

Non-clinical pharmacokinetics studies using radio-labeled compounds

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Drug Development Solutions Center

ADME Group Manager

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About Sekisui Medical

- ✓ 57 years of ADME experience
- ✓ Various animals (mice to monkeys)
- ✓ One stop shop; Radiolabel your compound
- ✓ Commitment to animal welfare

AAALAC Full Accreditation

International accreditation of animal welfare (AAALAC)



Agenda

- 1. What is Radiolabeled Compound ?**
- 2. Preparation of Dosing Formulation**
- 3. ADME Study Items**
- 4. Application of ADME Study Using
Radiolabeled Compound**

1. What is Radiolabeled Compound ?

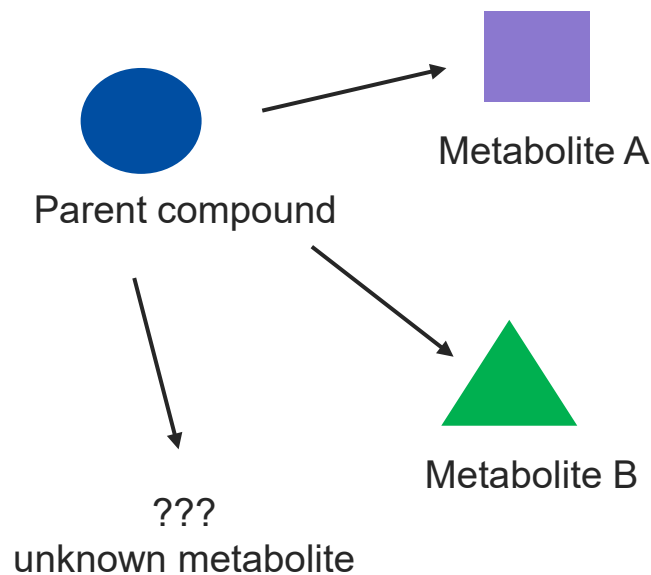
Nuclide	Radiation	Half life	Energy	Specific activity (maximum)	Measurement
^{14}C	β ray	5700 year	156 KeV	2.32 GBq/mmol	Liquid scintillation counter (LSC)
	<ul style="list-style-type: none"> ➤ Labeling carbon skeletons of a drug candidate ➤ Metabolically stable positions will be selected as labeling positions ➤ Commonly used for radiolabeling small molecules 				
^3H	β ray	12.3 year	18.6 KeV	1.07 TBq/mmol	Liquid scintillation counter (LSC)
	<ul style="list-style-type: none"> ➤ Higher sensitivity ➤ Less expensive than ^{14}C labeling ➤ Commonly used for explanatory 				

< Other nuclides >

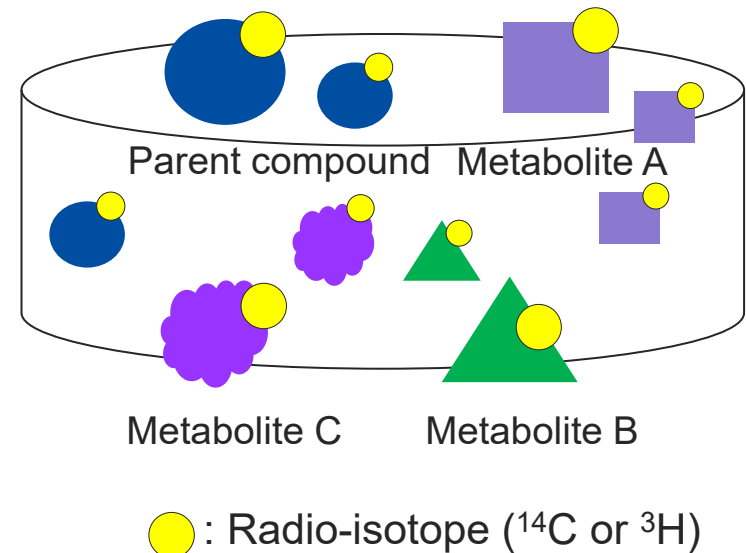
Nuclides such as ^{125}I , ^{111}In and ^{35}S may also be selected depending on the properties of the compound.

Measurement of radioactivity enables quantitative tracing of the total amount of compound including metabolites

< Unlabeled Compounds >



Labeled compounds



The use of radiolabeled compounds provides a more detailed ADME profile

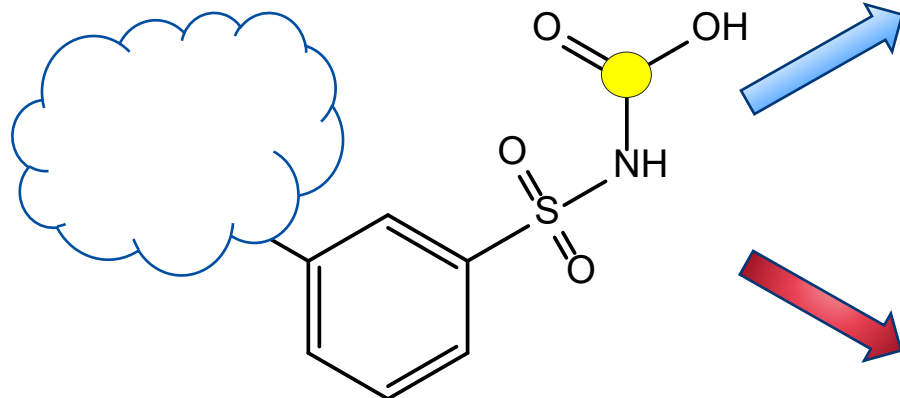
Specific radio activity (MBq/mg or MBq/mmol)

: Intensity of radio-activity per mass of a compound

Maximum specific radio activity per a ^{14}C
: 2.32 GBq/mmol

In the case of Molecular weight for 400

Theoretically 5.80 MBq/mg
(2.32 GBq/MW: 400)



Labeling index: about 70%

Measured value: 4.00 MBq/mg

● : Radioactive label (^{14}C)

Not all compounds are ^{14}C -labeled at the time of labeled synthesis

2. Preparation of Dosing Formulation

[Determination of the Radioactivity Dose]

- Our standard radioactivity dose is "3.7 MBq/kg"
(adjust appropriately considering detection limit, etc.)

$$\boxed{100 \mu\text{Ci (3.7 MBq)}} / \boxed{10 \text{ mg} / 5 \text{ mL}} / \text{kg}$$

Radioactivity Dose Dose Amount / Dose Volume

[Determination of Dose Amount / Dose Volume]

- Selected in consideration of PK and toxicity studies

Dosing formulation: 100 μCi (3.7 MBq)/10 mg/5 mL

The labeled compound and the unlabeled compound are weighed by a precision balance, and mixed.

In the case of Specific activity is “4.00 MBq/mg”

1. $3.7 \text{ MBq} / 4.00 \text{ MBq/mg}$
= 0.925 mg (amount of labeled compound)
2. $10 \text{ mg} - 0.925 \text{ mg}$
= 9.075 mg (compensated for by unlabeled compound)



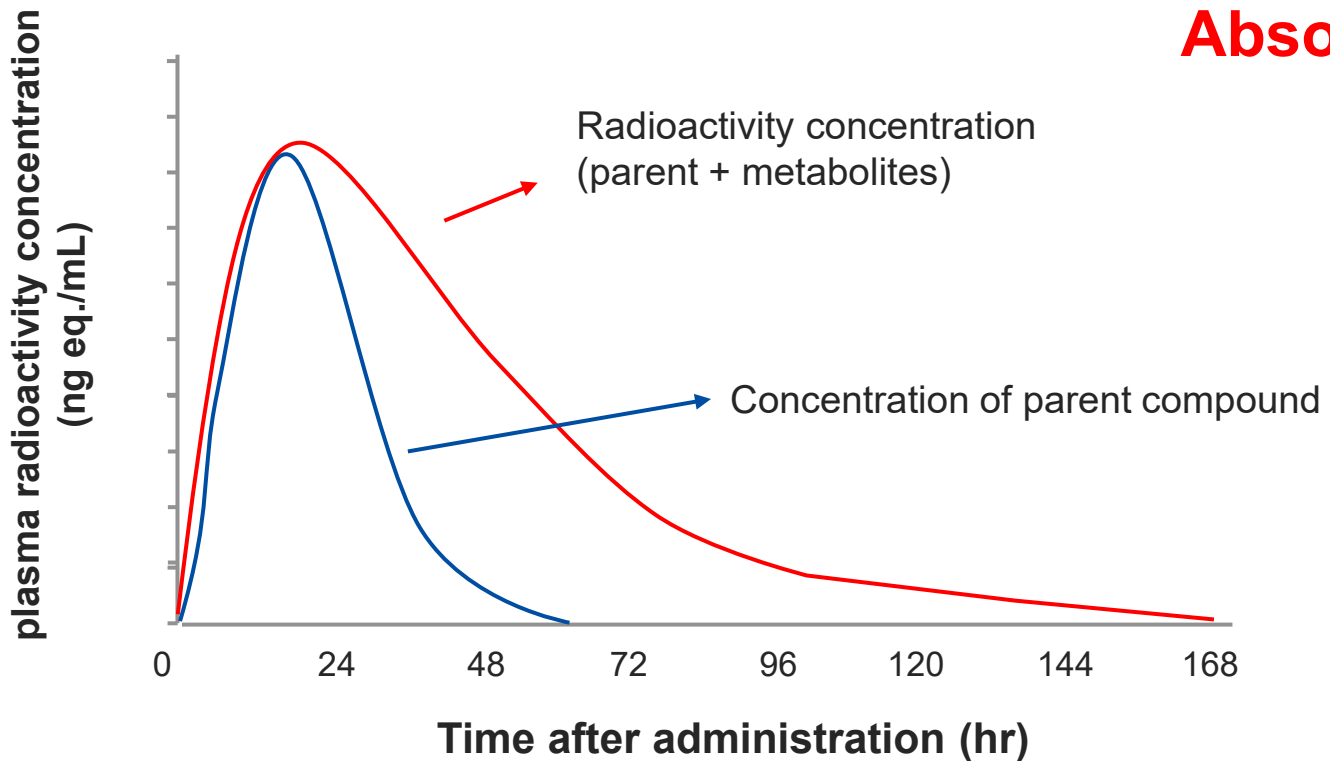
Prepare the dosing formulation by suspending or dissolving using the vehicle for dosing formulation.

1. A portion of the dosing formulation was collected and radioactivity is measured using LSC.
2. If the dosing formulation meets acceptance criteria (radiochemical purity, radioactivity concentration etc.), it will be administered to animals.

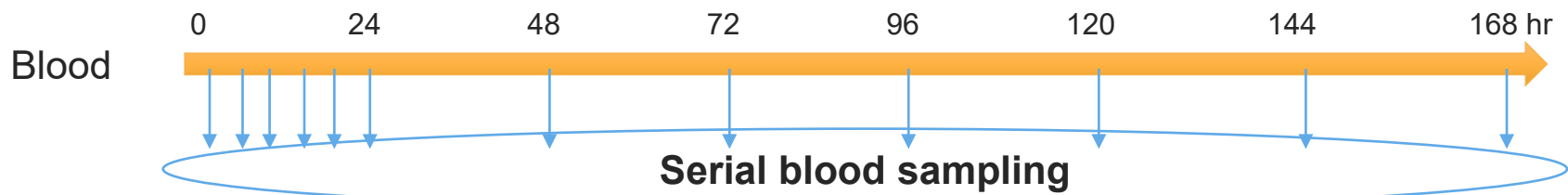
3. ADME Study Item

- Radioactivity Concentrations in Blood and Plasma: **Absorption**
- Urinary and Fecal Excretion: **Excretion**
- Biliary Excretion: **Excretion**
- Enterohepatic Circulation: **Excretion**
- Tissue Distribution: **Distribution**
(Tissue Excision/Quantitative Whole Body Autoradiography)
- Metabolite profile and Metabolite identification: **Metabolism**

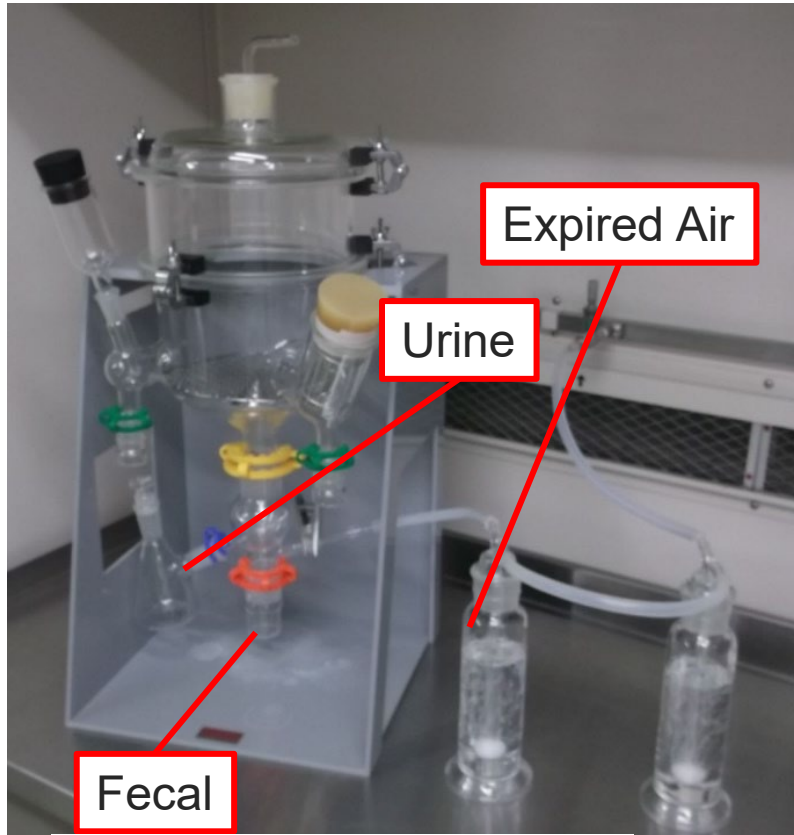
Absorption



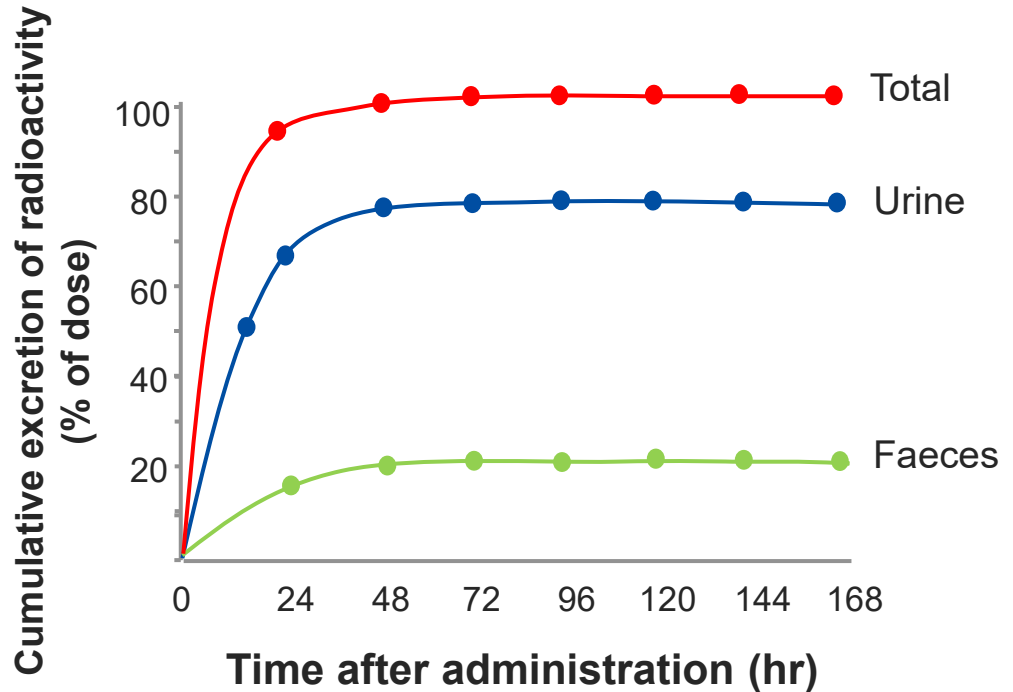
Sampling time points



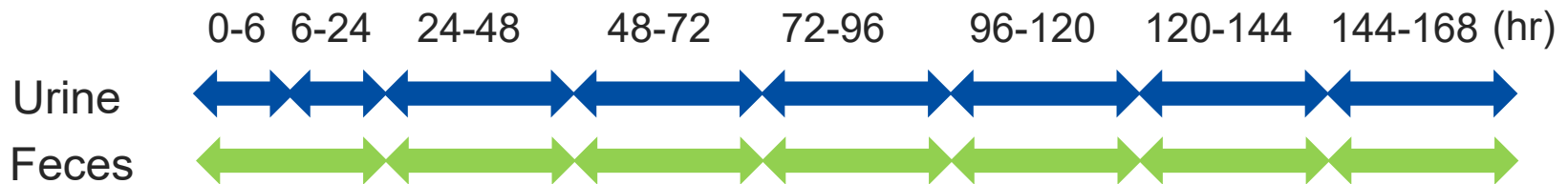
Measure radioactivity concentration in plasma to calculate pharmacokinetic parameters



Cumulative excretion of radioactivity in urine and feces after a single administration of radiolabeled compound

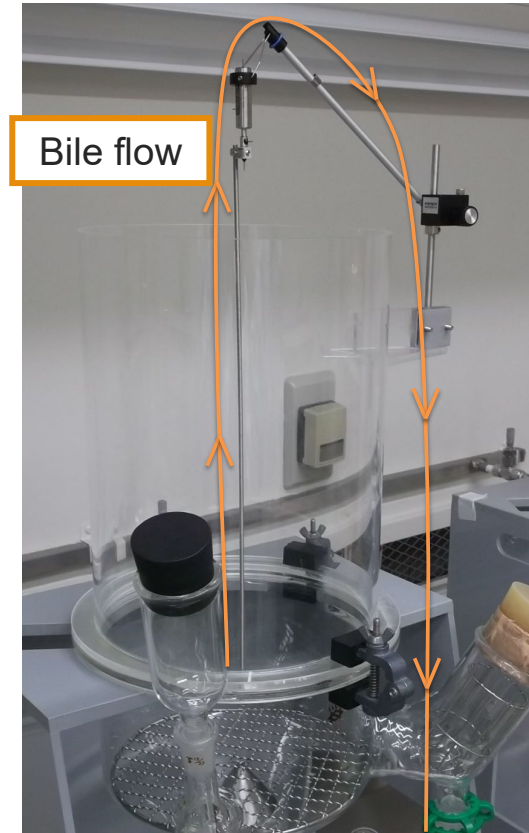
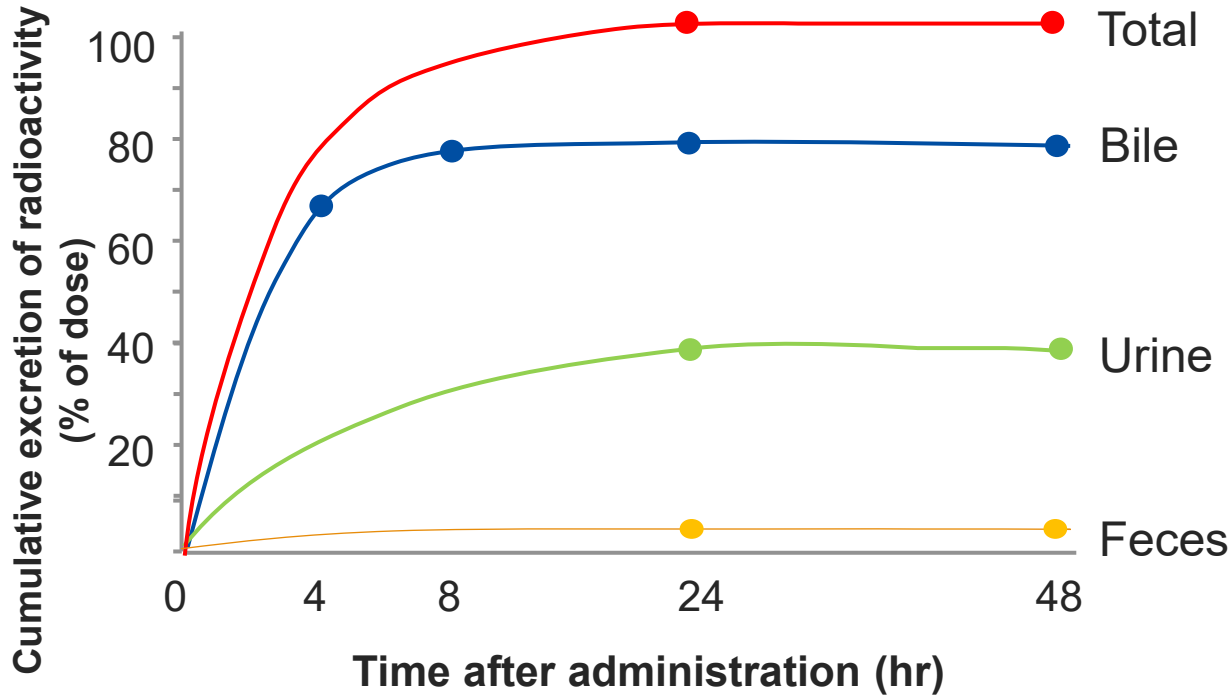


Sampling time points

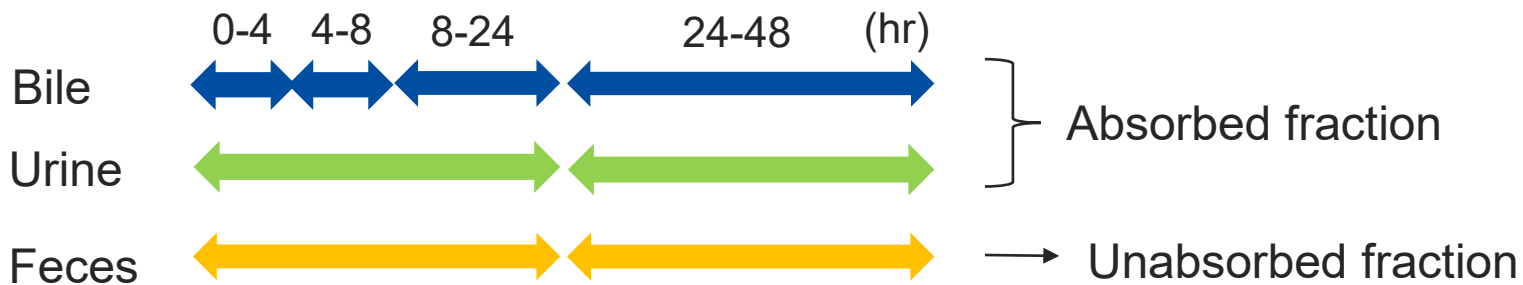


7 days after administration and more than 95% of dose

Cumulative excretion of radioactivity in bile, urine and feces after a single administration of radiolabeled compound



Sampling time points



Excretion

- High biliary excretion rate **and**
- The urinary excretion rate: intact > bile duct cannulation



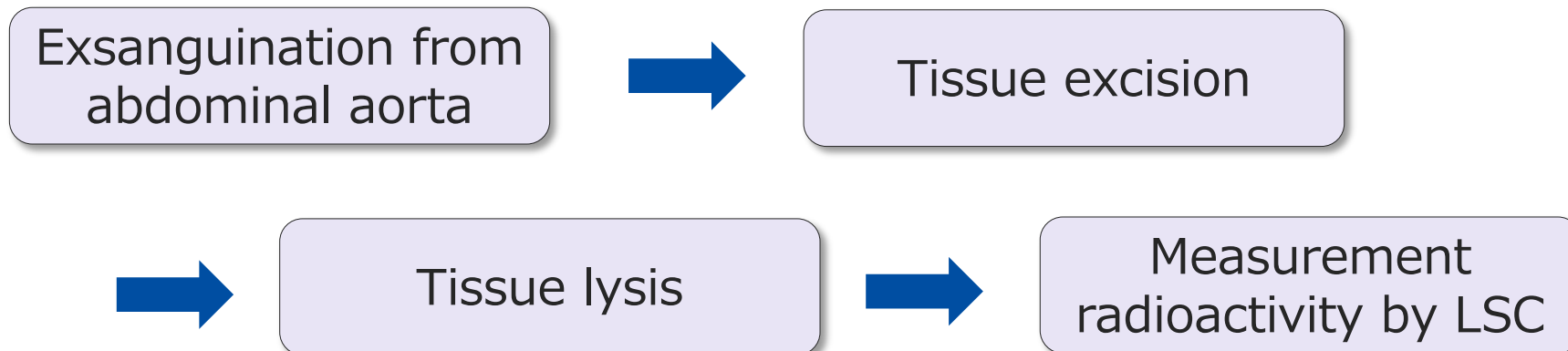
Consideration of Enterohepatic circulation study

Details

- Use of bile from biliary excretion studies
- Bile is injected into the duodenum
- Standard volume of bile is 0.1 mL
- Minimum radioactivity dose is 0.5 μCi (18.5 kBq)
- Infusion dosing is also possible

Distribution

➤ Tissue excision technique

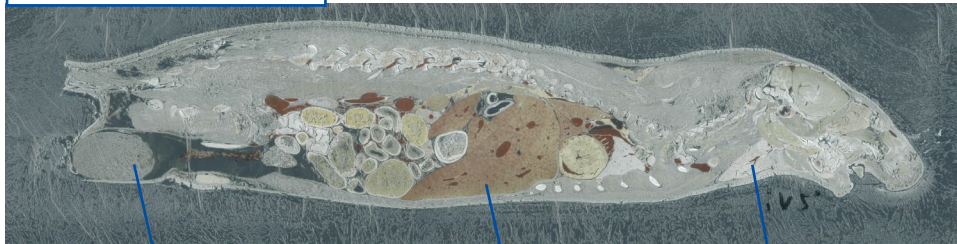


➤ Tissue list (example)

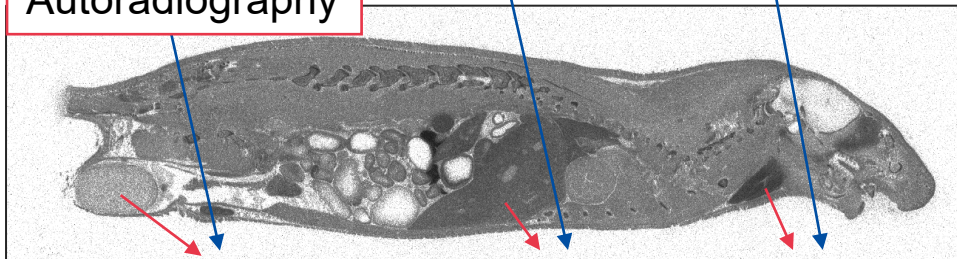
1.	Plasma	11.	Heart	21.	Skin
2.	Blood	12.	Lung	22.	Bone marrow
3.	Cerebrum	13.	Liver	23.	Artery
4.	Cerebellum	14.	Kidney	24.	Mesenteric lymph node
5.	Pituitary gland	15.	Adrenal gland	25.	Prostate gland
6.	Eyeball	16.	Spleen	26.	Testis
7.	Harderian gland	17.	Pancreas	27.	Epididymis
8.	Thyroid gland	18.	Skeletal muscle	28.	Stomach
9.	Mandibular gland	19.	Fat	29.	Small intestine
10.	Thymus	20.	Brown fat	30.	Large intestine

Distribution

Animal section



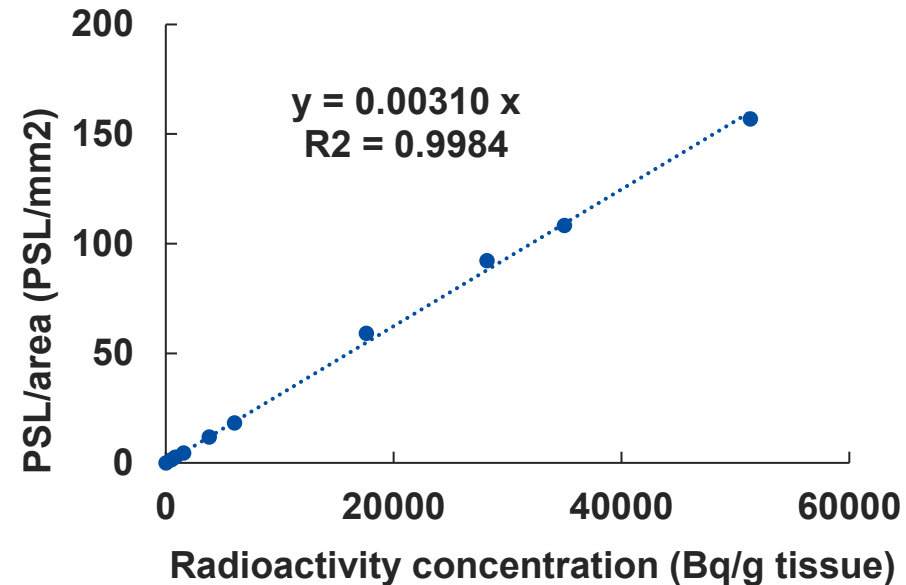
Autoradiography



testes

Liver

submandibular gland



Quantification: Calibration curve

X-axis: radioactivity concentration in standard

Y-axis: PSL (Photo-stimulated luminescence) value per unit area of standard

- **Select ROI (Region of interest) based on autoradiography**
- **Identify the PSL value of each tissues**
- **Convert to radioactivity concentration using calibration curve**

Typically cover 30 tissues in 3 animal sections

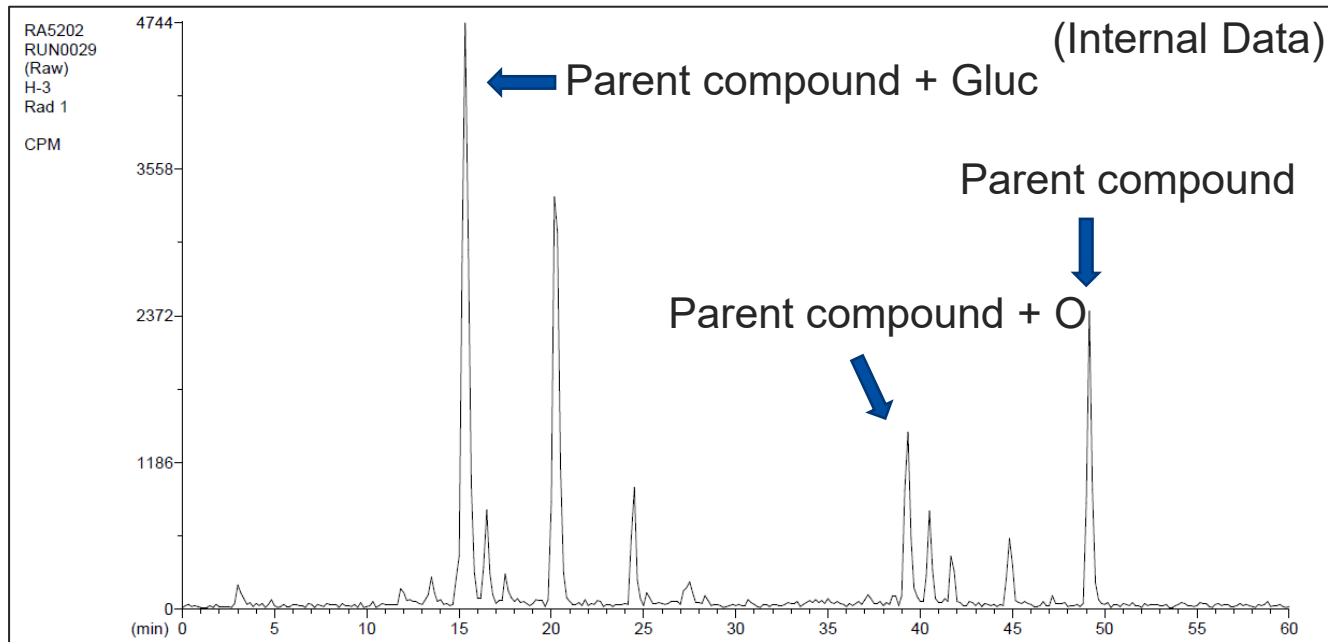
- ✓ Urinary and fecal excretion data in rodents
- ✓ Distribution data in rodents (**pigmented** and albino)
- ✓ Dose (mg)
- ✓ Radioactivity dose (Bq)
- ✓ Body weight (kg)



Effective dose (Sv/Bq) in human is estimated.

Metabolism

Radiochromatogram in biological samples

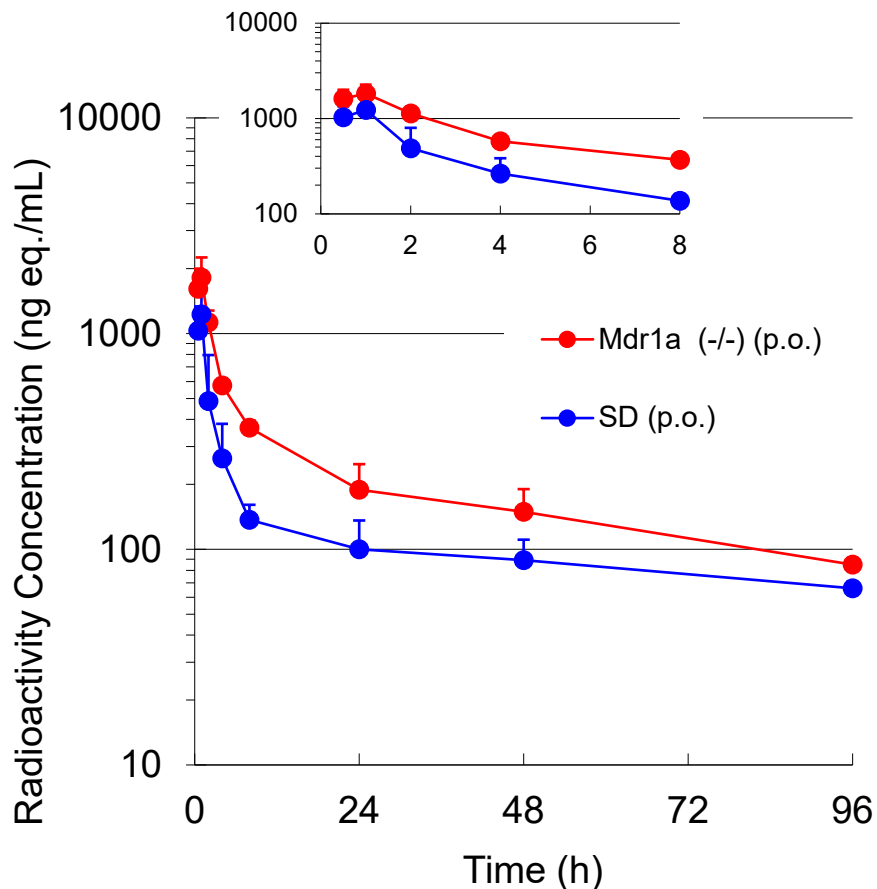


- Using the biological samples (Plasma, Urine, Feces, Bile etc.)
- Quantitative analysis of metabolites using HPLC with radioactive detector (HPLC-RAD)
- Metabolite identification using high resolution MS

4. Application of ADME Study Using Radiolabeled Compound

- Mdr1a-knockout [Mdr1a (-/-)] and SD rat, 7 week
- ³H-Qunidine, PO
- 0.25 - 4 MBq/10 mg/kg
- Radioactivity concentration in plasma (Absorption)
- Radioactivity concentration in tissues (Distribution)
- Autoradiography (Distribution)

Results (Plasma concentration, Absorption)



Pharmacokinetic parameters			
	Mdr1a (-/-) (p.o.)	SD (p.o.)	Ratio
C _{max} (ng/mL)	1819 ± 439	1229 ± 386	1.48
T _{max} (hr)	1 ± 0	1 ± 0	1.00
t _{1/2} (hr)	79 ± 45	93 ± 24	0.85
AUC _{0-last} (ng eq.·h/mL)	20428 ± 1924	11099 ± 2877	1.84
AUC _{0-∞} (ng eq.·h/mL)	30119 ± 4092	19949 ± 124.1	1.51

The radioactivity concentration in plasma after oral administration of ³H-quinidine at a dose of 10 mg/kg to fasting male Mdr1a-knockout rat and SD rat .

Results (Tissue concentration, Distribution)

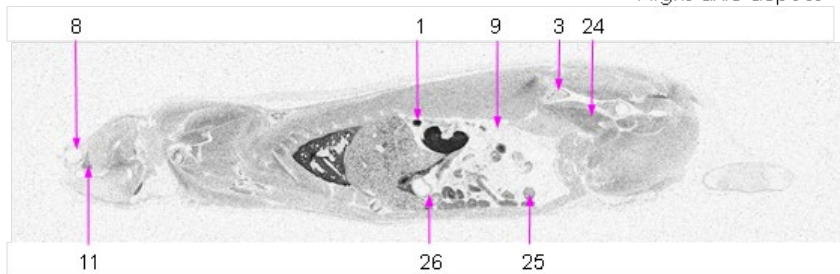
Tissue (5 min)	Radioactivity concentration (ng eq. of Quinidine/g or mL)				Kp ratio Mdr1a(-/-) / SD
	Mdr1a (-/-) (i.v.)		SD (i.v.)		
Plasma	2877	(1.00)	1978	(1.00)	1.00
Brain	4110	(1.43)	1314	(0.66)	2.17
Cerebrospinal fluid	449	(0.16)	94	(0.05)	3.20
Heart	27182	(9.45)	17308	(8.75)	1.08
Lung	73582	(25.58)	78637	(39.76)	0.64
Liver	10125	(3.52)	7865	(3.98)	0.88
Kidney	26690	(9.28)	19496	(9.86)	0.94
Spleen	22610	(7.86)	19111	(9.66)	0.81
Pancreas	65132	(22.64)	35037	(17.71)	1.28
Testis	2389	(0.83)	1342	(0.68)	1.22
Stomach	24454	(8.50)	19598	(9.91)	0.86
Duodenum	21965	(7.63)	21529	(10.88)	0.70
Small intestine	16296	(5.66)	16585	(8.38)	0.68
Large intestine	15020	(5.22)	9077	(4.59)	1.14

The radioactivity concentrations in tissues 5 min after intravenous administration of ³H-quinidine at a dose of 10 mg/kg to fasting male Mdr1a-knockout rat and SD rat .

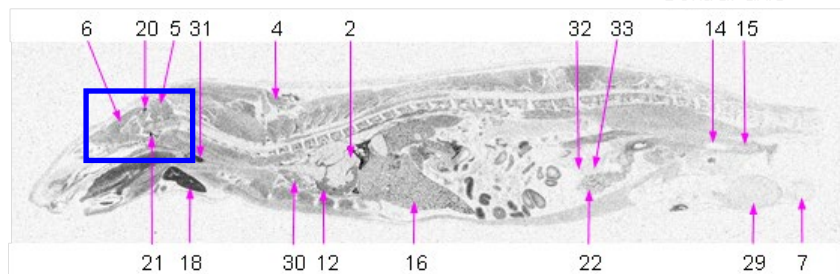
Results (Autoradiography, Distribution)

Mdr1a(-/-)

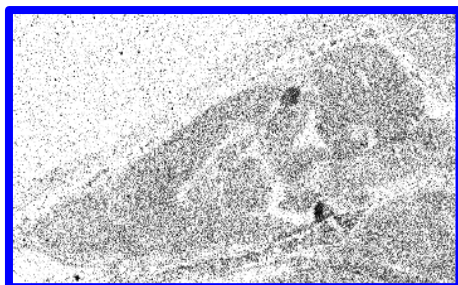
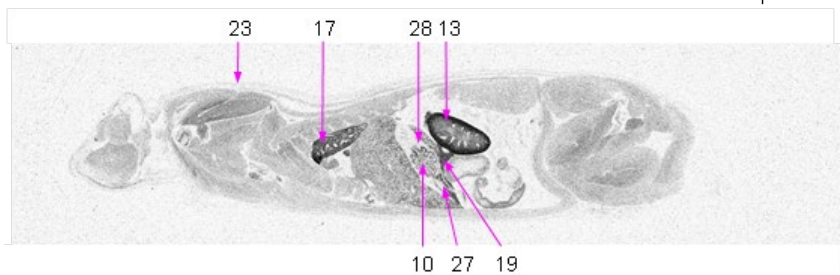
Right axis aspect



Central axis

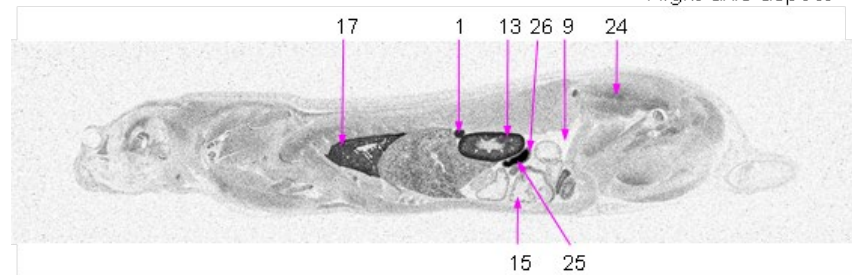


Left axis aspect

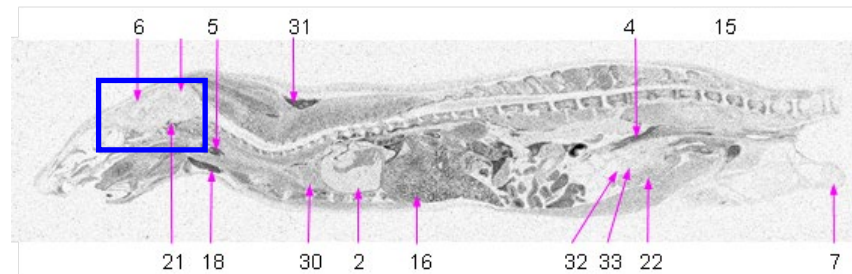


SD

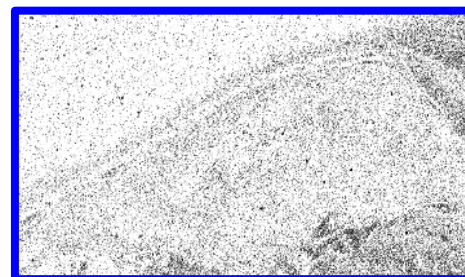
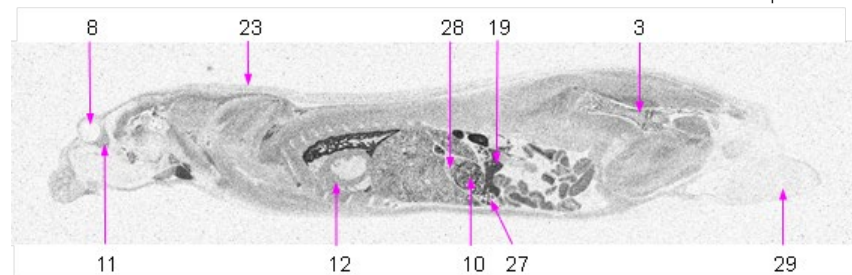
Right axis aspect



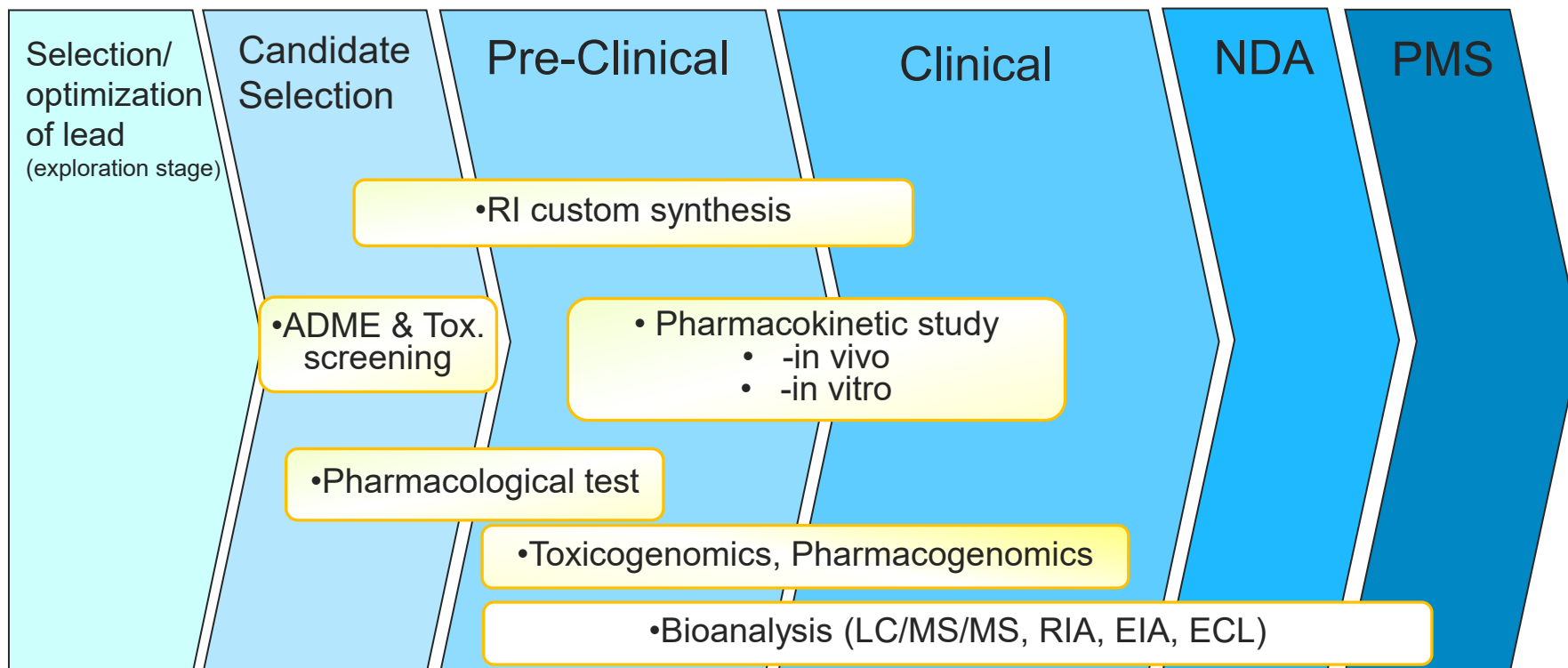
Central axis



Left axis aspect



Our services



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