Supplementary Information

Increased levels of the oxidative stress biomarker 8-iso-prostaglandin $F_{2\alpha}$ in wastewater associated with tobacco use

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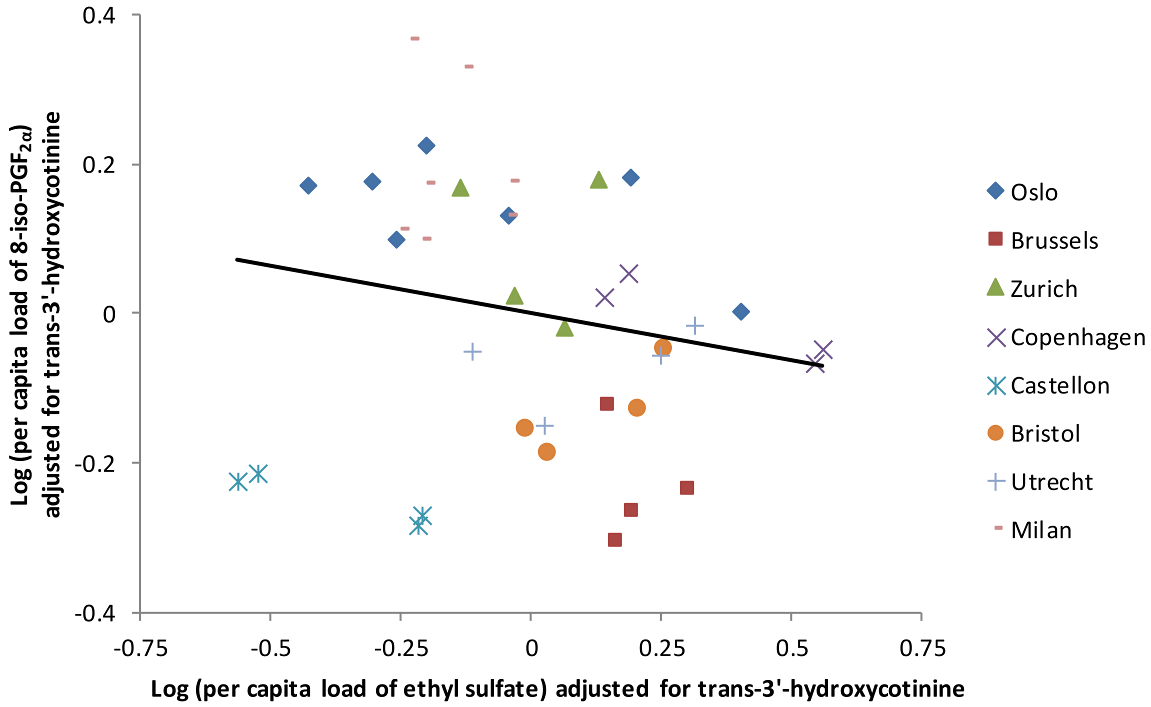
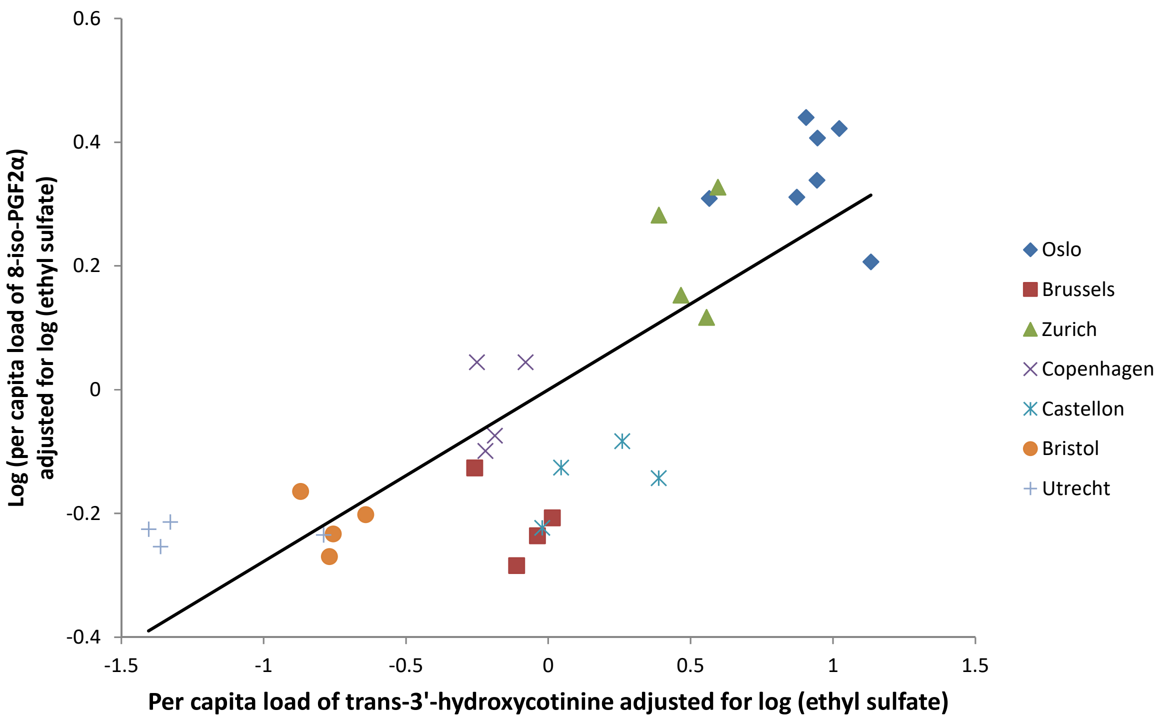


Supplementary Figure S1. Partial regression plot between log 8-iso-$PGF_{2\alpha}$ and log ethyl sulfate from the multiple linear regression analysis based on both weekday and weekend. 8-iso-$PGF_{2\alpha}$ data for Oslo was taken from our previous report[1].



Supplementary Figure S2. Partial regression plot between log 8-iso-$PGF_{2\alpha}$ and trans-3'-hydroxycotinine from the multiple linear regression analysis performed after excluding Milan data. 8-iso-$PGF_{2\alpha}$ data for Oslo was taken from our previous report[1].

Supplementary Table S1. Concentration of biomarkers and sewage flow in participating cities during the sampling period.

City	Population	Sampling start	Sampling end	Day	Sewage volume (m^3/day)	8-iso-$PGF_{2\alpha}$ (ng/L)	ethyl sulfate (ng/mL)	trans-3′-hydroxycotinine (ng/mL)
Brussels (BE)	953987	20/03/2015	21/03/2015	Fri	234906	10.6	22.4	9.0
	953987	21/03/2015	22/03/2015	Sat	233096	11.9	29.8	9.5
	953987	23/03/2015	24/03/2015	Mon	234774	9.4	20.3	8.6
	953987	24/03/2015	25/03/2015	Tue	359951	8.7	12.0	5.2
Zurich (CH)	410000	18/03/2015	19/03/2015	Wed	157084	15.2	12.9	7.3
	410000	19/03/2015	20/03/2015	Thu	161005	15.4	9.6	6.8
	410000	21/03/2015	22/03/2015	Sat	200010	18.0	6.3	5.6
	410000	22/03/2015	23/03/2015	Sun	243013	14.6	9.3	4.5
Copenhagen (DK)	531000	10/03/2015	11/03/2015	Tue	150936	17.2	16.9	7.6
	531000	11/03/2015	12/03/2015	Wed	147175	17.8	18.2	7.2
	531000	14/03/2015	15/03/2015	Sat	137793	16.6	49.5	8.6
	531000	15/03/2015	16/03/2015	Sun	137244	15.6	47.5	8.4
Castellon (ES)	180690	27/03/2015	28/03/2015	Fri	43728	11.9	9.9	10.3
	180690	28/03/2015	29/03/2015	Sat	38301	13.8	5.0	10.4
	180690	30/03/2015	31/03/2015	Mon	37469	11.2	9.9	9.9
	180690	31/03/2015	01/04/2015	Tue	40476	11.5	4.0	8.8
Milan (IT)	1100000	04/02/2015	05/02/2015	Wed	403960	9.9	4.1[b]	2.7
	1100000	05/02/2015	06/02/2015	Thu	673970	11.0	3.1[b]	1.9
	1100000	06/02/2015	07/02/2015	Fri	660810	10.6	2.3[b]	1.4
	1100000	07/02/2015	08/02/2015	Sat	469900	9.8	5.0[b]	2.1
	1100000	08/02/2015	09/02/2015	Sun	395410	10.2	5.7[b]	2.3
	1100000	09/02/2015	10/02/2015	Mon	423340	11.2	3.9[b]	2.5
	1100000	10/02/2015	11/02/2015	Tue	424210	10.0	3.6[b]	2.7
Utrecht (NL)	300000	04/03/2015	05/03/2015	Wed	47740	14.6	10.7	4.5
	300000	05/03/2015	06/03/2015	Thu	45030	15.9	18.9	9.0

	300000	07/03/2015	08/03/2015	Sat	46030	15.7	26.4	5.5
	300000	08/03/2015	09/03/2015	Sun	46900	16.7	29.8	5.3
Hamar (NO)	55000	14/11/2014	17/11/2014	Fri-Sun	66207	10.3	NA[c]	NA[c]
	55000	17/11/2014	18/11/2014	Mon	24881	11.8	NA[c]	NA[c]
	55000	21/11/2014	24/11/2014	Fri-Sun	80516	9.6	NA[c]	NA[c]
	55000	25/11/2014	26/11/2014	Tue	42669	9.4	NA[c]	NA[c]
	55000	28/11/2014	01/12/2014	Fri-Sun	80488	10.5	NA[c]	NA[c]
	55000	03/12/2014	04/12/2014	Wed	25153	11.9	NA[c]	NA[c]
	55000	05/12/2014	08/12/2014	Fri-Sun	63992	11.3	NA[c]	NA[c]
	55000	11/12/2014	12/12/2014	Thu	21655	11.3	NA[c]	NA[c]
Oslo (NO)	580639	05/11/2014	06/11/2014	Wed	491702	14.8	NA[c]	NA[c]
	580639	07/11/2014	10/11/2014	Fri-Sun	1313833	14.6	NA[c]	NA[c]
	580639	12/11/2014	13/11/2014	Wed	416186	15.6	NA[c]	NA[c]
	580639	14/11/2014	17/11/2014	Fri-Sun	1299286	15.3	NA[c]	NA[c]
	580639	19/11/2014	20/11/2014	Wed	268451	15.1	NA[c]	NA[c]
	580639	21/11/2014	24/11/2014	Fri-Sun	851342	17.0	NA[c]	NA[c]
	580639	26/11/2014	27/11/2014	Wed	361952	14.6	NA[c]	NA[c]
	580639	28/11/2014	01/12/2014	Fri-Sun	779316	13.9	NA[c]	NA[c]
	580639	11/03/2015	12/03/2015	Wed	332093	19.8[a]	5.1[b]	5.3
	580639	12/03/2015	13/03/2015	Thu	306596	19.1[a]	4.3[b]	5.8
	580639	13/03/2015	14/03/2015	Fri	275737	20.0[a]	9.1[b]	6.6
	580639	14/03/2015	15/03/2015	Sat	255126	21.6[a]	15.4[b]	6.5
	580639	15/03/2015	16/03/2015	Sun	248717	19.4[a]	32.0[b]	8.3
	580639	16/03/2015	17/03/2015	Mon	252838	18.9[a]	5.7[b]	6.9
	580639	17/03/2015	18/03/2015	Tue	251013	23.3[a]	4.0[b]	7.1
Stavanger (NO)	240000	24/10/2014	27/10/2014	Fri-Sun	493562	13.9	NA[c]	NA[c]
	240000	06/11/2014	07/11/2014	Thu	118568	17.0	NA[c]	NA[c]
	240000	07/11/2014	10/11/2014	Fri-Sun	393481	15.5	NA[c]	NA[c]
	240000	17/11/2014	18/11/2014	Mon	87215	14.3	NA[c]	NA[c]
	240000	21/11/2014	24/11/2014	Fri-Sun	311918	16.8	NA[c]	NA[c]

	240000	27/11/2014	28/11/2014	Thu	118400	15.9	NA[c]	NA[c]
	240000	02/12/2014	03/12/2014	Tue	84802	17.7	NA[c]	NA[c]
	240000	05/12/2014	08/12/2014	Fri-Sun	397626	16.0	NA[c]	NA[c]
Tromsø (NO)	20000	04/02/2015	05/02/2015	Wed	6872	15.0	NA[c]	NA[c]
	20000	06/02/2015	09/02/2015	Fri-Sun	28507	16.4	NA[c]	NA[c]
	20000	10/02/2015	11/02/2015	Tue	11327	16.2	NA[c]	NA[c]
	20000	13/02/2015	16/02/2015	Fri-Sun	21276	17.4	NA[c]	NA[c]
	20000	18/02/2015	19/02/2015	Wed	8059	15.9	NA[c]	NA[c]
	20000	20/02/2015	23/02/2015	Fri-Sun	33915	15.0	NA[c]	NA[c]
	20000	24/02/2015	25/02/2015	Tue	7973	16.9	NA[c]	NA[c]
	20000	27/02/2015	02/03/2015	Fri-Sun	27837	16.0	NA[c]	NA[c]
Bristol (UK)	886650	12/03/2015	13/03/2015	Thu	197523	13.6	21.8	6.1
	886650	14/03/2015	15/03/2015	Sat	220687	11.1	18.6	6.4
	886650	15/03/2015	16/03/2015	Sun	193194	10.1	13.4	6.3
	886650	16/03/2015	17/03/2015	Mon	197493	10.6	11.9	6.1

[a]Data from Ryu et al. (2015)[1]

[b]Data from Ryu et al. (2016)[2]

[c]Not analysed.

Supplementary Table S2. LC-MS/MS conditions for the analysis of 8-iso-$PGF_{2\alpha}$ in Milan samples.

HPLC pump	Agilent 1200 Series (Santa Clara, CA, USA)	
Autosampler	4 °C	
Injection volume	4 µL	
Column	XSELECT[TM] CSH[TM] C18 column (2.1 × 100 mm, 2.5 µm) (Waters, Milford, MA, USA)	
Column oven	Room temperature	
Eluent A	0.05% acetic acid in water	
Eluent B	acetonitrile	
Flow rate	0.16 mL/min	
Gradient	Linear	
Composition	Time (min)	%B
	0	2
	5	70
	9	98
	13	98
	14	2
	21	2

Mass Spectrometer	API 5500 QqQ (AB Sciex, Thornhill, Ontario, Canada)
Interface	Turbo Ion Spray
Polarity	Negative
Scan type	Multiple reaction monitoring (MRM)
Ion spray voltage (IS)	-4500 V
Source temperature	350 °C
Curtain gas (CUR)	25
Collision gas (CAD)	7
Ion source gas 1 (GS 1)	40
Ion source gas 2 (GS 2)	45
Declustering potential (DP)	-80
Entrance potential (EP)	-10

Compound	Precursor ion	CXP[a]	CE[b]	Product ion
8-iso-$PGF_{2\alpha}$	m/z 353	-13	-34	m/z 193
			-31	m/z 247
8-iso-$PGF_{2\alpha}$-d4	m/z 357	-15	-35	m/z 197

[a]Collision cell exit potential.
[b]Collision energy.

Supplementary Table S3. Summary of validation results from the analytical methods used in the present study.

Compound	Linear range (ng/mL)	R^2	LOQ[a] (ng/L)	Recovery (%)	RSD[b] (%)	Reference
8-iso-$PGF_{2\alpha}$	0.1 – 100	0.999	0.3	103 – 113	3 – 7	Ryu et al. (2015)[1]
trans-3′-hydroxycotinine	0 – 600	0.999	1.9	87	8	Senta et al. (2015)[3]
ethyl sulfate	2 – 200	0.998	1000	98	7	Reid et al. (2011)[4]

[a]Limit of quantification
[b]Relative standard deviation

Supplementary Table S4. Parameters used in Monte Carlo simulations to estimate daily mass loads (mg/day/1000 inhabitants) of 8-iso-$PGF_{2\alpha}$[a].

	Sampling uncertainty (U_s)	Measured Concentration (C)	Daily volume of wastewater (V)	Population (P)	Per capita daily mass load (L)	Total uncertainty (U_t)
Norwegian cities	Normal (1, $\frac{0.05}{\sqrt{8}}$)	Normal (C, 0.068*C)	Normal (V, 0.2*V)	Normal (P, 0.2*P)	$U_s \cdot \frac{C \cdot V}{P}$	$\frac{SD (L)}{Mean (L)}$
Milan	Normal (1, $\frac{0.05}{\sqrt{7}}$)	Normal (C, 0.068*C)				
The rest	Normal (1, $\frac{0.05}{\sqrt{4}}$)	Normal (C, 0.068*C)				

[a]Normal (mean, SD) specifies a normal distribution with the entered mean and standard deviation.

References

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