Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supplementary Information

Human CYP2A6 catalyzes the oxidation of 6:2 fluorotelomer alcohol

Oluwadamilola Daramola[1], Amy A. Rand[1]

[1]Department of Chemistry and Institute of Biochemistry, Carleton University, 1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada

Email address: amy.rand@carleton.ca

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Spiked concentration (ng/mL)	Recovered concentration (ng/mL)	Recovery (%)
1000	1020 ± 20	102 ± 2.0
4000	3800 ± 41	95 ± 1.0
8000	7120 ± 31	89 ± 0.4
15000	9900 ± 100	66 ± 0.7
50000	27500 ± 81	55 ± 0.2

Table S1. Spike and recovery data for 6:2 FTOH. Recovery values were obtained in triplicate after 2 hour incubations in 100 mM phosphate buffer and the NADPH recombinant system.

Treatment	Rate relative to control (%)
Uninhibited control	100.0 ± 5.3
Chloramphenicol (CYP2C19), 30 μM	87.5 ± 8.3
Diallyl disulfide (CYP2E1), 68 μM	97.2 ± 12.7
Sulfaphenazole (CYP2C9), 30 μM	95.2 ± 7.0
Quinidine (CYP2D6), 30 μM	84.1 ± 10.2
Ketoconazole (CYP3A4), 18 μM	58.4 ± 7.3
Tranylcypromine, HCl (CYP2A6), 50 μM	40.7 ± 5.3
1-Aminobenzotriazole (General CYP inhibitor), 75 μM	62.9 ± 1.0

Table S2. Relative metabolic rates of 6:2 FTOH conversion in the presence of competitive CYP inhibitors. The starting concentration of 6:2 FTOH is 10000 ng/mL. Standard deviations are derived from n=3 replicates.

Treatment	Rate relative to control (%)
Uninhibited control	100.0 ± 2.4
Chloramphenicol (CYP2C19), 30 μM	120.7 ± 0.9
Diallyl disulfide (CYP2E1), 68 μM	100.2 ± 0.3
Sulfaphenazole (CYP2C9), 30 μM	101.7 ± 1.8
Quinidine (CYP2D6), 30 μM	99.0 ± 4.0
Ketoconazole (CYP3A4), 18 μM	60.4 ± 7.3
Tranylcypromine, HCl (CYP2A6), 50 μM	37.6 ± 4.1

1-Aminobenzotriazole (General CYP inhibitor), 75 μM	66.1 ± 11.2

Table S3. Relative metabolic rates of 6:2 FTOH conversion in the presence of competitive inhibitors. The starting concentration of 6:2 FTOH is 1000 ng/mL. Standard deviations are derived from n=3 replicates.

	6:2 FTOH levels over time (ng/mL)			
Treatment	17 hr	20 hr	25 hr	31 hr
Active CYP2A6	4811 ± 11	4709 ± 20	4520 ± 10	4464 ± 15
Tranylcypromine, HCl, 17.5 μM	4523 ± 5	4510 ± 25	4502 ± 13	4497 ± 13
Tranylcypromine, HCl, 35 μM	4493 ± 17	4492 ± 13	4493 ± 15	4488 ± 20
Blank	4497 ± 20	4500 ± 19	4499 ± 20	4493 ± 17

Table S4. 6:2 FTOH conversion by human recombinant CYP2A6 with and without the inhibitor tranylcypromine. The initial concentration of 6:2 FTOH is 10000 ng/mL. The concentration of CYP2A6 is 1.5 μg/mL. Standard deviations are acquired from n = 3 replicates.

Treatment	6:2 FTOH loss rate
Active CYP2A6	282.2 ± 56.0
Tranylcypromine, HCl, 17.5 μM	21.2 ± 5.2
Tranylcypromine, HCl, 35 μM	3.9 ± 1.9
Blank	2.0 ± 6.5

Table S5. Metabolic rates of inhibited and uninhibited 6:2 FTOH conversion in the presence of human recombinant CYP2A6. The initial concentration of 6:2 FTOH is 10000 ng/mL. The concentration of CYP2A6 is 1.5 μg/mL. Standard deviations are acquired from n = 3 replicates.

	6:2 FTOH levels over time (ng/mL)			
Treatment	17 hr	20 hr	25 hr	31 hr
Active CYP2A6	948 ± 3	919 ± 11	875 ± 5	822 ± 3
Tranylcypromine, HCl, 35 μM	1090 ± 3	1090 ± 6	1085 ± 5	1083 ± 3
Blank	1111 ± 15	1109 ± 20	1110 ± 10	1107 ± 13

Table S6. 6:2 FTOH conversion by human recombinant CYP2A6 with and without the inhibitor tranylcypromine. The initial concentration of 6:2 FTOH is 1000 ng/mL. The concentration of CYP2A6 is 1.5 μg/mL. Standard deviations are acquired from n = 3 replicates.

Treatment	6:2 FTOH loss rate
Active CYP2A6	100.8 ± 3.3
Tranylcypromine, HCl, 35 µM	6.0 ± 5.3
Blank	2.0 ± 6.0

Table S7. Metabolic rates of inhibited and uninhibited 6:2 FTOH conversion in the presence of human recombinant CYP2A6. The initial concentration of 6:2 FTOH is 1000 ng/mL. The concentration of CYP2A6 is 1.5 μg/mL. Standard deviations are acquired from n = 3 replicates.

Treatment	Concentration (ng/mL)	6:2 FTOH loss rate (ng/mL min)	Specific rate (ng/min mg)
Uninhibited control	1000	12.1 ± 1.3	60.5
	2000	24.0 ± 0.2	119.9
	4000	37.8 ± 1.6	188.8
	7000	35.9 ± 1.5	179.6
	8000	43.7 ± 1.5	218.6
	9000	43.8 ± 3.3	218.9
	15000	52.3 ± 3.9	261.5
	20000	60.5 ± 1.9	302.4
	50000	64.0 ± 2.3	320.0
Tranylcypromine, HCl	1000	6.7 ± 1.5	33.5
	2000	10.5 ± 1.0	52.4
	4000	21.6 ± 1.1	108.1
	7000	21.1 ± 0.8	105.5
	8000	30.1 ± 1.5	150.8
	9000	35.8 ± 3.0	178.9
	15000	46.5 ± 2.5	232.7
	20000	51.9 ± 1.9	259.3
	50000	56.1 ± 3.0	280.4

Table S8. Rate and specific rate of 6:2 FTOH transformation in human liver microsomes, in presence and absence of the CYP2A6 selective inhibitor tranylcypromine (50.0 μM). Standard deviations are acquired from n = 3 replicates.

		6:2 FTOH levels over time (ng/ml)					
Treatment	[6:2 FTOH] (ng/ml)	5 min	30 min	60 min	90 min	120 min	Loss Rate (ng/ml/min)
Active CYP3A4	10000	5780 ± 129	5164 ± 188	4571 ± 149	4755 ± 93	4113 ± 79	12.8
Heat inactivated control	10000	6340 ± 125	5866 ± 35	5459 ± 110	4983 ± 77	4756 ± 130	13.9
Active CYP3A4	1000	852 ± 52	747 ± 9	894 ± 21	842 ± 9	832 ± 19	0.21
Heat inactivated control	1000	846 ± 93	866 ± 68	823 ± 40	835 ± 41	811 ± 20	0.35

Table S9. 6:2 FTOH conversion by human recombinant CYP3A4. The initial concentration of 6:2 FTOH is 10000 or 1000 ng/mL. The concentration of CYP3A4 is 100 μg/mL. Standard deviations are acquired from n = 3 replicates.

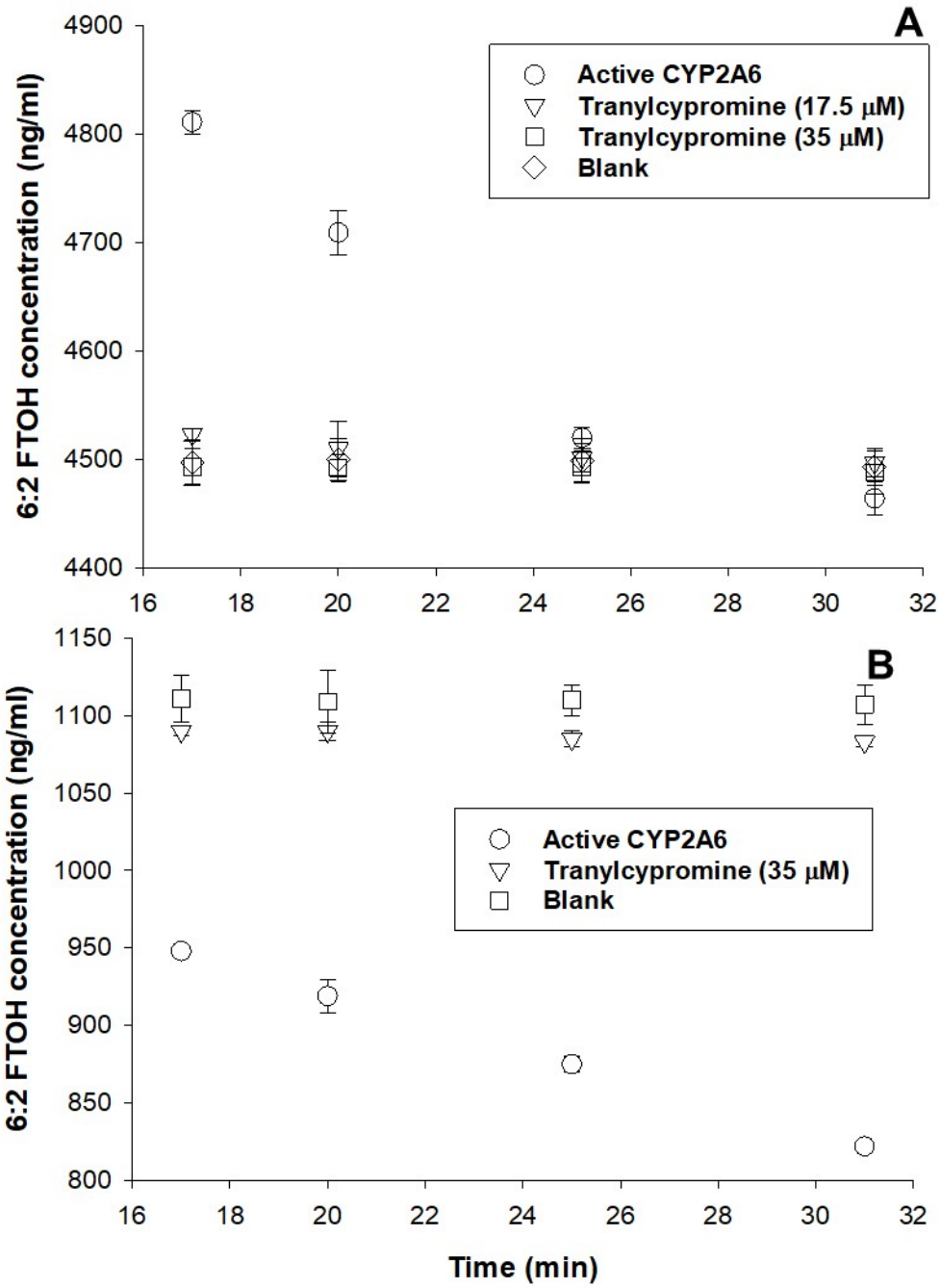

Figure S1. 6:2 FTOH metabolizes by human recombinant CYP2A6. 6:2 FTOH was incubated with human recombinant CYP2A6 (1.5 μg/mL) and a NADPH regeneration system in the presence and absence of the CYP2A6 selective inhibitor tranylcypromine. Panel A depicts the high concentration (10000 ng/mL) of 6:2 FTOH. Panel B depicts results with the low concentration (1000 ng/mL) of 6:2 FTOH. Blank assays were run in absence of enzyme. Standard deviations were derived from n=3 replicates. Raw data used for this figure are reported in supplemental Tables S4 and S6.

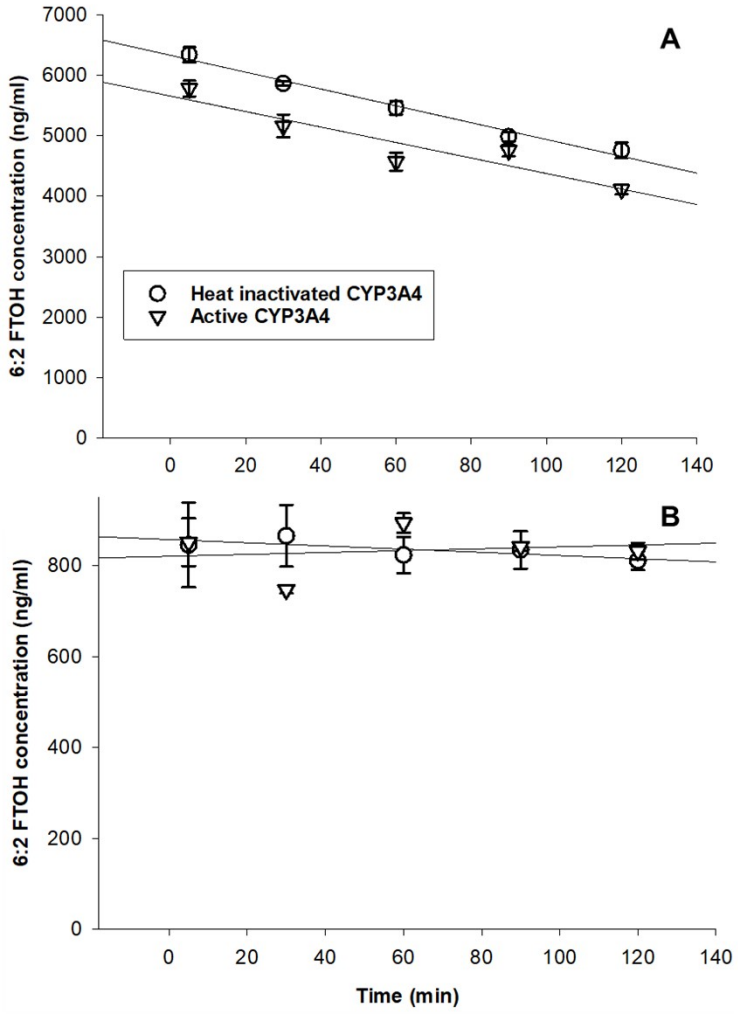

Figure S2. 6:2 FTOH does not metabolize by human recombinant CYP3A4. 6:2 FTOH was incubated with human recombinant CYP3A4 (100 μg/mL) and a NADPH regeneration system. As a negative control, the enzyme was heat inactivated. Panel A depicts the high concentration (10000 ng/mL) of 6:2 FTOH. Panel B depicts results with the low concentration (1000 ng/mL) of 6:2 FTOH. Standard deviations were derived from n=3 replicates. Raw data used for this figure are reported in supplemental Table S9.