Supporting Information

An Integrated Experimental and Physiologically Based Pharmacokinetic Modeling Study of Penicillin G in Heavy Sows

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Methods for Sample Collection, Preparation and Analysis

1. **Sample collection**

For blood collection, sows had blood collected from the left or right jugular vein using a 4 inch 16 gauge hypodermic needle and 12 mL Luer lock syringe. They were physically restrained with a hog snare and at each sample point 8 mL of blood was obtained. All blood samples for use in analysis were collected in glass 10 mL heparin tubes. Blood samples were mixed by inverting the tube, labeled with a unique identifier, and immediately placed on ice. The blood sample was centrifuged at 1000g for 15 minutes.

The tissue samples were collected during necropsies. These necropsies occurred at 1, 6, 14, and 28 days after final administration of procaine penicillin G (PPG) or sterile saline. Any gross pathological abnormalities were noted. Kidney, liver, semitendinosus/ semimembranosus muscle, and the right hip injection sites were collected and submitted for analysis. For injection sites, a two inch circumference around the final injection sites (right hip area) was dissected out for sampling.

For environmental samples, an unscented Swiffer pad was placed in a 50 mL vial containing 25 mL of physiologic saline until all liquid was absorbed by the Swiffer pad. The wet Swiffer was used to scrub a selected sampling area. Any excess fluid was mopped up by the pad, and the entire Swiffer pad was placed in a plastic bag. The fluids were extracted by digital manipulation of the pad, and were removed from the plastic bag. Then the fluids were poured back into the 50 mL vial and stored at -80°C until analysis.

For urine samples, free catch urine was collected from sows prior to euthanasia in a 50 mL Falcon tube. If the free catch sample was unable to be obtained antemortem, the bladder was expressed post-mortem by applying pressure to the flank and collecting the manually expressed

urine in a 50 mL Falcon tube. One sow was unable to be collected by either method so five mL of urine was aspirated from the urinary bladder using a 6 mL Luer Lock syringe with an attached 22 gauge × 3/4" needle. All samples were transferred to a non-additive red top tube and stored at -80 °C prior to analysis.

2. Sample extraction

Tissue samples, including liver, kidney, muscle and injection site, were extracted for LC-MS/MS analysis. Calibration standards for tissues were prepared using standard additions of penicillin G (potassium salt) with 2 grams of ground/processed blank tissue. Blank tissue refers to tissue with no known exposure to penicillin G. Final concentrations of penicillin G were 1, 5, 10, 20, 50, 100, 200, 500, 1000 ng/g. Standards were mixed using a vortex mixer and allowed to sit for 5 minutes. Internal standard, penicillin G-d7 ethylperidinium salt (Sigma, St. Louis, MO), was added to the standards/samples to give a final concentration of 500 ng/g. Ten mL of acetonitrile:water (4:1) was added and standards/samples were mixed using a multi-tube vortexer for 5 minutes. Samples/standards were then centrifuged at 2,500 rpm for 5 minutes. Supernatant was transferred to a 50 mL centrifuge tube containing 0.5 g of C18 sorbent. Ten mL of hexane saturated with acetonitrile was added. Samples/standards were vortexed for 1 minute and centrifuged at 3,500 rpm for 5 minutes. Hexane was then aspirated to waste. Two mL of samples/standards was evaporated to dryness, and then resuspended in 50 µL of 25% (v/v) acetonitrile:water and 150 µL water. Samples were transferred to an autosampler vial with glass insert, and centrifuged for 20 minutes at 2,400 rpm prior to LC-MS/MS analysis.

Plasma samples were thawed and centrifuged. Aliquots of 500 µL were transferred to test tubes. Standards were prepared by adding penicillin G to 500 µL of blank plasma to obtain final concentrations of 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 ng/mL. Quality control (QC) samples

were prepared by adding penicillin G to 500 µL of blank plasma to obtain final concentrations of 15, 150, 750 ng/mL. Internal standard, penicillin G-d7 ethylperidinium salt (Sigma), to give a final concentration of 40 ng/mL was added to each sample/standard. A volume of 2.5 mL acetonitrile was added to each standard/sample, followed by mixing with a vortex mixer, and then centrifugation for 20 min at 2,400 rpm. Supernatant was transferred to a test tube and evaporated to dryness using a stream of nitrogen. Standards/samples were reconstituted in 50 µL of 25% (v/v) acetonitrile in water and mixed with a vortex mixer. Then 150 µL of water was added and mixed with a vortex mixer. Standards/samples were transferred to an autosampler vial with glass insert, centrifuged for 20 min at 2,400 rpm and analyzed via LC-MS/MS.

Frozen urine standards/samples were thawed at room temperature. Standards for urine were prepared by spiking 150 µL of blank urine to concentrations of 10, 20, 50, 100, 200, 500, 1,000, 2,000, 5,000, 10,000, 20,000 and 50,000 ng/mL penicillin G. QC samples were prepared at concentrations of 30, 300, 3,000, and 30,000 ng/mL penicillin G by spiking 150 µL of urine. Internal standard, penicillin G-d7 ethylperidinium salt (Sigma), giving a final concentration of 3333 ng/mL was added to each standard/QC/sample and then diluted 1:8 with water. Standards/QCs/samples were transferred to an autosampler vial with glass insert, centrifuged for 20 minutes at 2,400 rpm and analyzed via LC-MS/MS. Samples that had a concentration higher than 10,000 ng/mL were diluted accordingly with water until their concentrations decreased enough to be in the range of the standard curve.

3. KIS test for kidney samples

The Kidney Inhibition Swab (KIS) test was also performed on porcine kidneys, according to test instructions. The juice from porcine kidney, with no known exposure to antibiotics, was used as the negative control. Positive control was prepared by mixing 0.8 ml of juice from

kidney with no known exposure to antibiotics with 0.5 ml of 50 ng/mL penicillin G. This solution of 50 ng/mL penicillin G, as well as other standards, with concentrations of 10, 20, 30 and 40 ng/mL were prepared in purified water by diluting a 1 µg/µl stock solution of penicillin G, potassium salt, in 60:40 acetonitrile:water. In each case, 0.5 ml of standard was mixed with 0.8 ml of kidney juice, not known to contain antibiotics. Following incubation, the agar color was compared to the colors shown on the interpretation card included with the KIS test supplies. Results were interpreted as positive or negative.

4. Urine sample analysis

Urine samples were prepared and analyzed using LC-MS/MS, Charm MRL and SNAP beta-lactam tests. For Charm MRL test, standards were prepared by diluting a 1 µg/µL stock solution of Penicillin G, potassium salt, in 60:40 acetonitrile:water with water purified using a Milli-Q water purification system (Millipore, Billerica, MA). Standards had final concentrations of 1, 5, 10, 20, 30, 40, 50, 60, 100 and 200 ng/mL. Purified water was also used as the negative control. The positive control was prepared by dissolving one tablet of penicillin G, supplied in the test kit, in 1.0 mL of purified water. Fifty µL of sample was diluted with 450 µL of dilution buffer supplied in the test kit. Three hundred µL of this mixture was applied to the sample pad on the test strip. The strip was incubated for 8 min at 55°C, visually examined and then inserted into a ROSA Reader from Charm Sciences Inc. (Lawrence, MA). Prior to analysis, positive and negative calibration strips, supplied by the manufacturer, were read as a daily performance check. Samples were reported as positive or negative, according to the ROSA Reader result, as long as the positive control gave a reading greater than +400 and the negative control gave a reading less than -400, according to test kit instructions.

The new SNAP beta-lactam test from IDEXX (Westbrook, ME) was used according to instructions. Urine samples were centrifuged at 1,200 g for 3 min prior to analysis. A disposable pipette, provided with each SNAP device, was used to draw up 450 µL (±50 µL) of sample. The sample was placed in the sample tube provided which contained a reagent pellet. After mixing thoroughly, the sample tube was incubated for 5 min at 45°C (±5°C). Following incubation, the sample was poured into the sample well on the SNAP device. As soon as the edge of the activation circle began to disappear, the activator was pushed down and the test device was left on the heater block for another 4 min. SNAP devices were verified visually and read using a SNAPshot Reader. Standards, with concentrations of 1, 5, 10, 20, 30, 40, 50 and 60 ng/mL were prepared in purified water by diluting a 1 µg/µL stock solution of penicillin G, potassium salt, in 60:40 acetonitrile:water. Purified water was used as negative control and to reconstitute the positive control, penicillin G, supplied in the test kit. Samples were reported as negative when the reading on the IDEXX SNAPshot Reader was 1.05 or lower, according to test kit instructions.

5. Environmental sample analysis

Environmental samples were also prepared and analyzed using Charm MRL and SNAP beta-lactam tests. For Charm MRL test, environmental samples were analyzed according to test kit instructions: 50 µL of sample was diluted with 450 µL of dilution buffer supplied in the test kit. Three hundred µL of this mixture was applied to the sample pad on the test strip. The strip was incubated for 8 min at 55 °C, visually examined and then inserted into a ROSA Reader. Purified water was used as the negative control. The positive control was prepared by dissolving one tablet of penicillin G, supplied in the test kit, in 1.0 ml of purified water. Prior to analysis, positive and negative calibration strips, supplied by the manufacturer, were read as a daily

performance check. Samples were reported as positive or negative, according to the ROSA Reader result, as long as the positive control gave a reading greater than +400 and the negative control gave a reading less than -400, according to test kit instructions.

For SNAP test, environmental samples were mixed using a vortex mixer then centrifuged at 15,000 rpm for 20 min prior to analysis. A disposable pipette, provided with each SNAP device, was used to draw up 450 µL (±50 µL) of sample. The sample was placed in the sample tube provided which contained a reagent pellet. After mixing thoroughly, the sample tube was incubated for 5 min at 45°C (±5°C). Following incubation, the sample was poured into the sample well on the SNAP device. As soon as the edge of the activation circle began to disappear, the activator was pushed down and the test device was left on the heater block for another 4 min. SNAP devices were verified visually and read using a SNAPshot Reader. Purified water was used as negative control and to reconstitute the positive control, penicillin G, supplied in the test kit. Samples were reported as negative when the reading on the IDEXX SNAPshot Reader was 1.05 or lower, according to test kit instructions.

Results of Plasma, Urine, Oral Fluid, and Environmental Samples

Penicillin G residue concentrations in the plasma samples of individual animals in the three treatment groups are provided in **Table S4a**, **Table S4b**, and **Table S4c**, respectively. Plasma samples of TG1 revealed penicillin G residues in 100% sows at Day 1 and Day 6. Ninety-two percent of sows sampled on Day 3 had detectable plasma residues. No residues were found in plasma samples in sows necropsied on Day 14 or Day 28. Residue concentrations decreased as time increased from last administration. In the negative control group, there was only one plasma sample with penicillin G residues, which may be due to false positive. TG3 had 100% residue detection in plasma samples at Day 1, Day 3 and Day 6. Seventy-five percent of sows had residues detected in plasma samples at Day 14, but no residues were identified at Day 28. Similar to the tissue residue pattern, extended residue detection was seen with the TG3 sows compared with TG1. Plasma detection of penicillin G residues by LC-MS/MS was more sensitive than detection of residues in kidney tissue by LC-MS/MS. Plasma detection level of penicillin G residues was similar to the level seen in the skeletal muscle. However, plasma concentrations were no indication of injection site concentrations.

Oral fluids were proposed to provide a practical sample of potential penicillin G contamination of the environment from treated sows. A previous study of oral fluid collection in individually housed sows revealed an oral fluid collection rate of only 23.2% of sows that were sampled on their first day of rope exposure (Pepin et al., 2015). The study also noticed an association with younger sows and a willingness to chew on ropes (Pepin et al., 2015). In the present study, ropes were hung in each pen during the 72 hour acclimatization period once a day for 30 minutes on each day of acclimatization. During this period only one sow in one pen chewed on ropes. Based on these observations and due to failure of sows in this study to provide

oral fluid samples, these data were not analyzed and environmental sampling was selected to be used as a more reliable assay for sampling the environment than oral fluids.

Results of environmental samples analyzed with the Charm MRL and SNAP rapid tests are reported in **Table S5.** Widely varying results were seen between the two assays. All environmental samples tested with Charm MRL were negative for penicillin G residues. This is in contrast to the SNAP tests with the same samples. At time 0, before any administration of PPG product, two positive tests were detected by SNAP test. On Day 1 after PPG administration 33% samples analyzed were positive on the SNAP test. All other samples were positive with the SNAP test except for one sow on Day 3. Based on these results SNAP appears to be a more sensitive assay for penicillin G residues. However, it is possible that some of the positives may be false positives. There was no consistency between the Charm MRL and SNAP tests or when correlated back to any of the other sample types. SNAP tests would be more useful than Charm MRL to use as a screening test for presence of penicillin G residues in the environment. Residues were consistently detected in the environment with the SNAP test, which indicates presence of penicillin G residues in the environment surrounding sows treated with PPG within at least 28 days. Based on these Charm MRL and SNAP results, using environmental sample testing to detect penicillin G residues in a group of sows may not be an accurate antemortem assessment.

Urine samples were analyzed by LC-MS/MS, SNAP and Charm MRL tests. Analysis with LC-MS/MS found penicillin G residues in 100% of available samples at Day 1 and Day 6 for TG1. All other time points for TG1 tested negative. The TG2 control group had no residues in urine samples in any sow at any time point. Penicillin G residues in TG3 sows were detected out to Day 14. All of sows at Day 1 and Day 6 had very concentrated residues. Fifty percent sows necropsied on Day 14 had penicillin G residues present in the urine, and no sows had

penicillin G residues present at Day 28. A complete list of urine analysis result is provided in **Table S6**. The Charm MRL tests that reported a positive urine sample correlated with the positive LC-MS/MS samples in TG1 with residues present at least 6 days post-administration of PPG. All but one positive sample on the Charm MRL test agreed with the positive LC-MS/MS samples of TG3, indicating residues present at least 14 days post-administration. TG2 Charm MRL tests had two samples test positive, which did not agree with the LC-MS/MS and should not be present in a truly negative animal. These samples that tested positive were present in one sow sampled at Day 1 and one sampled a Day 14. The SNAP tests performed on these two particular samples were also positive. The SNAP test for TG2 also had three more positive tests, one on Day 1, one on day 6 and one on Day 14. SNAP tests performed on TG1 sows agreed with the Charm MRL and LC-MS/MS except for two sows on Day 14 and one sow on Day 28. SNAP test results for TG3 also correlated well with the Charm MRL and LC-MS/MS results. There were two more positive tests on the SNAP than the Charm MRL, one sow at Day 14 and another at Day 28.

Supplementary Figures

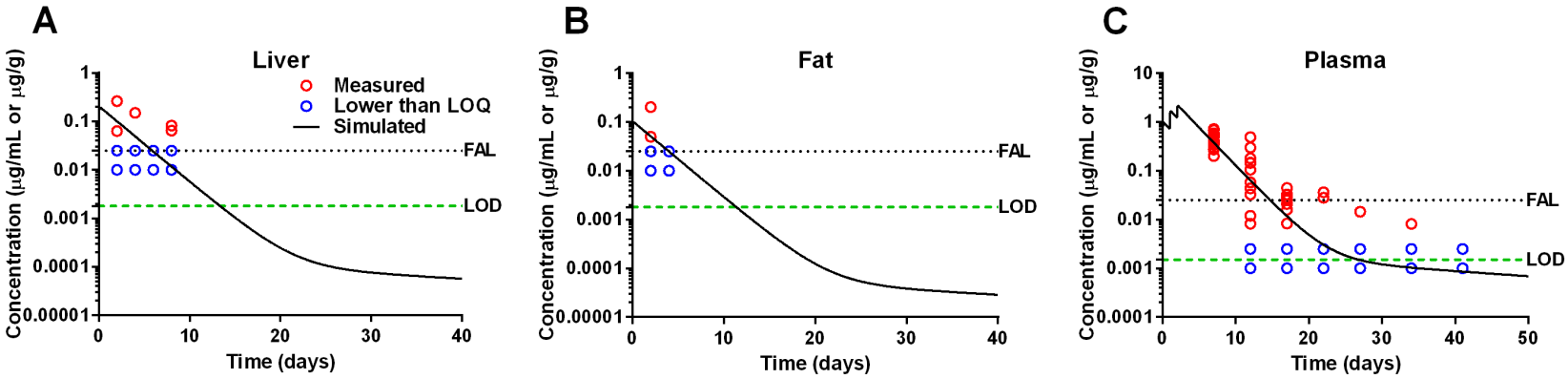

Figure S1. Calibration of the heavy sow PBPK model. Comparison of model simulations (solid line) and observed data (red circles) for concentrations of penicillin G in the liver, fat and plasma of heavy sows exposed to PPG via single IM injection (32.5 mg/kg, A, B) and repeated 3 doses of IM injections (32.5 mg/kg, C). Experimental data (individual data points) are from previous studies: panel A and B (Apley et al. 2009); panel C (Lupton et al. 2014). The data points less than LOQ were marked with the 0.5-fold and 0.2-fold LOQ using blue circles. The values of LOQs are summarized in Table 1. The FSIS action limit (FAL) is shown using the dotted line. FAL for penicillin G in heavy sows is 25 ng/g (FSIS, 2013). The limit of detection (LOD) is shown on each panel using green dash line. LODs for the liver and fat are 1.8 ng/g, and LOD for the plasma is 1.5 ng/g (Lupton et al., 2014).

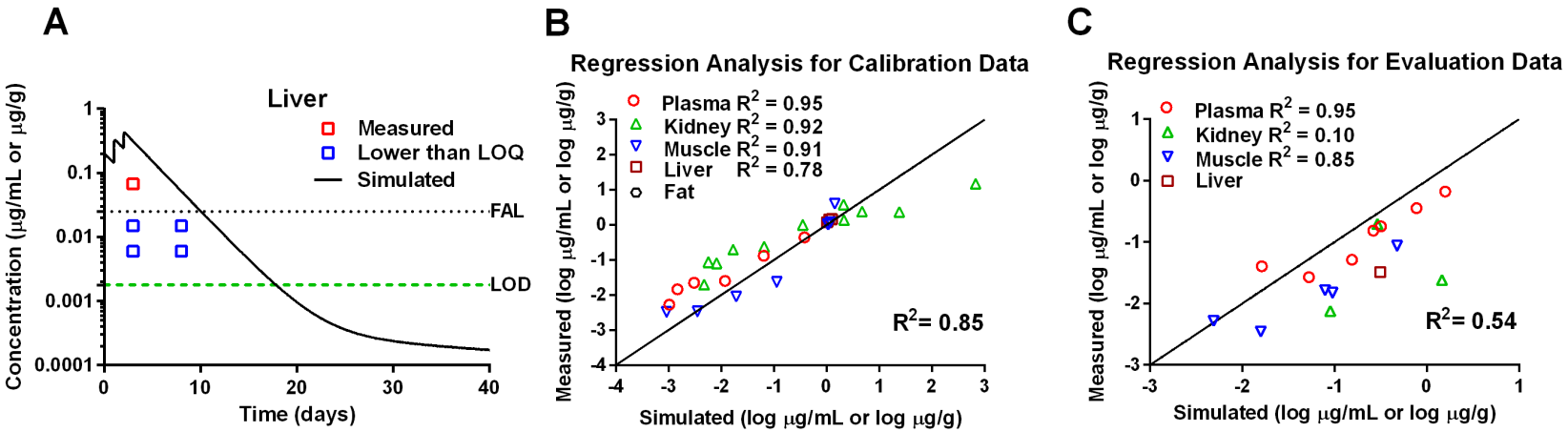

Figure S2. Evaluation and regression analysis of the heavy sow PBPK model simulation results. Comparison of model simulations (solid line) and observed data (red squares) for concentrations of penicillin G in the liver of heavy sows exposed to PPG with repeated 3 doses of IM injections (32.5 mg/kg, A). The data points less than LOQ were marked with the 0.5-fold and 0.2-fold LOQ using blue squares. The values of LOQs are summarized in Table 1. The FSIS action limit (FAL) is shown using the dotted line. FAL for penicillin G in heavy sows is 25 ng/g (FSIS, 2013). The limit of detection (LOD) is shown on each panel using green dash line. LODs for the liver is 0.2 ng/g. The results of regression analysis for calibration datasets are shown in panel B and for evaluation datasets are shown in panel C. The determination coefficients (R^2) for individual tissue and for pooled tissue data are also shown (B and C).

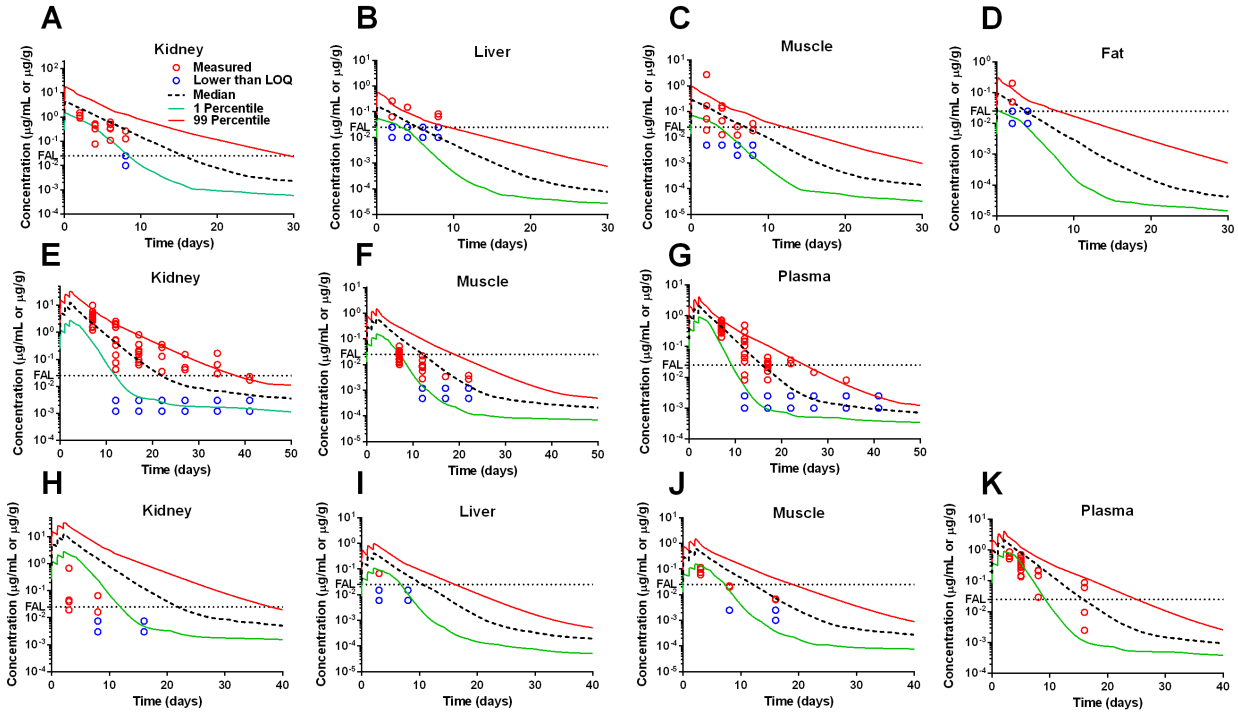

Figure S3. Monte Carlo simulations of penicillin G concentrations in plasma and tissues of heavy sows using the population PBPK model. The commonly used extralabel dose (5× label dose, 32.5 mg/kg) with single IM injection (A-D) and with 3 repeated IM injections (E-K) were simulated as the therapeutic scenarios for heavy sows. Each of the simulations was run for 1,000 iterations. The median, 1th and 99th percentiles of simulated results were plotted. The pharmacokinetic data (red and blue circles) from the previous studies (A-D) (Apley et al., 2009), (E-G) (Lupton et al., 2014) and current study (H-K) were plotted with the population PBPK model simulation results. The data points less than LOQ were marked with the 0.5-fold and 0.2-fold LOQ using blue squares. The FSIS action limit (FAL) is shown on each of panels using the dotted line. The extended withdrawal intervals were determined when tissue concentrations of penicillin G deplete to be below FAL for the 99th percentile of the population.

Supplementary Tables

Table S1. Study animal weights and necropsy group allocation information.

		Treatment Group 1		Treatment Group 2		Treatment Group 3	
Necropsy Group	**Pen Number**	**Sow ID**	**Weight (lb)**	**Sow ID**	**Weight (lb)**	**Sow ID**	**Weight (lb)**
G1	**1**	N/A	N/A	468	525.5	447	554
(day 1)	**2**	342	536.5	463	452	441	384
	3	473	508.5	462	536.5	444	512.5
	4	339	554.5	471	496	453	516
G2	**1**	470	586.5	456	456	450	489.5
(day 6)	**2**	446	564.5	466	564.5	467	567.5
	3	345	582.5	452	575.5	455	590.5
	4	474	592.5	350	588.5	346	640.5
G3	**1**	472	499	458	593.5	443	571.5
(day 14)	**2**	349	587.5	347	494.5	457	441
	3	344	471.5	451	469.5	454	539
	4	440	461.5	445	524.5	461	446
G4	**1**	340	509	459	594.5	341	515
(day 28)	**2**	348	492.5	460	608.5	442	590
	3	448	542	343	334	464	489
	4	465	506.5	469	525.5	449	505

Note: Sows in Treatment Group 1 (TG1) and Treatment Group 3 (TG3) received a dose of 3,000 IU/lb (6.5 mg/kg) and 15,000 IU/lb (32.5 mg/kg) procaine penicillin G IM for three consecutive days. Sows in Treatment Group 2 (TG2) received a dose of sterile saline equal to the average volume of procaine penicillin G administered to TG1 and TG3, but not exceeding 20 mL. Necropsy groups were named as Group 1 (G1), Group 2 (G2), Group 3 (G3) and Group 4 (G4), respectively.

Table S2. A summary of urine production rates for swine.

Breed	Body Weight (kg)	Urine Production Rate (L/h/kg)	Reference
Danish landrace pigs	12-18	0.012	[1]
Yorkshire/Duroc cross swine	21.3	0.003	[2]
Yorkshire gilts	40	0.006	[3]
Gestating sow	200	0.0003	[4]
Sow	196	0.002	[5]

Note: Published data using laboratory pigs, market-age swine and farm-raised sows were used to calculate the estimated range of urine production rate for sows, due to limited data available. The large variabilities may be because of different raising conditions, breeds, animal ages and urine collection methods. [1] Deding et al. 2006; [2] Hannon et al. 1990; [3] Patience et al. 1987; [4] Hamilton et al. 1997; [5] Chastain et al. 1999.

Table S3. Kidney Inhibition Swab (KIS) Test on kidney tissues.

		Treatment Group 1			Treatment Group 2			Treatment Group 3		
Necropsy Group	**Pen Number**	**Sow ID**	**LC-MS/MS**	**KIS Result**	**Sow ID**	**LC-MS/MS**	**KIS Result**	**Sow ID**	**LC-MS/MS**	**KIS Result**
G1 (day 1)	**1**	N/A	N/A	N/A	468	<LOQ	NEG	447	19.4	POS
	2	342	<LOQ	NEG	463	<LOQ	NEG	441	38.8	POS
	3	473	31.7	NEG	462	<LOQ	NEG	444	44.7	POS
	4	339	16.4	POS	471	<LOQ	NEG	453	679.9	POS
G2 (day 6)	**1**	470	<LOQ	NEG	456	<LOQ	NEG	450	64.9	POS
	2	446	96.4	NEG	466	<LOQ	NEG	467	16.3	NEG
	3	345	<LOQ	NEG	452	<LOQ	NEG	455	<LOQ	NEG
	4	474	<LOQ	NEG	350	<LOQ	NEG	346	<LOQ	POS
G3 (day 14)	**1**	472	<LOQ	NEG	458	<LOQ	NEG	443	<LOQ	NEG
	2	349	<LOQ	NEG	347	<LOQ	NEG	457	<LOQ	NEG
	3	344	<LOQ	NEG	451	<LOQ	NEG	454	<LOQ	NEG
	4	440	<LOQ	NEG	445	<LOQ	NEG	461	<LOQ	NEG
G4 (day 28)	**1**	340	<LOQ	NEG	459	<LOQ	NEG	341	<LOQ	NEG
	2	348	48.4	NEG	460	<LOQ	NEG	442	<LOQ	NEG
	3	448	<LOQ	NEG	343	<LOQ	NEG	464	<LOQ	NEG
	4	465	<LOQ	NEG	469	<LOQ	NEG	449	<LOQ	NEG

Note: Results were reported as positive (POS) or negative (NEG). A positive or negative result was determined by a color change. LC-MS/MS kidney values from Table 3 are included for comparison and reported in ng/g.

Table S4a. Plasma procaine penicillin G concentrations (ng/mL) in Treatment Group 1 (TG1) after IM administration at 3,000 IU/lb.

Necropsy Group	Pen Number	Sow ID	Days Post-Administration					
			0	1	3	6	14	28
G1 (day 1)	1							
	2	342	<LOQ	169				
	3	473	<LOQ	247.5				
	4	339	<LOQ	124				
G2 (day 6)	1	470	<LOQ		23	15.8		
	2	446	<LOQ		85.3	40.2		
	3	345	<LOQ		24.5	7.4		
	4	474	<LOQ		100.3	43.8		
G3 (day 14)	1	472	<LOQ		22.7		<LOQ	
	2	349	<LOQ		136.6		<LOQ	
	3	344	<LOQ		20.1		<LOQ	
	4	440	<LOQ		71.8		<LOQ	
G4 (day 28)	1	340	<LOQ		42.2			<LOQ
	2	348	<LOQ		<LOQ			<LOQ
	3	448	<LOQ		18.2			<LOQ
	4	465	7.5		70.1			<LOQ

Note: Concentrations that were below the level of quantification (LOQ) (5 ng/mL) was designated "<LOQ".

Table S4b. Plasma procaine penicillin G concentrations (ng/mL) in Treatment Group 2 (TG2) after IM administration of sterile saline at the volume equivalent of 9,000 IU/lb procaine penicillin G and not exceeding 20 mL.

Necropsy Group	Pen Number	Sow ID	Days Post-Administration					
			0	1	3	6	14	28
G1 (day 1)	1	468	<LOQ	<LOQ				
	2	463	<LOQ	<LOQ				
	3	462	<LOQ	<LOQ				
	4	471	<LOQ	<LOQ				
G2 (day 6)	1	456	<LOQ		<LOQ	8.2		
	2	466	<LOQ		<LOQ	<LOQ		
	3	452	<LOQ		<LOQ	<LOQ		
	4	350	<LOQ		<LOQ	<LOQ		
G3 (day 14)	1	458	<LOQ		<LOQ		<LOQ	
	2	347	<LOQ		<LOQ		<LOQ	
	3	451	<LOQ		<LOQ		<LOQ	
	4	445	<LOQ		<LOQ		<LOQ	
G4 (day 28)	1	459	<LOQ		<LOQ			<LOQ
	2	460	<LOQ		<LOQ			<LOQ
	3	343	<LOQ		<LOQ			<LOQ
	4	469	24.9		<LOQ			<LOQ

Note: Concentrations that were below the level of quantification (LOQ) (5 ng/mL) was designated "<LOQ".

Table S4c. Plasma procaine penicillin G concentrations (ng/mL) in Treatment Group 3 (TG3) after IM administration at 15,000 IU/lb.

Necropsy Group	Pen Number	Sow ID	Days Post-Administration					
			0	1	3	6	14	28
G1 (day 1)	1	447	<LOQ	641.3				
	2	441	<LOQ	620.3				
	3	444	<LOQ	871.9				
	4	453	<LOQ	520.4				
G2 (day 6)	1	450	<LOQ		336.3	213.1		
	2	467	<LOQ		401	149.5		
	3	455	<LOQ		470.6	29.5		
	4	346	<LOQ		299.4	220.6		
G3 (day 14)	1	443	<LOQ		226		88.2	
	2	457	<LOQ		411.6		<LOQ	
	3	454	<LOQ		713.6		61.2	
	4	461	<LOQ		298.9		9.6	
G4 (day 28)	1	341	<LOQ		277			<LOQ
	2	442	<LOQ		552.4			<LOQ
	3	464	<LOQ		145.9			<LOQ
	4	449	<LOQ		136.2			<LOQ

Note: Concentrations that were below the level of quantification (LOQ) (5 ng/mL) was designated "<LOQ".

Table S5. Environmental sample procaine penicillin G residues as determined by Charm MRL and SNAP beta-lactam tests.

			Charm MRL						SNAP					
			Days Post-Administration						Days Post-Administration					
Necropsy Group	Pen Number	Sow ID	0	1	3	6	14	28	0	1	3	6	14	28
G1 (day 1)	1	468, 447	NEG	NEG					NEG	N/A				
	2	342, 463, 441	NEG	NEG					NEG	NEG				
	3	473, 462, 444	NEG	NEG					NEG	POS				
	4	339, 471, 453	NEG	NEG					NEG	NEG				
G2 (day 6)	1	470, 456, 450	NEG		NEG	NEG			NEG		POS	POS		
	2	446, 466, 467	NEG		NEG	NEG			NEG		POS	POS		
	3	345, 452, 455	NEG		NEG	NEG			NEG		POS	POS		
	4	474, 350, 346	NEG		NEG	NEG			NEG		POS	POS		
G3 (day14)	1	472, 458, 443	NEG		NEG		NEG		NEG		POS		POS	
	2	349, 347, 457	NEG		NEG		NEG		NEG		POS		POS	
	3	344, 451, 454	NEG		NEG		NEG		POS		POS		POS	
	4	440, 445, 461	NEG		NEG		NEG		POS		POS		POS	
G4 (day 28)	1	340, 459, 341	NEG		NEG			NEG	NEG		POS			POS
	2	348, 460, 442	NEG		NEG			NEG	NEG		POS			POS
	3	448, 343, 464	NEG		NEG			NEG	NEG		POS			POS
	4	465, 469, 449	NEG		NEG			NEG	NEG		NEG			POS

Note: Charm test values were assigned "positive" if greater than +400 and "negative" if lower than -400. SNAP test values were assign "positive" with a reading of 1.06 or higher and "negative" with a reading of 1.05 or lower.

Table S6. Urine procaine penicillin G concentrations (ng/mL) as determined by LC-MS/MS, Charm MRL, and SNAP beta-lactam test kit.

		Treatment Group 1				Treatment Group 2				Treatment Group 3			
Necropsy Group	Pen Number	Sow ID	LC-MS/MS	Charm	SNAP	Sow ID	LC-MS/MS	Charm	SNAP	Sow ID	LC-MS/MS	Charm	SNAP
G1 (day 1)	1	N/A	N/A	N/A	N/A	468	<LOQ	NEG	NEG	447	1156912.8	POS	POS
	2	342	12488	POS	POS	463	<LOQ	POS	POS	441	654840	POS	POS
	3	473	31502.2	POS	POS	462	<LOQ	NEG	POS	444	313796.6	POS	POS
	4	339	37580.8	POS	POS	471	<LOQ	NEG	NEG	453	417324	POS	POS
G2 (day 6)	1	470	11270.8	POS	POS	456	<LOQ	NEG	NEG	450	48974.6	POS	POS
	2	446	869.2	POS	POS	466	<LOQ	NEG	NEG	467	26788.7	POS	POS
	3	345	2335.2	POS	POS	452	<LOQ	NEG	POS	455	16663.2	POS	POS
	4	474	N/A	POS	POS	350	<LOQ	NEG	NEG	346	56246.8	POS	POS
G3 (day 14)	1	472	<LOQ	NEG	NEG	458	<LOQ	POS	POS	443	<LOQ	POS	POS
	2	349	<LOQ	NEG	POS	347	<LOQ	NEG	NEG	457	<LOQ	NEG	POS
	3	344	<LOQ	NEG	NEG	451	<LOQ	NEG	POS	454	5419.4	POS	POS
	4	440	<LOQ	NEG	POS	445	<LOQ	NEG	NEG	461	1145.7	POS	POS
G4 (day 28)	1	340	<LOQ	NEG	POS	459	<LOQ	NEG	NEG	341	<LOQ	NEG	NEG
	2	348	N/A	NEG	NEG	460	<LOQ	NEG	NEG	442	<LOQ	NEG	NEG
	3	448	<LOQ	NEG	NEG	343	<LOQ	NEG	NEG	464	N/A	NEG	POS
	4	465	<LOQ	NEG	NEG	469	<LOQ	NEG	NEG	449	<LOQ	NEG	NEG

Note: Concentrations that were below the level of quantification (LOQ) (50 ng/mL) with LC-MS/MS was designated "LOQ". Charm test values were assigned "positive" if greater than +400 and "negative" if lower than -400. Snap test values were assign "positive" with a reading of 1.06 or higher and "negative" with a reading of 1.05 or lower.

Table S7. Normalized sensitivity coefficients (NSCs) of representative parameters using area under the concentration curves (AUCs) of penicillin G in plasma, liver, kidney and muscle of heavy sows as the dose metrics.

Parameters	AUCs of Penicillin Concentrations				
	AUCCV	AUCCL	AUCCK	AUCCM	UN
BW	-0.05	-0.06	-0.05	-0.05	L
QCC	-0.41	-0.40	0.03	-0.41	L
VLC	-0.05	-0.06	-0.05	-0.05	L
QKC	-0.41	-0.41	0.03	-0.41	L
Kim	0.83	0.83	0.83	0.83	H
PL	-0.05	0.94	-0.05	-0.05	H
PK	0.00	0.00	1.00	0.00	H
PM	-0.01	-0.01	-0.01	0.99	H
KmC	-0.05	-0.06	-0.05	-0.05	M
KurineC	-0.50	-0.50	-0.95	-0.50	M

Notes: Only parameters with at least one absolute value of NSC greater than 0.05 are shown in the table. AUCCV, AUCCL, AUCCK, and AUCCM represent 24-hour area under concentration curves of penicillin in plasma, liver, kidney and muscle, respectively. Please refer to **Table 2** for abbreviations of specific parameters. UN, uncertainty designation. L, M, and H stand for low, medium, and high uncertainty, respectively.

PBPK Model Code

Note: The Berkeley Madonna model code below is a physiologically based pharmacokinetic (PBPK) model for procaine penicillin G in heavy sows based on published PBPK model for procaine penicillin G in cattle and swine by Li et al., 2017. All parameter values used in the model for heavy sows are summarized in **Tables 2.**

```
{
Penicillin PBPK model for heavy sows (flow-limited model, linear metabolism equation, plasma protein binding)
The PBPK model code is based on the Penicillin PBPK model for cattle and swine published by Li et al. 2017
}

METHOD RK4

STARTTIME = 0
STOPTIME= 1200; 24
DT = 0.000125
DTOUT = 0.1

{Physiological Parameters}
; Blood Flow Rates
QCC = 8.543                    ; L/h/kg, Cardiac Output (1989 Hannon, 1982 Tranquilli)

; Fracion of blood flow to organs (unitless)
QLC = 0.273                    ; Fraction of blood flow to the liver (2015 Huang, 2016 Lin)
QKC = 0.116                    ; Fraction of blood flow to the kidneys (1989 Hannon, 1982 Tranquilli)
QMC = 0.293                    ; Fraction of blood flow to the muscle (1983 Lundeen)
QFC = 0.128                    ; Fraction of blood flow to the fat (2015 Huang, 2016 Lin)
QLuC = 1                       ; Fraction of blood flow to the lung (2016 Lin)
QRC = 1-QLC-QKC-QFC-QMC        ; Fraction of blood flow to the rest of body (total sum equals to 1)

; Tissue Volumes
BW = 209.7                     ; Body Weight (kg)

; Fractional organ tissue volumes (unitless)
VLC = 0.011                    ; Fractional liver tissue (1991 Fugate)
VKC = 0.0024                   ; Fractional kidney tissue (1991 Fugate)
VMC = 0.355                    ; Fractional muscle tissue (1987 Amstrong)
VFC = 0.235                    ; Fractional fat tissue (1986 Doornebal)
VLuC = 0.010                   ; Fractional lung tissue (2016 Lin)
VvenC = 0.044                  ; Venous blood volume, fraction of blood volume (2016 Lin)
VartC = 0.016                  ; Arterial blood volume, fraction of blood volume (2016 Lin)
VRC = 1-VLC-VKC-VFC-VMC-VLuC-VvenC-VartC          ; Fractional rest of body (total sum equals to 1)

{Mass Transfer Parameters (Chemical-Specific Parameters)}
; Partition Coefficients (PC, tissue:plasma)
PL = 0.2                       ; Liver:plasma PC (0.157, Tsuji et al., 1983, Table 4, in rats)
PK = 10.000                    ; Kidney:plasma PC (3.70, Tsuji et al., 1983, Table 4, in rats)
PM = 0.300                     ; Muscle:plasma PC (0.062, Tsuji et al., 1983, Table 4, in rats)
PF = 0.100                     ; Fat:plasma PC
PLu = 0.180                    ; Lung:plasma PC (0.157, Tsuji et al., 1983, Table 4, in rats)
PR = 0.479                     ; Rest of body:plasma PC (Cao et al. 2012, Table 1, estimated value in human)

{Kinetic Constants}
; IM Absorption Rate Constants
Kim = 0.015                    ; /h, IM absorption rate constant
```

```
Frac = 0.010                    ; unitless, this parameter is mainly used for long-acting formulation. For conventional formulation, this parameter value is set at a low value of 0.01.
Kdiss = 0.001                   ; /h

; Percentage Plasma Protein Binding unitless
PB = 0.366; Percentage of drug bound to plasma proteins (1965 Keen)
Free = 1-PB

; Metabolic Rate Constant
KmetC = 0.05 ; h/kg

; Urinary Elimination Rate Constants
KurineC = 0.8 ; L/h/kg

{Parameters for Various Exposure Scenarios}
PDOSEim = 32.5; (mg/kg)

{Cardiac output and blood flow to tissues (L/h)}
QC = QCC*BW ; Cardiac output
QL = QLC*QC ; Liver
QK = QKC*QC ; Kidney
QF = QFC*QC ; Fat
QM = QMC*QC ; Muscle
QLu = QLuC*QC ; Lung
QR = QRC*QC ; Rest of body

{Tissue volumes (L)}
VL = VLC*BW ; Liver
VK = VKC*BW ; Kidney
VF = VFC*BW ; Fat
VM = VMC*BW ; Muscle
VLu = VLuC*BW ; Lung
VR = VRC*BW ; Rest of body
Vven = VvenC*BW ; Venous Blood
Vart = VartC*BW ; Arterial Blood

; Metabolic rate constant, /h
Kmet = KmetC*BW

; Urinary Elimination Rate Constant, L/h
Kurine = KurineC*BW

{Dosing}
; Dosing caculation based on BW
DOSEim = PDOSEim* BW            ; (mg)

; Dosing, repeated doses
tinterval = 24                  ; Varied dependent on the exposure paradigm (h)
Tdoses = 3                      ; The number of injections for multiple oral gavage

dosingperiod = if time < Tdoses*tinterval-DT then 1 else 0

; Dosing, IM, intramuscular
Rinputim = pulse(DOSEim,0,tinterval)*dosingperiod
Rpenim = Rinputim*(1-Frac);
Rppgim = Rinputim*Frac;
```

```
Rim = Kim*Amtsiteim
d/dt(Absorbim) = Rim
init Absorbim = 0
d/dt(Amtsiteim) = Rpenim- Rim + Kdiss* DOSEppgim
init Amtsiteim = 0
d/dt(DOSEppgim) = Rppgim-Kdiss* DOSEppgim
init DOSEppgim = 0

{Penicillin distribution in each compartment}
; Penicillin in venous blood compartment
RV = (QL*CVL+QK*CVK+QF*CVF+QM*CVM+QR*CVR+Rim)-QC*CV          ; RV the changing rate in
the venous blood (mg/h)
d/dt(AV) = RV                                    ; AV the amount of the drug in the venous blood (mg)
init AV = 0
CV = AV/Vven                                     ; CV drug concentration in the venous blood (mg/L)
RA = QC*(CVLu-CAfree)                            ; RA the changing rate in the arterial blood (mg/h)
d/dt(AA)= RA
init AA = 0                                      ; AA the amount of the drug in the arterial blood (mg)
CA = AA/Vart                                     ; CAfree concentration of unbound drug in the arterial blood
(mg/L)
CAfree = CA*Free
d/dt(AUCCV) = CV                                 ; AUCCV AUC of drug concentration in the venous blood
(mg*h/L)
init AUCCV = 0

ABlood = AA+AV

; Penicillin in liver compartment, flow-limited model
RL = QL*(CAfree-CVL)-Rmet                        ; RL the changing rate of the amount of drug in liver (mg/h)
d/dt(AL) = RL                                    ; AL amount of drug in liver (mg)
init AL = 0
CL = AL/VL                                       ; CL drug concentration in liver (mg/L)
CVL= AL/(VL*PL)                                  ; CVL drug concentration in venous blood from liver (mg/L)

; Metabolism of Penicillin in liver compartment
Rmet = Kmet*CL*VL                                ; Rmet the metabolic rate in liver (mg/h)
d/dt(Amet) = Rmet                                ; Amet the amount of drug metabolized in liver (mg)
init Amet = 0

; Penicillin in kidney compartment, flow-limited model
RK = QK*(CAfree-CVK)-Rurine                      ; RK the changing rate of the amount of drug in kidney (mg/h)
d/dt(AK) = RK                                    ; AK amount of drug in kidney (mg)
init AK = 0;
CK = AK/VK                                       ; CK drug concentration in kidney (mg/L)
CVK = AK/(VK*PK);
d/dt(AUCCK) = CK                                 ; AUCCK AUC of drug concentration in kidney (mg*h/L)
init AUCCK = 0

; Penicillin urinary excretion
Rurine = Kurine*CVK
d/dt(Aurine) = Rurine
init Aurine = 0

; Penicillin in muscle compartment, flow-limited model
RM = QM*(CAfree-CVM)                             ; RM the changing rate of the amount of drug in muscle
(mg/h)
```

```
d/dt(AM)= RM                                    ; AM amount of the drug in muscle (mg)
init AM = 0
CM = AM/VM                                      ; CM drug concentration in muscle (mg/L)
CVM = AM/(VM*PM)
d/dt(AUCCM) = CM
init AUCCM = 0

; Penicillin in fat compartment, flow-limited model
RF = QF*(CAfree-CVF)                            ; RF the changing rate of the amount of drug in fat (mg/h)
d/dt(AF) = RF                                   ; AF amount of the drug in fat (mg)
init AF = 0
CF = AF/VF                                      ; CF drug concentration in fat (mg/L)
CVF = AF/(VF*PF)
d/dt(AUCCF) = CF                                ; AUCCF AUC of drug concentration in fat (mg*h/L)
init AUCCF = 0

; Penicillin in the compartment of rest of body, flow-limited model
RR = QR*(CAfree-CVR)            ; Rrest the changing rate of the amount of drug in the rest of the body (mg/h)
d/dt(AR) = RR                   ; Arest amount of the drug in the rest of the body (mg)
init AR = 0
CR = AR/VR                      ; Crest drug concentration in the rest of the body (mg/L)
CVR = AR/(VR*PR)
d/dt(AUCCR) = CR                ; AUCCrest AUC of drug concentration in the rest of the body (mg*h/L)
init AUCCR = 0

; Penicillin in lung compartment, flow-limited model
RLu = QLu*(CV-CVLu)             ; RLu the changing rate of the amount of drug in the lung (mg/h)
d/dt(ALu) = RLu                 ; ALu amount of the drug in the lung (mg)
init ALu = 0
CLu = ALu/VLu                   ; CLu drug concentration in the rest of the lung (mg/L)
CVLu = ALu/(VLu*PLu)
d/dt(AUCCLu) = CLu              ; AUCCLu AUC of drug concentration in the lung (mg*h/L)
init AUCCLu = 0

{Mass balance equations}
Qbal = QC-QM-QR-QF-QK-QL
Tmass = ABlood+AM+ALu+AR+AF+AK+AL+Aurine+Amet
Input = Absorbim
Bal = Input-Tmass
```

Population PBPK Model Code

Note: The Berkeley Madonna model code below is a population physiologically based pharmacokinetic (PBPK) model for procaine penicillin G in heavy sows based on published population PBPK model for procaine penicillin G in cattle and swine. All parameter values used for population model in swine and cattle are summarized in **Tables 2.**

```
{
Monte Carlo Analysis based on Penicillin PBPK model for heavy sows (flow-limited model, linear metabolism equation, plasma protein binding)
The PBPK model code is based on the Penicillin PBPK model for swine and cattle published by Li et al. 2017
}

METHOD RK4

STARTTIME = 0
STOPTIME= 1200                  ; h, 24
DT = 0.000125
DTOUT = 0.1

{Physiological Parameters}
; Blood Flow Rates
QCC = 8.543                     ; L/h/kg, Cardiac Output (1989 Hannon, 1982 Tranquilli)

; Fracion of blood flow to organs (unitless)
QLC = 0.273                     ; Fraction of blood flow to the liver (2015 Huang, 2016 Lin)
QKC = 0.116                     ; Fraction of blood flow to the kidneys (1989 Hannon, 1982 Tranquilli)
QMC = 0.293                     ; Fraction of blood flow to the muscle (1983 Lundeen)
QFC = 0.128                     ; Fraction of blood flow to the fat (2015 Huang, 2016 Lin)
QLuC = 1                        ; Fraction of blood flow to the lung (2016 Lin)
QrestC = 0.190                  ; Fraction of blood flow to the rest of body (total sum equals to 1)

; Tissue Volumes
BW = 223.062                    ; Body Weight (kg) (1989 Hannon, 1982 Tranquilli, 1983 Lundeen, 1986
Doornebal)

; Fractional organ tissue volumes (unitless)
VLC = 0.011                     ; Fractional liver tissue (1991 Fugate)
VKC = 0.002                     ; Fractional kidney tissue (1991 Fugate)
VMC = 0.355                     ; Fractional muscle tissue (1987 Amstrong)
VFC = 0.235                     ; Fractional fat tissue (1986 Doornebal)
VLuC = 0.010                    ; Fractional lung tissue (2016 Lin)
VvenC = 0.044                   ; Venous blood volume, fraction of blood volume (2016 Lin)
VartC = 0.016                   ; Arterial blood volume, fraction of blood volume (2016 Lin)
VrestC = 0.326                  ; Fractional rest of body (total sum equals to 1)

{Mass Transfer Parameters (Chemical-Specific Parameters)}
; Partition Coefficients (PC, tissue:plasma)
PL = 0.2                        ; Liver:plasma PC (0.157, Tsuji et al., 1983, Table 4, in rats)
PK = 10.000                     ; Kidney:plasma PC (3.70, Tsuji et al., 1983, Table 4, in rats)
PM = 0.3                        ; Muscle:plasma PC (0.062, Tsuji et al., 1983, Table 4, in rats)
PF = 0.1                        ; Fat:plasma PC
PLu = 0.180                     ; Lung:plasma PC (0.157, Tsuji et al., 1983, Table 4, in rats)
Prest = 0.479                   ; Rest of body:plasma PC (Cao et al. 2012, Table 1, estimated value in human)

{Kinetic Constants}
```

```
; IM Absorption Rate Constants
Kim = 0.015                    ; /h, IM absorption rate constant
Frac = 0.010                   ; unitless, this parameter is mainly used for long-acting formulation. For
conventional formulation, this parameter value is set at a low value of 0.01.
Kdiss = 0.001                  ; /h

; Percentage Plasma Protein Binding unitless
PB = 0.366                     ; Percentage of drug bound to plasma proteins (1965 Keen)
Free = 1-PBm                   ; Percentage of drug not bound to plasma protein

{Metabolic Rate Constant}
KmC = 0.05                     ; metabolic rate constant

; Urinary Elimination Rate Constants
KurineC = 0.8                  ; L/h/kg

{Parameters for Various Exposure Scenarios}
PDOSEim = 32.5                 ; (mg/kg)

{Variances of Parameters}
QCC_sd = 1.910                 ; Standard deviation of QCC
QLC_sd = 0.08175               ; Standard deviation of QLC
QKC_sd = 0.01733               ; Standard deviation of QKC
QMC_sd = 0.04216               ; Standard deviation of QMC
QFC_sd = 0.03825               ; Standard deviation of QFC
QrestC_sd = 0.05712            ; Standard deviation of QrestC
BW_sd = 38.15                  ; Standard deviation of Body Weight
VLC_sd = 4.039e-3              ; Standard deviation of VLC
VKC_sd = 8.078e-4              ; Standard deviation of VKC
VMC_sd = 2.494e-3              ; Standard deviation of VMC
VFC_sd = 1.802e-2              ; Standard deviation of VFC
VLuC_sd = 3e-3                 ; Standard deviation of VLuC
VrestC_sd = 9.778e-2           ; Standard deviation of VrestC
VvenC_sd = 1.332e-2            ; Standard deviation of VvenC
VartC_sd = 4.68e-3             ; Standard deviation of VartC
PL_sd = 8e-2                   ; Standard deviation of PL
PK_sd = 4                      ; Standard deviation of PK
PM_sd =1.2e-1                  ; Standard deviation of PM
PF_sd = 4e-2                   ; Standard deviation of PF
PLu_sd = 7.2e-2                ; Standard deviation of PLu
Prest_sd = 1.916e-1            ; Standard deviation of Prest
Kim_sd = 6e-3                  ; Standard deviation of Kim
Frac_sd = 1e-3                 ; Standard deviation of Frac
Kdiss_sd = 4e-4                ; Standard deviation of Kdiss
PB_sd = 0.1464                 ; Standard deviation of PB
KmC_sd = 2e-2                  ; Standard deviation of KmC
KurineC_sd = 0.32              ; Standard deviation of KurineC

{Generation of Parameters based on Normal Distribution}
; Generation of Parameters based on Normal Distribution

init QCCm = Normal(QCC, QCC_sd)          ; Generation of the QCCm based on normal distribution
init QLCm = Normal(QLC, QLC_sd)          ; Generation of the QLCm based on normal distribution
init QKCm = Normal(QKC, QKC_sd)          ; Generation of the QKCm based on normal distribution
init QFCm = Normal(QFC, QFC_sd)          ; Generation of the QFCm based on normal distribution
init QMCm = Normal(QMC, QMC_sd)          ; Generation of the QMCm based on normal distribution
```

```
init QrestCm = Normal(QrestC, QrestC_sd)          ; Generation of the QrestCm based on normal distribution
init BWm = Normal(BW, BW_sd)                      ; Generation of the BWm based on normal distribution
init VLCm = Normal(VLC, VLC_sd)                   ; Generation of the VLCm based on normal distribution
init VKCm = Normal(VKC, VKC_sd)                   ; Generation of the VKCm based on normal distribution
init VMCm = Normal(VMC, VMC_sd)                   ; Generation of the VMCm based on normal distribution
init VFCm = Normal(VFC, VFC_sd)                   ; Generation of the VFCm based on normal distribution
init VLuCm = Normal(VLuC, VLuC_sd)                ; Generation of the VLuCm based on normal distribution
init VrestCm = Normal(VrestC, VrestC_sd)          ; Generation of the VrestCm based on normal distribution
init VvenCm = Normal(VvenC, VvenC_sd)             ; Generation of the VvenCm based on normal distribution
init VartCm = Normal(VartC, VartC_sd)             ; Generation of the VvartCm based on normal distribution

; Assignment of the Values to Parameters
next QCCm = QCCm                                  ; Assignment of the first created value to QCCm, without this
step QCCm will change at each integration time step
next BWm=BWm ;

; Creation of Adjust Factor
AdjustF = QLCm+QKCm+QFCm+QMCm+QrestCm                                   ; Adjust factor to keep the
sum of blood flow fractions to 1
AdjustF1=VLCm+VKCm+VMCm+VFCm+VLuCm+VrestCm+VvenCm+VartCm                ; Adjustment factor to
make sure the sum of fractions of organ tissue volumes to be 1

; Creation of Adjusted Parameters
next QLCm = QLCm/AdjustF                          ; Adjustment of QLCm based on the adjust factor
next QKCm = QKCm/AdjustF                          ; Adjustment of QKCm
next QFCm = QFCm/AdjustF                          ; Adjustment of QFCm
next QMCm = QMCm/AdjustF                          ; Adjustment of QMCm
next QrestCm = QrestCm/AdjustF                    ; Adjustment of QrestCm
next VLCm=VLCm/AdjustF1                           ; Adjustment of VLCm based on the adjust factor
next VKCm=VKCm/AdjustF1                           ; Adjustment of VKCm based on the adjust factor
next VMCm=VMCm/AdjustF1                           ; Adjustment of VMCm based on the adjust factor
next VFCm=VFCm/AdjustF1                           ; Adjustment of VFCm based on the adjust factor
next VLuCm=VLuCm/AdjustF1                         ; Adjustment of VLuCm based on the adjust factor
next VrestCm=VrestCm/AdjustF1                     ; Adjustment of VrestCm based on the adjust factor
next VvenCm=VvenCm/AdjustF1                       ; Adjustment of VvenCm based on the adjust factor
next VartCm=VartCm/AdjustF1                       ; Adjustment of VartCm based on the adjust factor

{Lognormal Transformation of Parameters}
PL_ln = logn(PL^2/(PL_sd^2+PL^2)^0.5)             ; Lognormal transformation of PL values
PL_lnsd = (logn(1+PL_sd^2/PL^2))^0.5
PK_ln = logn(PK^2/(PK_sd^2+PK^2)^0.5)             ; Lognormal transformation of PK values
PK_lnsd = (logn(1+PK_sd^2/PK^2))^0.5
PM_ln = logn(PM^2/(PM_sd^2+PM^2)^0.5)             ; Lognormal transformation of PM values
PM_lnsd = (logn(1+PM_sd^2/PM^2))^0.5
PF_ln = logn(PF^2/(PF_sd^2+PF^2)^0.5)             ; Lognormal transformation of PF values
PF_lnsd = (logn(1+PF_sd^2/PF^2))^0.5
PLu_ln = logn(PLu^2/(PLu_sd^2+PLu^2)^0.5)         ; Lognormal transformation of PLu values
PLu_lnsd = (logn(1+PLu_sd^2/PLu^2))^0.5
Prest_ln = logn(Prest^2/(Prest_sd^2+Prest^2)^0.5) ; Lognormal transformation of Prest values
Prest_lnsd = (logn(1+Prest_sd^2/Prest^2))^0.5
Kim_ln = logn(Kim^2/(Kim_sd^2+Kim^2)^0.5)         ; Lognormal transformation of Kim value
Kim_lnsd = (logn(1+Kim_sd^2/Kim^2))^0.5
Frac_ln = logn(Frac^2/(Frac_sd^2+Frac^2)^0.5)     ; Lognormal transformation of Frac value
Frac_lnsd = (logn(1+Frac_sd^2/Frac^2))^0.5
Kdiss_ln = logn(Kdiss^2/(Kdiss_sd^2+Kdiss^2)^0.5) ; Lognormal transformation of Kdiss value
Kdiss_lnsd = (logn(1+Kdiss_sd^2/Kdiss^2))^0.5
```

```
PB_ln = logn(PB^2/(PB_sd^2+PB^2)^0.5)                                   ; Lognormal transformation of PB
PB_lnsd = (logn(1+PB_sd^2/PB^2))^0.5
KmC_ln = logn(KmC^2/(KmC_sd^2+KmC^2)^0.5)                               ; Lognormal transformation of KmC
KmC_lnsd = (logn(1+KmC_sd^2/KmC^2))^0.5
KurineC_ln = logn(KurineC^2/(KurineC_sd^2+KurineC^2)^0.5)               ; Lognormal transformation of KurineC
KurineC_lnsd = (logn(1+KurineC_sd^2/KurineC^2))^0.5

{Creation of Parameters based on Lognormal Distribution}
init PLm = exp(Normal(PL_ln, PL_lnsd)) next PLm = PLm                   ; Generation of PLm based
on lognormal distribution
init PMm = exp(Normal(PM_ln, PM_lnsd)) next PMm = PMm                   ; Generation of PMm
init PFm = exp(Normal(PF_ln, PF_lnsd)) next PFm = PFm                   ; Generation of PFm
init PKm = exp(Normal(PK_ln, PK_lnsd)) next PKm = PKm                   ; Generation of PKm
init PLum = exp(Normal(PLu_ln, PLu_lnsd)) next PLum = PLum              ; Generation of PLum
init Prestm = exp(Normal(Prest_ln, Prest_lnsd)) next Prestm = Prestm     ; Generation of Prestm
init Kimm = exp(Normal(Kim_ln, Kim_lnsd)) next Kimm = Kimm              ; Generation of Kimm
init Fracm = exp(Normal(Frac_ln, Frac_lnsd)) next Fracm = Fracm          ; Generation of Fracm
init Kdissm = exp(Normal(Kdiss_ln, Kdiss_lnsd)) next Kdissm = Kdissm    ; Generation of Kdissm
init PBm = exp(Normal(PB_ln, PB_lnsd)) next PBm = PBm                   ; Generation of PBm
init KmCm = exp(Normal(KmC_ln, KmC_lnsd)) next KmCm = KmCm              ; Generation of KmCm
init KurineCm = exp(Normal(KurineC_ln, KurineC_lnsd)) next KurineCm = KurineCm  ; Generation of KurineCm

{limit the parameter values within the lower and upper bounds of 95% confident interval}
limit BWm >= 148.293
limit BWm <= 297.832
limit QCCm >= 4.8
limit QCCm <= 12.287
limit VartCm >= 0.006
limit VartCm <= 0.025
limit VvenCm >= 0.018
limit VvenCm <= 0.071
limit VLCm >= 0.003
limit VLCm <= 0.019
limit VKCm >= 0.001
limit VKCm <= 0.004
limit VMCm >= 0.351
limit VMCm <= 0.360
limit VFCm >= 0.200
limit VFCm <= 0.270
limit VLuCm >= 0.004
limit VLuCm <= 0.016
limit VrestCm >=0.134
limit VrestCm <=0.518
limit QLCm >=0.112
limit QLCm <=0.433
limit QKCm >=0.082
limit QKCm <=0.150
limit QMCm >=0.211
limit QMCm <=0.376
limit QFCm >=0.053
limit QFCm <=0.202
limit QrestCm >=0.078
limit QrestCm <=0.302
limit Kimm >=0.007
limit Kimm <= 0.030
limit Fracm >= 0.008
```

```
limit  Fracm <= 0.012
limit  Kdissm >=0.0004
limit  Kdissm <= 0.002
limit  PLm >= 0.087
limit  PLm <= 0.395
limit  PKm >= 4.364
limit  PKm <= 19.756
limit  PMm >= 0.131
limit  PMm <= 0.593
limit  PFm >= 0.044
limit  PFm <= 0.198
limit  PLum >= 0.079
limit  PLum <= 0.356
limit  Prestm >= 0.209
limit  Prestm <= 0.946
limit  KmCm >=0.022
limit  KmCm <=0.099
limit  PBm >=0.160
limit  PBm <=0.723
limit  KurineCm >= 0.349
limit  KurineCm <= 1.580

{Cardiac output and blood flow to tissues (L/h)}
QC = QCCm*BWm                    ; Cardiac output
QL = QLCm*QC                     ; Liver
QK = QKCm*QC                     ; Kidney
QF = QFCm*QC                     ; Fat
QM = QMCm*QC                     ; Muscle
QLu = QLuC*QC                    ; Lung
QR = QrestCm*QC                  ; Rest of body

{Tissue volumes (L)}
VL = VLCm*BWm                    ; Liver
VK = VKCm*BWm                    ; Kidney
VF = VFCm*BWm                    ; Fat
VM = VMCm*BWm                    ; Muscle
VLu = VLuCm*BWm                  ; Lung
VR = VrestCm*BWm                 ; Rest of body
Vven = VvenCm*BWm                ; Venous Blood
Vart = VartCm*BWm                ; Arterial Blood

; Metabolic rate constant, /h
Kmet = KmCm*BWm

; Urinary Elimination Constant, L/h
Kurine = KurineCm*BWm

{Dosing}
; Dosing calculation based on BW
DOSEim = PDOSEim*BWm             ; (mg)

; Dosing, repeated doses
tinterval = 24                   ; Varied dependent on the exposure paradigm (h)
Tdoses = 3                       ; The number of injections for multiple oral gavage

dosingperiod = if time < Tdoses*tinterval-DT then 1 else 0
```

```
; Dosing, IM, intramuscular
Rinputim = pulse(DOSEim,0,tinterval)*dosingperiod
Rpenim = Rinputim*(1-Fracm)
Rppgim = Rinputim*Fracm
Rim = Kimm*Amtsiteim
d/dt(Absorbim) = Rim
init Absorbim = 0
d/dt(Amtsiteim) = Rpenim- Rim + Kdissm* DOSEppgim
init Amtsiteim = 0
d/dt(DOSEppgim) = Rppgim-Kdissm* DOSEppgim
init DOSEppgim = 0

{Penicillin distribution in each compartment}
; Penicillin in venous blood compartment
RV = (QL*CVL+QK*CVK+QF*CVF+QM*CVM+QR*CVR+Rim)-QC*CV      ; RV the changing rate in the
venous blood (mg/h)
d/dt(AV) = RV                                ; AV the amount of the drug in the venous blood (mg)
init AV = 0
CV = AV/Vven                                 ; CV drug concentration in the venous blood (mg/L)
RA = QC*(CVLu-CAfree)                        ; RA the changing rate in the arterial blood (mg/h)
d/dt(AA)= RA
init AA = 0                                  ; AA the amount of the drug in the arterial blood (mg)
CA = AA/Vart                                 ; CAfree concentration of unbound drug in the arterial blood (mg/L)
CAfree = CA*Free
d/dt(AUCCV) = CV                             ; AUCCV AUC of drug concentration in the venous blood (mg*h/L)
init AUCCV = 0

ABlood = AA+AV

; Penicillin in liver compartment, flow-limited model
RL = QL*(CAfree-CVL)-Rmet                    ; RL the changing rate of the amount of drug in liver (mg/h)
d/dt(AL) = RL                                ; AL amount of drug in liver (mg)
init AL = 0
CL = AL/VL                                   ; CL drug concentration in liver (mg/L)
CVL= AL/(VL*PLm)                             ; CVL drug concentration in venous blood from liver (mg/L)
d/dt(AUCCL) = CL                             ; AUCCL area under the curve of drug concentration in liver (mg*h/L)
init AUCCL = 0

; Metabolism of Penicillin in liver compartment
Rmet = Kmet*CL*VL                            ; Rmet the metabolic rate in liver (mg/h)
d/dt(Amet) = Rmet                            ; Amet the amount of drug metabolized in liver (mg)
init Amet = 0

; Penicillin in kidney compartment, flow-limited model
RK = QK*(CAfree-CVK)-Rurine                  ; RK the changing rate of the amount of drug in kidney (mg/h)
d/dt(AK) = RK                                ; AK amount of drug in kidney (mg)
init AK = 0
CK = AK/VK                                   ; CK drug concentration in kidney (mg/L)
CVK = AK/(VK*PKm)
d/dt(AUCCK) = CK                             ; AUCCK AUC of drug concentration in kidney (mg*h/L)
init AUCCK = 0

; Penicillin urinary excretion
Rurine = Kurine*CVK                          ; mg/h
d/dt(Aurine) = Rurine
```

```
init Aurine = 0

; Penicillin  in muscle compartment, flow-limited  model
RM = QM*(CAfree-CVM)                    ; RM the changing rate of the amount of drug in muscle (mg/h)
d/dt(AM)= RM                            ; AM amount of the drug in muscle (mg)
init AM = 0
CM = AM/VM                              ; CM drug concentration in muscle (mg/L)
CVM  = AM/(VM*PMm)
d/dt(AUCCM) = CM
init AUCCM  = 0

; Penicillin  in fat compartment, flow-limited  model
RF = QF*(CAfree-CVF)                    ; RF the changing rate of the amount of drug in fat (mg/h)
d/dt(AF) = RF                           ; AF amount of the drug in fat (mg)
init AF = 0
CF = AF/VF                              ; CF drug concentration in fat (mg/L)
CVF  = AF/(VF*PFm)
d/dt(AUCCF) = CF                        ; AUCCF  AUC of drug concentration in fat (mg*h/L)
init AUCCF  = 0

; Penicillin  in the compartment of rest of body, flow-limited  model
RR = QR*(CAfree-CVR)            ; Rrest the changing rate of the amount of drug in the rest of the body (mg/h)
d/dt(AR) = RR                   ; Arest amount of the drug in the rest of the body (mg)
init AR = 0
CR = AR/VR                      ; Crest drug concentration in the rest of the body (mg/L)
CVR  = AR/(VR*Prestm)
d/dt(AUCCR) = CR                ; AUCCrest  AUC of drug concentration in the rest of the body (mg*h/L)
init AUCCR  = 0

; Penicillin  in lung compartment, flow-limited  model
RLu = QLu*(CV-CVLu)             ; RLu the changing rate of the amount of drug in the lung (mg/h)
d/dt(ALu) = RLu                 ; ALu amount of the drug in the lung (mg)
init ALu = 0
CLu = ALu/VLu                   ; CLu drug concentration in the rest of the lung (mg/L)
CVLu  = ALu/(VLu*PLum)
d/dt(AUCCLu) = CLu              ; AUCCLu  AUC of drug concentration in the lung (mg*h/L)
init AUCCLu  = 0

{Mass balance equations}
Qbal = QC-QM-QR-QF-QK-QL
Tmass = ABlood+AM+ALu+AR+AF+AK+AL+Aurine+Amet
Input = Absorbim
Bal = Input-Tmass
```

Supplementary References

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