- Supplemental Data -

Lechner C, Ishiguro N, Fukuhara A, Shimizu H, Ohtsu O, Takatani M, Wasio I, Yamamura N, Kusuhara H (2015): Impact of Experimental Conditions on the Evaluation of Interactions between Multidrug and Toxin Extrusion Proteins and Candidate Drugs. *Drug Metabolism & Disposition*

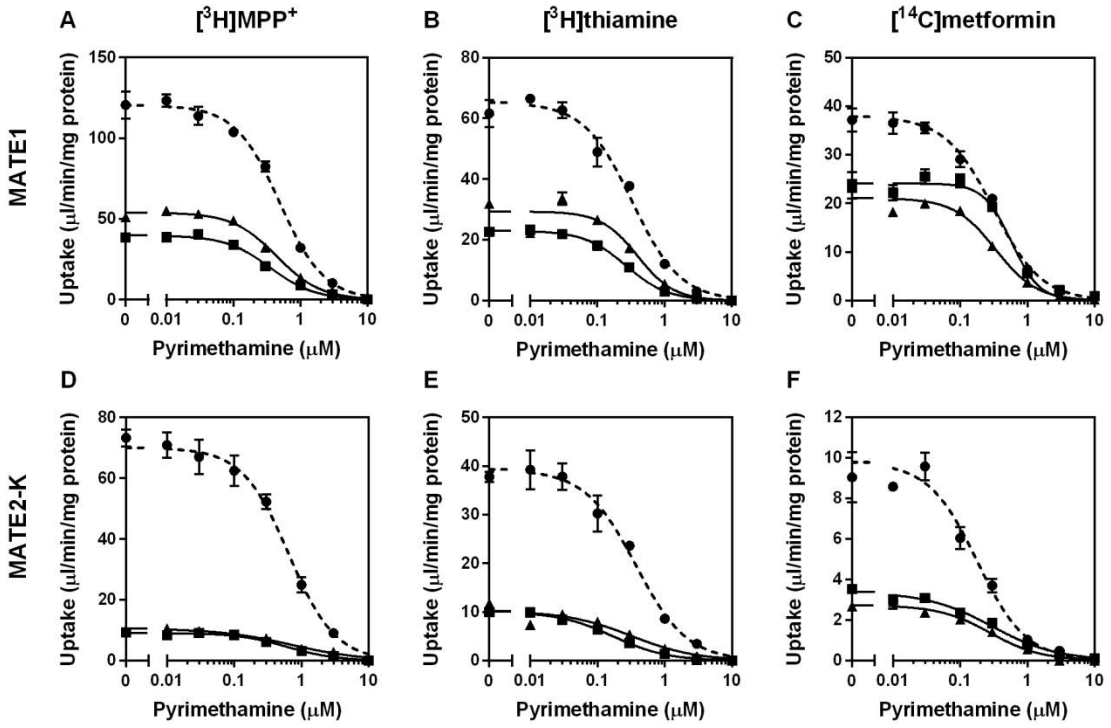

Fig. 1. Inhibitory effect of pyrimethamine on the uptake of [^{3}H]MPP$^+$, [^{3}H]thiamine, and [^{14}C]metformin at different assay conditions. Uptake of [^{3}H]MPP$^+$ (1 µM, 1 min) (A, D), [^{3}H]thiamine (1 µM, 1 min) (B, E), and [^{14}C]metformin (10 µM, 1 min) (C, F) was determined in the absence and presence of pyrimethamine (0.003-3 µM). All experiments were conducted with HEK293-MATE1 (A-C) and HEK293-MATE2-K cells (D-F) and at three different assay conditions: Condition A (K^+-based incubation medium, intracellular acidification by NH_4Cl) (closed circles), condition B (K^+-based incubation medium, without intracellular acidification) (closed squares), and condition C (Na^+-based incubation medium, without intracellular acidification) (closed triangles). Each point represents the mean value ± S.E. of triplicate measures from one experiment.

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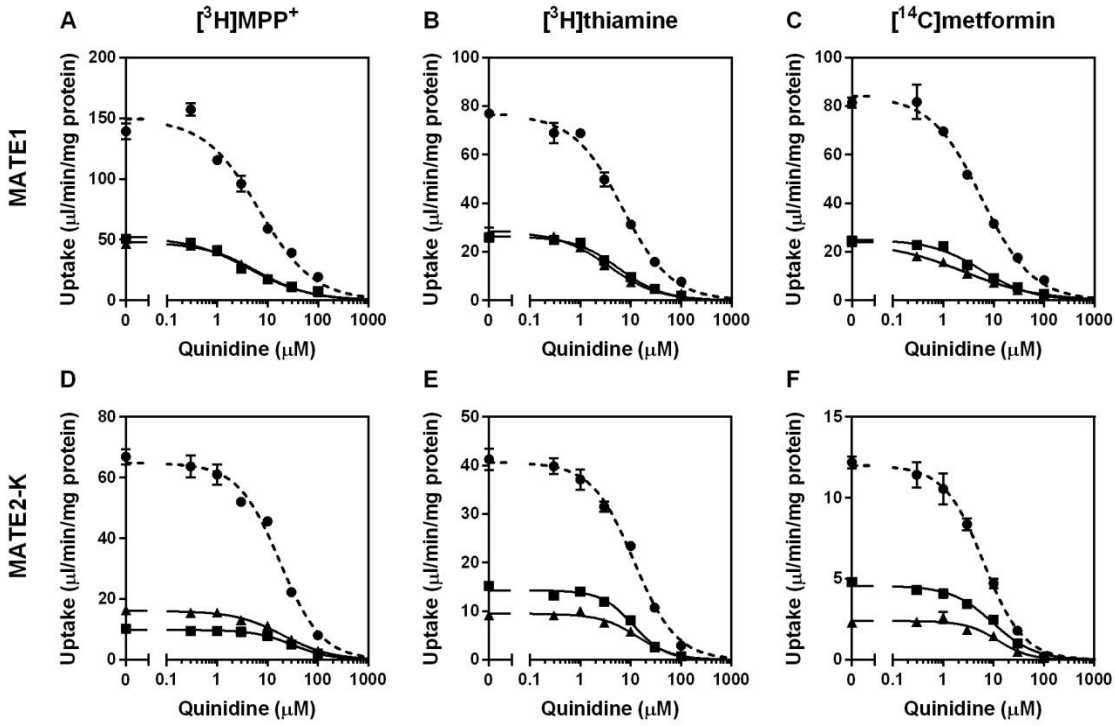

Fig. 2. Inhibitory effect of quinidine on the uptake of [^{3}H]MPP$^+$, [^{3}H]thiamine, and [^{14}C]metformin at different assay conditions. Uptake of [^{3}H]MPP$^+$ (1 μM, 1 min) (A, D), [^{3}H]thiamine (1 μM, 1 min) (B, E), and [^{14}C]metformin (10 μM, 1 min) (C, F) was determined in the absence and presence of quinidine (0.3-100 μM). All experiments were conducted with HEK293-MATE1 (A-C) and HEK293-MATE2-K cells (D-F) and at three different assay conditions: Condition A (K^+-based incubation medium, intracellular acidification by NH_4Cl) (closed circles), condition B (K^+-based incubation medium, without intracellular acidification) (closed squares), and condition C (Na^+-based incubation medium, without intracellular acidification) (closed triangles). Each point represents the mean value ± S.E. of triplicate measures from one experiment.

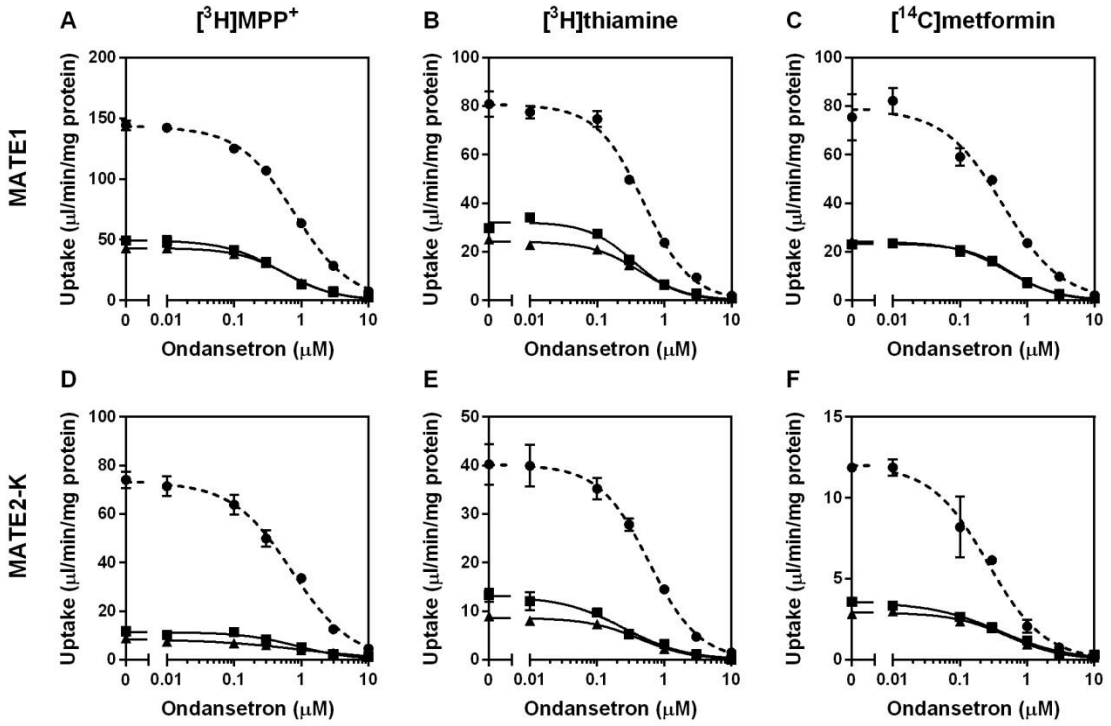

Fig. 3. Inhibitory effect of ondansetron on the uptake of [^{3}H]MPP$^+$, [^{3}H]thiamine, and [^{14}C]metformin at different assay conditions. Uptake of [^{3}H]MPP$^+$ (1 μM, 1 min) (A, D), [^{3}H]thiamine (1 μM, 1 min) (B, E), and [^{14}C]metformin (10 μM, 1 min) (C, F) was determined in the absence and presence of ondansetron (0.01-10 μM). All experiments were conducted with HEK293-MATE1 (A-C) and HEK293-MATE2-K cells (D-F) and at three different assay conditions: Condition A (K^+-based incubation medium, intracellular acidification by NH_4Cl) (closed circles), condition B (K^+-based incubation medium, without intracellular acidification) (closed squares), and condition C (Na^+-based incubation medium, without intracellular acidification) (closed triangles). Each point represents the mean value ± S.E. of triplicate measures from one experiment.

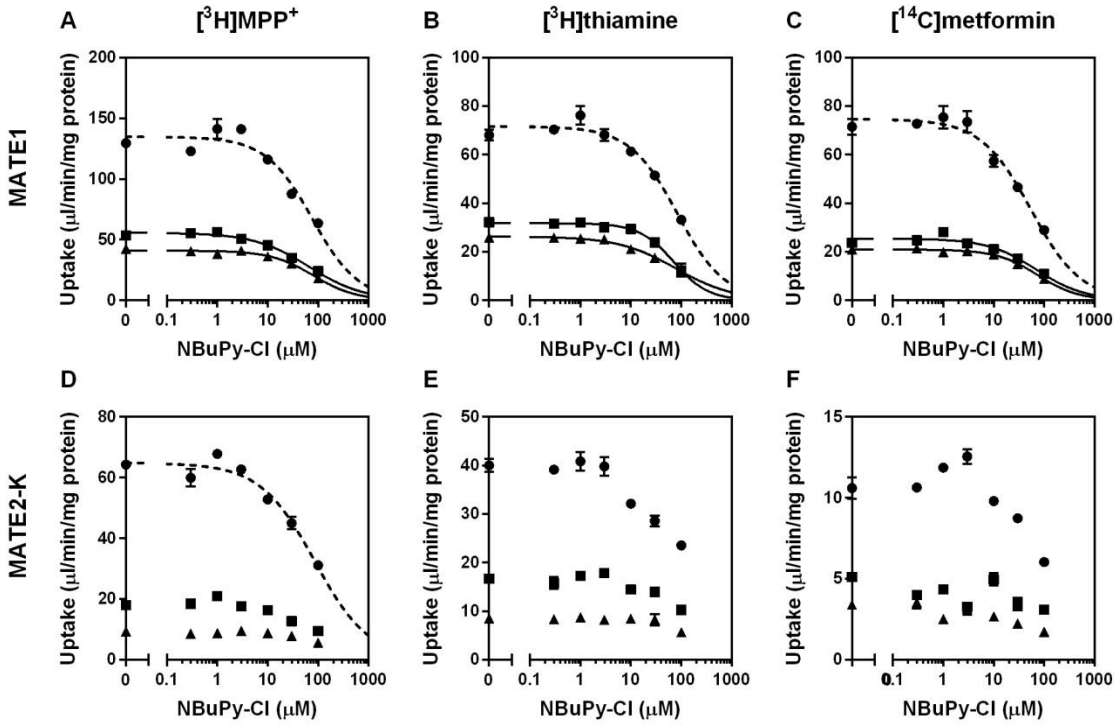

Fig. 4. Inhibitory effect of NBuPy-Cl on the uptake of [^{3}H]MPP$^+$, [^{3}H]thiamine, and [^{14}C]metformin at different assay conditions. Uptake of [^{3}H]MPP$^+$ (1 μM, 1 min) (A, D), [^{3}H]thiamine (1 μM, 1 min) (B, E), and [^{14}C]metformin (10 μM, 1 min) (C, F) was determined in the absence and presence of NBuPy-Cl (0.3-100 μM). All experiments were conducted with HEK293-MATE1 (A-C) and HEK293-MATE2-K cells (D-F) and at three different assay conditions: Condition A (K^+-based incubation medium, intracellular acidification by NH_4Cl) (closed circles), condition B (K^+-based incubation medium, without intracellular acidification) (closed squares), and condition C (Na^+-based incubation medium, without intracellular acidification) (closed triangles). Each point represents the mean value ± S.E. of triplicate measures from one experiment.

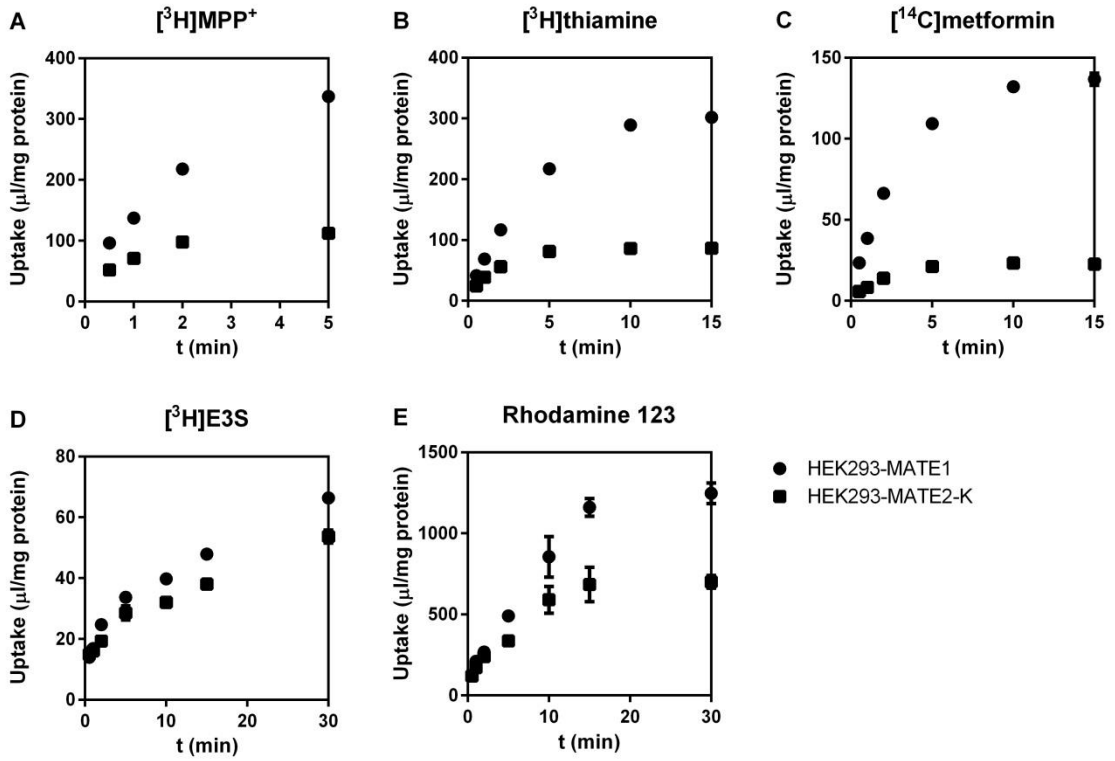

Fig. 5. Time-dependent uptake of [^{3}H]MPP$^+$ (A), [^{3}H]thiamine (B), [^{14}C]metformin (C), [^{3}H]E3S (D) and rhodamine 123 (E) by HEK293 cells expressing MATE1 or MATE2-K and control cells. Uptake of [^{3}H]MPP$^+$ (1 µM), [^{3}H]thiamine (1 µM), [^{14}C]metformin (10 µM), [^{3}H]E3S (10 µM) and rhodamine 123 (1 µM) was determined in HEK293-MATE1 (closed circles), HEK293-MATE2-K (closed squares) at condition A (see Table 1). Transporter-mediated uptake was calculated by subtracting the uptake in HEK293-mock cells from that in transporter expressing cells. Each point represents the mean value ± S.E. of triplicate measures from one experiment.

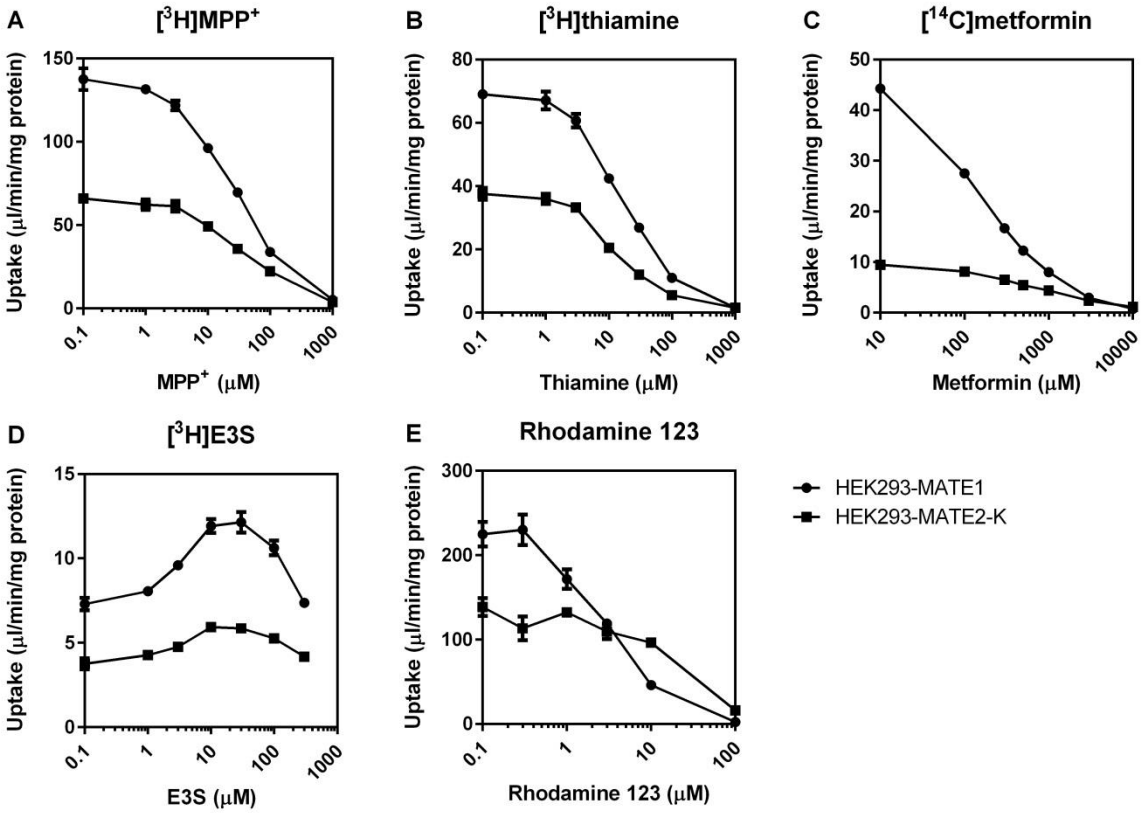

Fig. 6. Concentration-dependent uptake of [^{3}H]MPP$^+$ (A), [^{3}H]thiamine (B), [^{14}C]metformin (C), [^{3}H]E3S (D) and rhodamine 123 (E) by HEK293 cells expressing MATE1 or MATE2-K. Uptake of [^{3}H]MPP$^+$ (0.1 – 1000 µM, 1 min), [^{3}H]thiamine (0.1 - 1000 µM, 1 min), [^{14}C]metformin (10 - 10000 µM, 1 min), [^{3}H]E3S (0.1 – 300 µM, 2 min) and rhodamine 123 (0.1 - 100 µM, 2 min) was determined in HEK293-MATE1 (closed circles), HEK293-MATE2-K (closed squares) at condition A (see Table 1). Transporter-mediated uptake was calculated by subtracting the uptake in HEK293-mock cells from that in transporter expressing cells. Each point represents the mean value ± S.E. of triplicate measures from one experiment.