

IN VIVO PK / BBB

Obtaining early stage pharmacokinetic (PK) and blood-brain barrier (BBB) data for evaluation of new chemical entities is a prerequisite for successful animal pharmacology and toxicology studies. Quantitative measures of drug exposure are key components needed for the sound interpretation of preclinical efficacy studies. PK data can also help in the design or species selection of preclinical toxicology studies.

Clients provide crucial information on the physicochemical properties of the test compound and Cerep's experience in animal dosing and sample collection ensures quality data. According to in-house SOPs, blood and/or tissue (e.g. brain) samples are obtained from test animals following dose administration (IV, PO, IP, IM, SC, IN). Both serial sampling (for *in vivo* rat or mouse PK) and parallel sampling (for *in vivo* mouse PK, *in vivo* rat or mouse BBB, combined *in vivo* rat or mouse PK-BBB) are available. Samples are analyzed using bioanalytical methods developed at Cerep. In general, a whole PK study includes both in-life portion and bioanalytical work. Either a whole PK or a portion of a whole PK study (in-life or bioanalytical) can be performed at Cerep.

The data are used to generate concentration versus time curves and allow the determination of fundamental PK parameters.

Cassette dosing is available with up to 5 compounds in the same dosing solution.

- ▶ *In vivo* rat or mouse PK
- ▶ *In vivo* rat or mouse BBB
- ▶ Combined *in vivo* PK-BBB
- ▶ *In vivo* rat renal clearance
- ▶ *In vivo* rat biliary clearance

FORMULATION

▶ FORMULATION FOR IV DOSING

The solubility of the test compound is first evaluated in phosphate-buffered saline, pH 7.4 (PBS) by visual inspection. PBS is used as the vehicle if the compound is soluble at the target concentration. Other vehicles that are compatible with IV dosing may be evaluated if the compound is not completely soluble in PBS. Such vehicles include DMSO, Solutal® HS 15, and Cremophor EL among others. Customized formulation can be accommodated as well. IM, SC, or IN dosing uses the same formulation protocol as IV dosing.

▶ FORMULATION FOR PO DOSING

The solubility of the test compound is first evaluated in PBS. PBS is used as the vehicle if the compound is soluble at the target concentration. DMSO/ Solutal® HS 15/PBS (5/5/90, v/v/v), or DMSO/1% methylcellulose (5/95, v/v) may be used as the vehicle if the test compound is not completely soluble in PBS at the target concentration. Customized formulation can be accommodated as well. IP dosing uses the same formulation protocol as PO dosing.

TYPICAL STUDY DESIGN FOR *IN VIVO* RAT AND MOUSE PK

▶ *IN VIVO* RAT AND MOUSE PK

Animal handling and dosing

. **Rat:** male, CD IGS, jugular vein-cannulated, 180-250 grams

. **Mouse:** male, CD-1, 20 - 30 grams

Minimal of 2 days of acclimation prior to dosing

Not fasted prior to dosing

Two routes of administration (IV and PO¹)

Single dose (1 mg/kg for IV and 5 mg/kg for PO)

Dosing technique and dosing volume:

. IV bolus, typically 5 mL/kg:

. **Rat:** through jugular vein catheter

. **Mouse:** through tail vein injection

. PO, gastric gavage, typically 5 mL/kg

Number of replicates:

. **Rat:** n=3 cannulated rats per route per compound

. **Mouse (parallel sampling²):** n=3 mice per time point

. **Mouse (serial sampling²):** n=3 mice per route per compound

Control animals:

(for blank plasma collection)

. **Rat:** n = 1 non-cannulated rat per route per compound

. **Mouse:** n=2-3 mice per compound

¹ Other available dosing routes are IP, SC, IM, and IN.

² A comparison of serial and parallel blood sampling from mice is presented on page 3

▶ *IN VIVO* RAT AND MOUSE BBB

Animal handling and dosing

. **Rat:** Male, CD IGS, 180-250 grams

. **Mouse:** Male, CD1, 20-30 grams

Minimal of 2 days of acclimation prior to dosing

Not fasted prior to dosing

One route of administration (IV by default)

Single dose (1 mg/kg for IV)

Dosing: IV bolus, typically 5 mL/kg:

. **Rat:** through tail vein injection

. **Mouse:** through tail vein injection

Number of replicates:

. n=3 animals per time-point

Control animals:

(for blank brain and blank plasma collection)

. **Rat:** n=2 rats per route per compound

. **Mouse:** 5 mice per route per compound

► IN VIVO RAT AND MOUSE PK

blood collection

Eight time points:

- . IV: 5, 15, 30, 60, 120, 240, 360 and 1440 min
- . PO: 15, 30, 60, 120, 240, 360, 480 and 1440 min

(Seven time points for mouse serial sampling)

Blood samples are collected (300-400 µL per sample) in heparin coated polypropylene tubes and kept on ice:

- . **Rat:** from the jugular vein catheter
 - . **Mouse (parallel sampling):** by cardiac puncture
- Blood samples are collected (<30 µL per sample) using heparin coated polypropylene tubes. Aliquot of 15 µL blood sample is transferred to another tube and kept on ice. Blood samples are analyzed directly for test compound concentration:

- . **Mouse (serial sampling):** via saphenous vein

Plasma samples are obtained by centrifugation (2,000 xg for 15 min at 4°C) of blood samples within 1 hour of collection.

Plasma or blood samples are stored frozen (-20°C) until further processing.

Bile or urine collection (if applicable)

Seven time intervals:

- . **Bile:** 0-30, 30-60, 60-120, 120-180, 180-240, 240-300, and 300-360 min
- . **Urine:** 0-30, 30-60, 60-120, 120-180, 180-240, 240-300, and 300-360 min

Bile samples are collected from the bile duct catheter using bile duct cannulated rats. Urine samples are collected using metabolic cages. The volume of bile or urine in each collection is also recorded.

Note: Renal and biliary clearance studies are performed using IV dosing route.

► IN VIVO RAT AND MOUSE BBB

blood collection

Three time points: 30, 60 and 180 min

Blood is collected by cardiac puncture (300-400 µL per sample) in heparin coated polypropylene tubes and kept on ice

Plasma samples are obtained by centrifugation (2,000 xg for 15 min at 4°C) of blood samples within 1 hour of collection.

Plasma samples are stored frozen (-20°C) until further processing

Brain collection

Three time points: 30, 60 and 180 min

Immediately after blood collection, the whole brain is quickly removed, rinsed with cold saline (0.9 % NaCl, g/mL), surface vasculature ruptured, blotted with dry gauze or tissue, weighed. The whole brain is homogenized within 1 hour after collection. Each brain is homogenized in ice cold phosphate-buffered saline, pH 7.4 (3 mL per rat brain or 1.5 mL per mouse brain). The homogenate is then stored at -70°C until further processing

Note: In addition to brain collection, other tissue collection is also available for tissue distribution study, such as liver, muscle, skin, and adipose tissue.

■ QUANTITATIVE BIOANALYSIS

Plasma, blood or brain samples are processed using the extraction procedure and then analyzed using the HPLC-MS/MS or HPLC-MS method established during analytical method development. Plasma, blood or brain concentrations are determined relative to the respective calibration curves.

■ DELIVERABLES

- Plasma or blood concentrations (ng/mL) and brain concentrations (ng/g tissue) when applicable are tabulated.
- The ratio of brain concentration to plasma concentration for BBB study
- Plots of plasma/blood concentration of compound versus time are constructed for PK study
- Fundamental pharmacokinetic parameters such as AUC_{last}, AUC_{INF}, T_{1/2}, CL, V_Z, V_{SS}, T_{max} and C_{max} are obtained from the non-compartmental analysis of the plasma/blood data using WinNonlin. Bioavailability (F), if applicable, is also calculated and reported.
- The plasma/blood sample and brain homogenate (when applicable) preparation method and the HPLC-MS/MS method for each compound are reported.
- Formulation, animal body weight and dosing record are also reported.

■ COMPOUND REQUIREMENTS

Typically, a minimum of 25 mg (for rat PK) or 20 mg (for mouse parallel sampling PK) and of 1.5 mg (for rat BBB) or 10 mg (for mouse serial sampling PK or mouse BBB) of dry powder, free acid/base equivalent, is required for each test compound for a PK study involving both IV and PO routes. The exact amount of compound required would depend on the actual dose, and study design, and whether formulation is to be performed at Cerep.

■ TURNAROUND TIME

Typically, data are delivered within 2 weeks upon receipt of the compounds and animals. This includes analytical method development, animal dosing and quantitative bioanalysis.

■ CUSTOMIZATION

Study design is flexible to meet client needs. Some examples of customization include: different strains of animals, fasting animals for oral dosing, tail vein injection for rat IV dosing, customized doses and sampling time points, and customized formulation vehicles. A customization fee may be applied.

COMPARISON OF MOUSE SERIAL AND PARALLEL SAMPLING EXPERIMENTAL DESIGNS

Mice are one of the most commonly used animal models in the drug discovery stage. Because of their small body size, parallel blood sampling is generally used in mouse PK studies, i.e. each mouse is subject to only one blood draw through cardiac puncture.

However, the parallel sampling can generate several issues. First, the number of animals used could be substantial, depending on the number of time points and replicates. Consequently, a greater quantity of test compound would be needed for administration to the larger number of animals required for the study. At the drug discovery stage, there are many tests and evaluations which need to be performed and the quantity of compound available may be a limiting factor. Furthermore, each concentration-time profile is not generated from an individual animal. Individual variance amongst animals may affect the PK parameters obtained, and calculated. To address these issues, we have developed a serial blood sampling technique for mouse PK studies.

Below is the comparison of serial and parallel sampling experimental designs and results

■ SERIAL SAMPLING

A total of 3 animals are used for each independent experiment. Each mouse is administered with test compound via tail vein injection. Blood samples are collected at 5, 15, 30, 60, 120 (or 180), 360, and 1440 min from each animal via saphenous vein, with 20-30 μ L withdrawn at each time point. Fifteen microliters of each blood sample are then used for quantitative bioanalysis by HPLC-MS/MS.

■ PARALLEL SAMPLING

Total of 21 animals are used for each independent experiment. Each mouse is administered with test compound via tail vein injection. Blood samples are collected at 5, 15, 30, 60, 120 (or 180), 360, or 1440 min via cardiac puncture (typically 300 μ L is collected from each animal, 3 animals per each time point). Fifteen microliters of each blood sample are then used for quantitative bioanalysis by HPLC-MS/MS.

The fundamental pharmacokinetic parameters (half-life, clearance, volume of distribution and AUC) are obtained from the non-compartmental analysis using WinNonlin.

■ RESULTS AFTER IV ADMINISTRATION TO MICE (5 mg/kg)

| ► Antipyrine | Sampling | Parallel | Serial | Concentration-time profile |
|-------------------|---|--------------|--------------|----------------------------|
| | T _{1/2} (min) | 34±7 | 48±4 | |
| | Cl (mL/min/kg) | 91±8 | 92±6 | |
| | V _z (mL/kg) | 4459±921 | 6360±635 | |
| | V _{ss} (mL/kg) | 3485±677 | 5235±387 | |
| | AUC _{last} (min*ng/mL) | 55168±4779 | 55183±3702 | |
| | AUC _{INF} (min*ng/mL) | 55771±4953 | 56705±4310 | |
| | <i>Results are expressed as mean ± SE, from 3 independent experiments</i> | | | |
| ► Caffeine | Sampling | Parallel | Serial | Concentration-time profile |
| | T _{1/2} (min) | 32±2 | 32±2 | |
| | Cl (mL/min/kg) | 23±4 | 25±2 | |
| | V _z (mL/kg) | 1075±186 | 1138±116 | |
| | V _{ss} (mL/kg) | 1092±172 | 1189±116 | |
| | AUC _{last} (min*ng/mL) | 210218±29722 | 200123±15971 | |
| | AUC _{INF} (min*ng/mL) | 223145±29702 | 212309±16475 | |
| | <i>Results are expressed as mean ± SE, from 3 independent experiments</i> | | | |
| ► Ciprofloxacin | Sampling | Parallel | Serial | Concentration-time profile |
| | T _{1/2} (min) | 545±69 | 516±43 | |
| | Cl (mL/min/kg) | 64±7 | 79±17 | |
| | V _z (mL/kg) | 50584±9329 | 60478±14198 | |
| | V _{ss} (mL/kg) | 39647±12871 | 69735±17865 | |
| | AUC _{last} (min*ng/mL) | 68044±8942 | 67194±13594 | |
| | AUC _{INF} (min*ng/mL) | 79785±8805 | 87396±15383 | |
| | <i>Results are expressed as mean ± SE, from 3 independent experiments</i> | | | |
| ► Dextrometorphan | Sampling | Parallel | Serial | Concentration-time profile |
| | T _{1/2} (min) | 37±5 | 43±3 | |
| | Cl (mL/min/kg) | 195±12 | 142±12 | |
| | V _z (mL/kg) | 10506±2021 | 8784±953 | |
| | V _{ss} (mL/kg) | 8638±1597 | 6752±587 | |
| | AUC _{last} (min*ng/mL) | 24024±2297 | 37251±3959 | |
| | AUC _{INF} (min*ng/mL) | 25764±1538 | 37786±3949 | |
| | <i>Results are expressed as mean ± SE, from 3 independent experiments</i> | | | |

| ► Erythromycin | Sampling | Parallel | Serial | Concentration-time profile |
|----------------|---|----------------|----------------|----------------------------|
| | T _{1/2} (min) | 21±2 | 25±3 | |
| | Cl (mL/min/kg) | 169±37 | 194±19 | |
| | V _z (mL/kg) | 5339±1771 | 6786±947 | |
| | V _{ss} (mL/kg) | 4457±736 | 7180±985 | |
| | AUClast (min*ng/mL) | 32416±7575 | 26541±2374 | |
| | AUCINF (min*ng/mL) | 32941±7605 | 27722±2465 | |
| | <i>Results are expressed as mean ± SE, from 3 independent experiments</i> | | | |
| ► Midazolam | Sampling | Parallel | Serial | Concentration-time profile |
| | T _{1/2} (min) | 13±1 | 20±1 | |
| | Cl (mL/min/kg) | 191±8 | 164±11 | |
| | V _z (mL/kg) | 3520±529 | 4654±316 | |
| | V _{ss} (mL/kg) | 2347±321 | 3472±302 | |
| | AUClast (min*ng/mL) | 26104±1048 | 31327±2146 | |
| | AUCINF (min*ng/mL) | 26205±1106 | 31767±2230 | |
| | <i>Results are expressed as mean ± SE, from 3 independent experiments</i> | | | |
| ► Propranolol | Sampling | Parallel | Serial | Concentration-time profile |
| | T _{1/2} (min) | 100±18 | 80±7 | |
| | Cl (mL/min/kg) | 162±55 | 99±16 | |
| | V _z (mL/kg) | 21095±4505 | 11713±2249 | |
| | V _{ss} (mL/kg) | 13759±2166 | 10845±2295 | |
| | AUClast (min*ng/mL) | 36462±12070 | 60398±11090 | |
| | AUCINF (min*ng/mL) | 39434±13568 | 63836±12013 | |
| | <i>Results are expressed as mean ± SE, from 3 independent experiments</i> | | | |
| ► Sulfadiazine | Sampling | Parallel | Serial | Concentration-time profile |
| | T _{1/2} (min) | 270±5 | 312±20 | |
| | Cl (mL/min/kg) | 0.79±0.06 | 0.76±0.03 | |
| | V _z (mL/kg) | 308±25 | 336±14 | |
| | V _{ss} (mL/kg) | 318±27 | 344±12 | |
| | AUClast (min*ng/mL) | 6251918±467181 | 6387994±234482 | |
| | AUCINF (min*ng/mL) | 6401859±467785 | 6687976±296752 | |
| | <i>Results are expressed as mean ± SE, from 3 independent experiments</i> | | | |

Plasma concentration-time profiles and PK parameters obtained by the serial and parallel sampling protocols are in good concordance, especially for those compounds with low clearance values.

| | Advantages | Disadvantages |
|--------------------------|---|--|
| Serial sampling | <ul style="list-style-type: none"> ► Requires fewer animals ► Requires less test compound ► Able to compare individual differences | <ul style="list-style-type: none"> ► Small sample size, sufficient for only enough for one analysis ► Potential stress on animals and excessive blood loss |
| Parallel sampling | <ul style="list-style-type: none"> ► Large sample size, which can be used for reanalysis or other assays | <ul style="list-style-type: none"> ► Requires more animals ► Requires more test compound ► Unable to compare individual differences |

We have established a technique for serial sampling from one mouse. The average circulating blood volume for mice is 72 mL/kg (Diehl *et al*, 2001). For a 25 mg mouse, the blood volume would be 1.8 mL. The total blood volume we sampled from one mouse is around 175 µL throughout the study, about 10% of total circulating blood volume. The animals did not show any observed adverse signs, suggesting the amount of blood loss is tolerable to the animals.



FRANCE
Le Bois l'Évêque
86600 LLE L'EVESCAULT
tel. +33 (0)5 49 89 30 00

(Headquarters)
155 boulevard Haussmann
75008 PARIS
tel. +33 (0)1 45 64 44 60

USA
15318 N.E. 95th Street
REDMOND, WA 98052
tel. +1 (425) 895 8666

JAPAN
Namiki Shoji Co., Ltd.
Kenseishinjuku Bldg. 5-5-3
Shinjuku, Shinjuku-ku
TOKYO, 160-0022
tel. +81 (0)3 3354 4026
fax +81 (0)3 3352 2196

CHINA
Ai Di Sheng (Edison) Road 326,
302-1 room
Zhangjiang High-Tech Park
SHANGHAI
tel. +86 18702160370

■ **QUESTIONS OR CONCERNS?**
Please contact us: sales@cerep.com