

## Supplementary Information

Species	Study	Sex	Route	Dose (mg/kg)	Dose ( $\mu$ Ci/kg)	Dose Volume (mL/kg)	Formulation
Mouse	Cold PK	M/F	IV	5	--	5	70% PEG 400, 30% normal saline
	Cold PK	M/F	PO	250	--	10	100% PEG 400
	Mass Balance and $^{14}$ C-PK	M/F	IV	5	5	5	70% PEG 400, 30% normal saline
	Mass Balance and $^{14}$ C-PK	M/F	PO	250	500	10	100% PEG 400
Rat	Cold PK	M	IV	0.5	-	1	80% PEG 400, 20% normal saline
	$^{14}$ C-PK	M/F	IV	0.5	60	1	80% PEG 400, 20% normal saline
	Mass Balance	M/F	IV	0.5	40	1	80% PEG 400, 20% normal saline
	Cold PK	M	PO	5	--	5	100% PEG 400
	$^{14}$ C-PK	M/F	PO	5	96	5	100% PEG 400
	Mass Balance	M/F	PO	5	120	5	100% PEG 400
	Bile duct cannulated	M	PO	5	160	5	100% PEG 400
	QWBA	M	PO	5	160	5	100% PEG 400
Dog	Cold PK	M	IV	0.5	-	0.25	70% PEG 400, 30% normal saline
	Mass Balance and $^{14}$ C-PK	M/F	IV	0.5	10	0.25	70% PEG 400, 30% water
	Cold PK	M	PO	5	--	1	100% PEG 400
	Mass Balance and $^{14}$ C-PK	M/F	PO	5	20	1	100% PEG 400

Doses of empagliflozin and  $^{14}$ C-empagliflozin for PK and ADME studies

Species	Route	Dose (mg/kg)	Sex	$C_{max}$ ( $\mu$ M)	$t_{max}$ (h)	$AUC_{0-\infty}$ ( $\mu$ M $\cdot$ h)	$k_e$ (1/h)	$t_{1/2}$ (h)	MRT (h)	CL ‡ (mL/min/kg)	$V_{ss}$ ¶ (L/kg)	F (%)
Mouse	IV	5	M	-†	-	4.61	0.548	1.26	0.49	40.1	1.17	-
			F	-	-	5.60	1.06	0.65	0.43	33.0	0.858	-
	PO	250	M	97.7	0.67	207	0.124	5.59	2.52	44.6	6.74	89.8
			F	91.5	0.33	273	0.161	4.31	4.05	33.8	8.21	96.7
Rat	IV	0.5	M	-	-	1.32 $\pm$ 0.36	0.190 $\pm$ 0.68	3.64 $\pm$ 1.6	0.83 $\pm$ 0.51	14.8 $\pm$ 3.9	0.818 $\pm$ 0.71	-
	PO	5	M	0.724 $\pm$ 0.12	1	3.99 $\pm$ 0.65	0.110 $\pm$ 0.29	6.32 $\pm$ 2.3	7.18 $\pm$ 1.2	47.2 $\pm$ 7.4	20.0 $\pm$ 2.8	31.0 $\pm$ 5.1
Dog	IV	0.5	M	-	-	10.6 $\pm$ 1.3	0.111 $\pm$ 0.035	6.27 $\pm$ 2.1	5.32 $\pm$ 0.78	1.77 $\pm$ 0.20	0.568 $\pm$ 0.12	-
	PO	5	M	17.3 $\pm$ 1.6	1	93.7 $\pm$ 9.3	0.111 $\pm$ 0.017	6.25 $\pm$ 1.0	6.37 $\pm$ 0.61	1.98 $\pm$ 0.19	0.760 $\pm$ 0.11	89.0 $\pm$ 3.9

Mean pharmacokinetic parameters of empagliflozin in plasma from CD-1 mice, Wistar Han rats, and beagle dogs

† Not calculated. ‡ For oral dose group the value represents Clearance/F. ¶ For oral dose group the value represents  $V_z/F$ .

Species	Route	Dose (mg/kg)	Sex	C <sub>max</sub> (µM)	t <sub>max</sub> (h)	AUC <sub>0-∞</sub> (µM*h)	k <sub>e</sub> (1/h)	t <sub>1/2</sub> (h)	MRT (h)	CL ‡ (mL/min/kg)	V <sub>ss</sub> ¶ (L/kg)	F (%)
Mouse	IV	5	M	-†	-	11.1	0.709	0.98	1.26	16.6	1.25	-
			F	-	-	9.51	0.737	0.95	0.93	19.4	1.09	-
	PO	250	M	121	1	452	0.0878	7.89	6.46	20.4	7.92	81.5
			F	117	0.33	386	0.0578	12.0	7.93	23.9	11.4	81.2
Rat	IV	0.5	M	-	-	1.34 ± 0.24	1.03 ± 0.25	0.67 ± 0.15	0.57 ± 0.11	14.1 ± 2.7	0.487 ± 0.17	-
			F	-	-	1.51 ± 0.24	0.631 ± 0.21	1.10 ± 0.41	0.60 ± 0.11	12.5 ± 1.7	0.452 ± 0.11	-
	PO	5	M	1.27 ± 0.15	1	4.67 ± 0.89	0.395 ± 0.018	1.75 ± 0.08	2.88 ± 0.21	--	--	32.8 ± 6.3
			F	1.10 ± 0.18	1	4.20 ± 0.28	0.366 ± 0.043	1.89 ± 0.08	3.10 ± 0.39	--	--	25.8 ± 2.4
Dog	IV	0.5	M	-	-	10.2 ± 0.70	0.0315 ± 0.0056	22.0 ± 3.9	7.87 ± 1.4	1.76 ± 0.13	0.836 ± 0.19	-
			F	-	-	10.8 ± 0.20	0.0222 ± 0.0095	31.2 ± 13.0	10.9 ± 5.5	1.65 ± 0.03	1.08 ± 0.56	-
	PO	5	M	16.1 ± 0.78	1	101 ± 13	0.193 ± 0.0086	3.60 ± 0.16	5.07 ± 0.17	1.73 ± 0.22	-	102
			F	15.5 ± 1.1	1	96.3 ± 6.7	0.134 ± 0.049	5.16 ± 1.90	5.54 ± 0.48	1.80 ± 0.13	-	92.1

Mean pharmacokinetic parameters of empagliflozin-derived total radioactivity in plasma from CD-1 mice, Wistar Han rats, and beagle dogs

† Not calculated. ‡ For oral dose group the value represents Clearance/F. ¶ For oral dose group the value represents V<sub>z</sub>/F.

	Radioactivity concentration (µM) at different time point post-dose			
	1 h	8 h	24 h	72 h
Adrenal gland	0.653	ND	ND	ND
Bile	27.5	ND	ND	ND
Cecum	3.26	0.381	ND	ND
Esophagus	1.99	ND	ND	ND
Exorbital lacrimal gland	0.477	ND	ND	ND
Kidney	5.64	1.95	0.557	ND
Large intestine	0.815	ND	ND	ND
Liver	9.01	0.841	ND	ND
Lung	0.644	ND	ND	ND
Pancreas	0.495	ND	ND	ND
Preputial gland	0.921	ND	ND	ND
Prostate	0.588	ND	ND	ND
Renal cortex	6.42	2.97	1.01	ND
Renal medulla	4.55	0.937	0.170	ND
Salivary gland	0.584	ND	ND	ND
Skin	0.280	ND	ND	ND
Small intestine	0.753	ND	ND	ND
Spleen	0.351	BLQ	ND	ND
Stomach	0.575	ND	ND	ND
Urinary bladder	1.63	BLQ	ND	ND

Tissue radioactivity distribution after single oral dosing of 5 mg/kg [<sup>14</sup>C]-empagliflozin to male Long Evans rats