could not be reliably estimated

ANALYTE

Hydroxyurea

TK PARAMETERS

Cmax = Observed or Predicted Maximum plasma (or tissue) concentration
Tmax = Time at which Cmax predicted or observed occurs
Lambda_z = Non-compartmental analysis (NCA) terminal elimination rate constant, NCA ke or kelim
Half_life = Lambda z Half life, t 1/2, the terminal elimination half-life based on non-compartmental analysis
AUC_0-T = Area under the plasma concentration versus time curve, AUC, from time ti (initial) to tf (final), AUClast
AUCinf = Area under the plasma concentration versus time curve, AUC, extrapolated to time equals infinity

TK PARAMETERS PROTOCOL

PLASMA

Gavage 18.8 Rat female dam F1-Group 1 GD17, Gavage 75 Rat female dam F2-Group 2 GD17**

Block 1 (Dam and Fetal group-Fetal Transfer) pregnant female rats were dosed twice on **one** day (doses 6 hours apart) on GD17. The doses (2 x 9.4 mg/kg for a total of 18.8 mg/kg/day after second dose or 2 x 37.5 mg/kg for a total of 75 mg/kg/day after second dose) were administered by gavage. Rat dam plasma, amniotic fluid, and whole fetus samples were collected from one dam per dose group at each of 10 time points (0 [predose], 5, 10, 20, 30, 40, 60, 90, 180 and 360 min) following each dose administration (first n=1 per time point and then second dose n=1 per time point). Whole fetus samples were pooled by litter. Time-mated pregnant rats were 200-240 g and 11-13 weeks old on GD0. For determination of gestational day, the day after breeding by the vendor was designated as GD0. Extracted and protein precipitated samples were analyzed by hydrophilic interaction liquid chromatography-tandem mass spectrometry.

AMNIOTIC FLUID

Gavage 18.8 Rat female dam F1-Group 1 GD17, Gavage 75 Rat female dam F2-Group 2 GD17**

Block 1 (Dam and Fetal group-Fetal Transfer) pregnant female rats were dosed twice on **one** day (doses 6 hours apart) on GD17. The doses (2 x 9.4 mg/kg for a total of 18.8 mg/kg/day after second dose or 2 x 37.5 mg/kg for a total of 75 mg/kg/day after second dose) were administered by gavage. Rat dam plasma, amniotic fluid, and whole fetus samples were collected from one dam per dose group at each of 10 time points (0 [predose], 5, 10, 20, 30, 40, 60, 90, 180 and 360 min) following each dose administration (first n=1 per time point and then second dose n=1 per time point). Whole fetus samples were pooled by litter. Extracted and protein precipitated samples were analyzed by hydrophilic interaction liquid chromatography-tandem mass spectrometry.

**F1 and F2 represent group names for dams and not generations

TK PARAMETERS PROTOCOL (cont'd)

FETUS, WHOLE

Gavage_18.8_Rat_female_dam_F1-Group 1_GD17, Gavage_75_Rat_female_dam_F2-Group 2_GD17**

Block 1 (Dam and Fetal group-Fetal Transfer) pregnant female rats were dosed twice on one day (doses 6 hours apart) on GD17. The doses (2 x 9.4 mg/kg for a total of 18.8 mg/kg/day after second dose or 2 x 37.5 mg/kg for a total of 75 mg/kg/day after second dose) were administered by gavage. Rat dam plasma, amniotic fluid, and whole fetus samples were collected from one dam per dose group at each of 10 time points (0 [predose], 5, 10, 20, 30, 40, 60, 90, 180 and 360 min) following each dose administration (first n=1 per time point and then second dose n=1 per time point). Whole fetus samples were pooled by litter. Extracted and protein precipitated samples were analyzed by hydrophilic interaction liquid chromatography-tandem mass spectrometry.

PLASMA

Gavage_18.8_Rat_female_dam_D1-Group 3_GD17-PND21, Gavage_75_Rat_female_dam_D2-Group 4_GD17-PND21

Block 2 (Juvenile and Adolescent group) pregnant female rats were dosed twice for 5 days (GD17-PND21) (doses 6 hours apart) on GD17-PND21. The doses (2 x 9.4 mg/kg for a total of 18.8 mg/kg/day or 2 x 37.5 mg/kg for a total of 75 mg/kg/day) were administered by gavage. Rat dam plasma samples were collected from one dam per dose group at each of 10 time points (0 [predose], 5, 10, 20, 30, 40, 60, 90, 180 and 360 min) on the last day of dosing. Blood was sampled from the dams in the Juvenile group by tail vein bleeding of one dam/group at each of 10 time points following the first dose administration on PND 21 and by cardiac puncture of one dam/group (same rats as used for the tail vein bleeding) at each of 10 time points following the second dose administration on PND 21. Pups and dams were matched by litter for each time point on PND 21. Time-mated pregnant rats were 200-240 g and 11-13 weeks old on GD0. For determination of gestational day, the day after breeding by the vendor was designated as GD0. Extracted and protein precipitated samples were analyzed by hydrophilic interaction liquid chromatography-tandem mass spectrometry.

**F1 and F2 represent group names for dams and not generations

TK PARAMETERS PROTOCOL (cont'd)

PLASMA

Gavage_18.8_Rat_female_pup_J1-Group 5_PND10-21, Gavage_18.8_Rat_male_pup_J1-Group 5_PND10-21, Gavage_75_Rat_female_pup_J2-Group 6_PND10-21, Gavage_75_Rat_male_pup_J2-Group 6_PND10-21

Block 2 (Juvenile and Adolescent group) Juvenile pups were housed with their mothers (Groups 3 or 4) and received exposure via their dams (dosed twice daily from GD17-PND21) and then from PND10-PND21 the pups were dosed themselves (twice daily). At PND4, the pups were culled to 5 per sex per litter. The doses (2 x 9.4 mg/kg for a total of 18.8 mg/kg/day or 2 x 37.5 mg/kg for a total of 75 mg/kg/day) were administered by gavage. Pups and dams were matched by litter for each time point on the last day of dosing, PND 21. Plasma samples were collected from one pup per sex per dose group (matched to the dam) at each of 10 time points (0 [predose], 5, 10, 20, 30, 40, 60, 90, 180 and 360 min) after the first dose and 10 time points again after the second dose. Extracted and protein precipitated samples were analyzed by hydrophilic interaction liquid chromatography-tandem mass spectrometry.

Gavage_18.8_Rat_female_pup_A1-Group 9_PND10-34, Gavage_18.8_Rat_male_pup_A1-Group 9_PND10-34,
Gavage_75_Rat_female_pup_A2-Group 10_PND10-34, Gavage_75_Rat_male_pup_A2-Group 10_PND10-34
For Gavage_18.8_Rat_female_dam_D3-Group 7_GD17-PND21, Gavage_75_Rat_female_dam_D4-Group 8_GD17-PND21 no samples were taken

Block 2 (Juvenile and Adolescent group) Adolescent pups were housed with their mothers (Groups 7 or 8) until weaning (on PND28) and received exposure via their dams (dosed twice daily from GD17-PND28) and then from PND10-PND34 the pups were dosed themselves (twice daily). At PND4, the pups were culled to 5 per sex per litter. The doses (2 x 9.4 mg/kg for a total of 18.8 mg/kg/day or 2 x 37.5 mg/kg for a total of 75 mg/kg/day) were administered by gavage. At weaning, dams were euthanized with no sampling and pups (8/litter) from a given litter were co-housed from wean until termination on PND 34. One pup per sex per dose (matched to the dam) was euthanized at each of 10 time points (0 [predose], 5, 10, 20, 30, 40, 60, 90, 180 and 360 min) following the first dose and 10 time points after the second dose on PND 34 (last day of dosing). Extracted and protein precipitated samples were analyzed by hydrophilic interaction liquid chromatography-tandem mass spectrometry.

TK PARAMETERS PROTOCOL (cont'd)

PLASMA

Gavage 18.8 Rat female dam D5-Group 11 GD17-PND14

Block 3 (Lactational transfer) Pregnant female rats were dosed twice a day (doses 6 hours apart) from GD17-PND14. The doses (2 x 9.4 mg/kg for a total of 18.8 mg/kg/day) were administered by gavage. Rat dam plasma samples were collected from one dam per dose group at each of 10 time points (0 [predose], 5, 10, 20, 30, 40, 60, 90, 180 and 360 min) on the last day of dosing. Blood was sampled from the dams by tail vein bleeding of one dam/group at each of 10 time points following the first dose administration on PND 14 and by cardiac puncture of one dam/group (same rats as used for the tail vein bleeding) at each of 10 time points following the second dose administration on PND 14. Blood was collected from two pups per sex which were matched by litter to one dam for each time point on PND 14. Time-mated pregnant rats were 200-240g and 11-13 weeks old on GD0. For determination of gestational day, the day after breeding by the vendor was designated as GD0. Extracted and protein precipitated samples were analyzed by hydrophilic interaction liquid chromatography-tandem mass spectrometry.

Gavage 18.8 Rat female pup P-Group 12, TK Gavage 18.8 Rat male pup P-Group 12

Block 3 (Lactational transfer) Dams (D5-Group 11) were dosed twice a day (doses 6 hours apart) from GD17-PND14. The doses (2 x 9.4 mg/kg for a total of 18.8 mg/kg/day) were administered by gavage. Pups were not dosed. Pups were culled 10 per litter, split as evenly as possible between sexes, on PND 4. On PND 14 (the last day dams were dosed) blood was sampled from 1 dam and 2 pups/sex at each of 10 time points (0 [predose], 5, 10, 20, 30, 40, 60, 90, 180 and 360 min) following each dose administration to the dams. Extracted and protein precipitated samples were analyzed by hydrophilic interaction liquid chromatography-tandem mass spectrometry.