

SUPPLEMENTAL INFORMATION

ESTIMATION OF CAFFEINE INTAKE FROM ANALYSIS OF CAFFEINE METABOLITES IN WASTEWATER

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Table S1. Excretory profile of caffeine and its metabolites**Caffeine (1,3,7-trimethylxanthine)**

References	Dose	Subjects treated	Duration (h)	Caffeine excretion (%)	SD
(Latini et al., 1981)	5 mg/kg	4	72	1.8	
(Dan-Shya et al., 1983)	Theophylline (7.5 mg/kg) and caffeine (7.5 mg/kg) 2 weeks later	6	60	3.7	1
(Callahan et al., 1982)	5 mg/kg (¹⁴ C-labeled caffeine)	10	48	1.1	0.59
(Callahan et al., 1983)	5 mg/kg (2- ¹⁴ C)caffeine	4 males 4 females oral contraceptives 4 ovulating females	96	1.46 2.61 1.33	0.4 1.19 0.45
(Blanchard et al., 1985)	5 mg/kg	5 (elderly) 7 (young)	24	1.93 2.35	0.57 2.05
(Scott et al., 1986)	123-369 mg 300-750 mg	15 pregnant 9 female	24	3.3 2.0	1.4 1.1
(Carrillo and Benitez, 1994)	300 mg	107	24	1.4	0.07

Paraxanthine (1,7-dimethylxanthine)

References	Dose	Subjects treated	Duration (h)	Paraxanthine excretion (%)	SD
(Latini et al., 1981)	5 mg/kg	4	72	5	
(Dan-Shya et al., 1983)	Theophylline (7.5 mg/kg) and caffeine (7.5 mg/kg) 2 weeks later	6	60	7.1	1.7
(Callahan et al., 1982)	5 mg/kg (¹⁴ C-labeled caffeine)	10	48	5.7	1.64
(Callahan et al., 1983)	5 mg/kg (2- ¹⁴ C)caffeine	4 males	96	5.39	1.63

1983)		4 females oral contraceptives 4 ovulating females		5.49 3.45	0.26 0.18
(Grant et al., 1983)	300 mg	68	24	4.8	2.4
(Blanchard et al., 1985)	5 mg/kg	5 (elderly) 7 (young)	24	3.37 3.49	1.47 1.87
(Scott et al., 1986)	123-369 mg 300-750 mg	15 pregnant 9 female	24	5.8 4.7	1.1 0.9
(Carrillo and Benitez, 1994)	300 mg	107	24	4.08	0.18

1-methylxanthine

References	Dose	Subjects treated	Duration (h)	1-methylxanthine excretion (%)	SD
(Latini et al., 1981)	5 mg/kg	4	72	16	
(Dan-Shya et al., 1983)	Theophylline (7.5 mg/kg) and caffeine (7.5 mg/kg) 2 weeks later	6	60	10	3
(Callahan et al., 1982)	5 mg/kg (¹⁴ C-labeled caffeine)	10	48	16.31	3.76
(Callahan et al., 1983)	5 mg/kg (2- ¹⁴ C)caffeine	4 males 4 females oral contraceptives 4 ovulating females	96	14.88 9.32 12.28	1.94 1.44 4.75
(Grant et al., 1983)	300 mg	68	24	10.1	4.1
(Blanchard et al., 1985)	5 mg/kg	5 (elderly) 7 (young)	24	8.9 9.48	5.4 3.7
(Scott et al., 1986)	123-369 mg 300-750 mg	15 pregnant 9 female	24	7.3 11.4	3.4 2.1
(Carrillo and Benitez, 1994)	300 mg	107	24	9.13	0.4

7-methylxanthine

References	Dose	Subjects treated	Duration (h)	7-methylxanthine excretion (%)	SD
(Latini et al., 1981)	5 mg/kg	4	72	8.5	
(Dan-Shya et al., 1983)	Theophylline (7.5 mg/kg) and caffeine (7.5 mg/kg) 2 weeks later	6	60	4	1.6
(Callahan et al., 1983)	5 mg/kg (2- ¹⁴ C)caffeine	4 males 4 females oral contraceptives 4 ovulating females	96	1.84 1.81 2.17	0.5 0.25 0.45
(Grant et al., 1983)	300 mg	68	24	2.5	1.4
(Blanchard et al., 1985)	5 mg/kg	5 (elderly) 7 (young)	24	2.32 2.4	1.18 1.45
(Scott et al., 1986)	123-369 mg 300-750 mg	15 pregnant 9 female	24	5 4	2.6 0.3
(Carrillo and Benitez, 1994)	300 mg	107	24	3.11	0.21

1-methyluric acid

References	Dose	Subjects treated	Duration (h)	1-methyluric acid excretion (%)	SD
(Latini et al., 1981)	5 mg/kg	4	72	51	
(Dan-Shya et al., 1983)	Theophylline (7.5 mg/kg) and caffeine (7.5 mg/kg) 2 weeks later	6	60	21	8
(Callahan et al., 1982)	5 mg/kg (¹⁴ C-labeled caffeine)	10	48	25.55	5.2
(Callahan et al., 1983)	5 mg/kg (2- ¹⁴ C)caffeine	4 males 4 females oral contraceptives 4 ovulating females	96	19.89 11.06 16.14	3.9 1.95 4.79
(Grant et al., 1983)	300 mg	68	24	11.8	5

(Blanchard et al., 1985)	5 mg/kg	5 (elderly) 7 (young)	24	38.12 22.05	14.23 4.69
(Scott et al., 1986)	123-369 mg 300-750 mg	15 pregnant 9 female	24	9.4 19.5	3.7 5.3
(Carrillo and Benitez, 1994)	300 mg	107	24	16.49	0.84

1,7-dimethyluric acid

References	Dose	Subjects treated	Duration (h)	1,7-dimethyluric acid excretion (%)	SD
(Latini et al., 1981)	5 mg/kg	4	72	8.5	
(Dan-Shya et al., 1983)	Theophylline (7.5 mg/kg) and caffeine (7.5 mg/kg) 2 weeks later	6	60	7.3	1
(Callahan et al., 1982)	5 mg/kg (¹⁴ C-labeled caffeine)	10	48	4.32	1.64
(Callahan et al., 1983)	5 mg/kg (2- ¹⁴ C)caffeine	4 males 4 females oral contraceptives 4 ovulating females	96	6.19 9 6.05	3.31 2.04 2.63
(Grant et al., 1983)	300 mg	68	24	6	1.9
(Blanchard et al., 1985)	5 mg/kg	5 (elderly) 7 (young)	24	12.56 7.81	1.99 3.36
(Scott et al., 1986)	123-369 mg 300-750 mg	15 pregnant 9 female	24	9.3 7.2	2.9 2.1
(Carrillo and Benitez, 1994)	300 mg	107	24	6.57	0.22

Theophylline (1,3-dimethylxanthine)

References	Dose	Subjects treated	Duration (h)	Theophylline excretion (%)	SD
(Blanchard et al., 1985)	5 mg/kg	5 (elderly) 7 (young)	24	0.48 0.77	0.31 0.69
(Scott et al., 1986)	123-369 mg 300-750 mg	15 pregnant 9 female	24	1.6 0.8	0.5 0.4
(Carrillo and Benitez, 1994)	300 mg	107	24	0.5	0.04

Theobromine (3,7-dimethylxanthine)

References	Dose	Subjects treated	Duration (h)	Theobromine excretion (%)	SD
(Latini et al., 1981)	5 mg/kg	4	72	3.2	
(Callahan et al., 1982)	5 mg/kg (¹⁴ C-labeled caffeine)	10	48	1.57	0.46
(Callahan et al., 1983)	5 mg/kg (2- ¹⁴ C)caffeine	4 males 4 females oral contraceptives 4 ovulating females	96	1.21 1.18 0.92	0.29 0.23 0.54
(Grant et al., 1983)	300 mg	68	24	1.1	0.6
(Blanchard et al., 1985)	5 mg/kg	5 (elderly) 7 (young)	24	1.22 2.04	0.79 1.96
(Scott et al., 1986)	123-369 mg 300-750 mg	15 pregnant 9 female	24	4.3 1.4	3.4 0.6
(Carrillo and Benitez, 1994)	300 mg	107	24	1.28	0.1

1,3-dimethyluric acid

References	Dose	Subjects treated	Duration (h)	1,3-dimethyluric acid excretion (%)	SD
(Latini et al., 1981)	5 mg/kg	4	72	4	
(Dan-Shya et al., 1983)	Theophylline (7.5 mg/kg) and caffeine (7.5 mg/kg) 2 weeks later	6	60	2.9	1
(Callahan et al., 1982)	5 mg/kg (¹⁴ C-labeled caffeine)	10	48	2.05	0.31
(Grant et al., 1983)	300 mg	68	24	1.2	0.4
(Blanchard et al., 1985)	5 mg/kg	5 (elderly) 7 (young)	24	3.37 2.73	0.89 0.86
(Scott et al., 1986)	123-369 mg 300-750 mg	15 pregnant 9 female	24	2.6 1.6	0.9 0.5
(Carrillo and Benitez, 1994)	300 mg	107	24	1.31	0.04

3,7-dimethyluric acid

References	Dose	Subjects treated	Duration (h)	3,7-dimethyluric acid excretion (%)	SD
(Dan-Shya et al., 1983)	Theophylline (7.5 mg/kg) and caffeine (7.5 mg/kg) 2 weeks later	6	60	1.2	0.5
(Carrillo and Benitez, 1994)	300 mg	98	24	0.16	0.13

3-methylxanthine

References	Dose	Subjects treated	Duration (h)	3-methylxanthine excretion (%)	SD
(Latini et al., 1981)	5 mg/kg	4	72	3.5	

(Dan-Shya et al., 1983)	Theophylline (7.5 mg/kg) and caffeine (7.5 mg/kg) 2 weeks later	6	60	2.3	0.3
(Callahan et al., 1982)	5 mg/kg (¹⁴ C-labeled caffeine)	10	48	2.09 1.98 2.12	0.56 0.24 0.51
(Grant et al., 1983)	300 mg	68	24	1.5	0.7
(Blanchard et al., 1985)	5 mg/kg	5 (elderly) 7 (young)	24	0.94 1.93	0.51 0.91
(Scott et al., 1986)	123-369 mg 300-750 mg	15 pregnant 9 female	24	5.6 2.6	3.2 0.7
(Carrillo and Benitez, 1994)	300 mg	107	24	1.7	0.11

Table S2. Main characteristics of the wastewater treatment plants (WWTPs) investigated

WWTPs investigated (country)	Mean daily flow rate (m³/day)	Population served by WWTP	Sampling dates (2015)
Bristol (UK)	209,289	886,650	10 – 16 March
Brussels (Belgium)	251,830	954,000	18 – 24 March
Castellón (Spain)	42,372	180,000	25 – 31 March
Copenhagen (Denmark)	144,558	530,000	10 – 16 March
Lugano (Switzerland)	44,386	103,560	25 – 31 March
Milan (Italy)	437,726	1,100,000	10 – 16 March
Oslo (Norway)	276,235	580,000	11 – 17 March
Porto (Portugal)	31,560	150,000	23 – 29 April
Utrecht (The Netherlands)	46,743	300,000	4 – 10 March
Zurich (Switzerland)	180,088	410,000	18 – 24 March

Table S3. Precursor and products ions of the analyzed compounds with the associated collision energies

Compound	Precursor ion (<i>m/z</i>)	Product ion 1 (<i>m/z</i>) and collision energy (eV)	Product ion 2 (<i>m/z</i>) and collision energy (eV)
caffeine	195.1	138 (25)	110 (30)
caffeine- ₃ C ¹³	198.1	140 (25)	-
Paraxanthine (1,7-dimethylxanthine)	181.1	124 (26)	96 (32)
1-methylxanthine	167.1	110 (25)	82 (33)
7-methylxanthine	167.1	124 (24)	150 (24)
1-methyluric acid	182.1	70.1 (30)	126.0 (24)
1,7-dimethyluric acid	197.1	140.1 (25)	69.1 (35)
1,7-dimethyluric acid-d ₃	200.1	140.1 (25)	-

Table S4. Linearities, recoveries, repeatability and quantification limits

Compound	Linearity range (ng/mL)	Coefficient of correlation (r^2)	Recovery (%)	Repeatability RSD (%)	MLQ (ng/L)
Caffeine*	0-600	0.9989	88	12	3.6
Paraxanthine (1,7-dimethylxanthine)*	0-600	0.9996	76	5	6.6
1-methylxanthine*	0-600	0.9996	72	14	6.1
7-methylxanthine*	0-600	0.9999	64	10	28.5
1-methyluric acid	0-600	0.9988	68	14	220
1,7-dimethyluric acid	0-600	0.9990	87	4	185

*(Senta et al., 2015)

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