WP2 - Qualitative and quantitative analysis of new psychoactive substances (NPS) in Europe, with focus on synthetic opioids and prescriptions opioids

Deliverable 10

Data analysis of prescription opioids and data triangulation

The list of biomarkers of prescription opioids initially selected for analysis in urban wastewater (Deliverable 5) was revised creating a more specific list for analysis in Italy by considering the most prescribed substances in our country (Deliverable 2) and the availability of analytical standards. The final list of the 14 biomarkers of prescription opioids selected for analysis in Italy is reported in Table 1. These substances were analysed in untreated urban wastewater by liquid chromatography-tandem mass spectrometry (LC-MS/MS) applying the quantitative method described in Deliverable 7.

Table 1. List of biomarkers of prescription opioids investigated in Italy.

Biomarkers to be monitored in wastewater	
Parent compound	***Metabolites***
Buprenorphine	Norbuprenorphine
Hydromorphone	
Naloxone	
Hydrocodone	
Oxycodone	Noroxycodone
Tapentadol	
Cis-tramadol	O-desmethyl tramadol
Codeine	
Morphine	
Methadone	EDDP

Area of study

Raw WW samples were collected at the entrance of urban wastewater treatment plants (WWTPs) in 8 Italian cities (Fig.1) in October-November 2020. The cities were chosen among the most representative (main cities in different regions) to cover all the national territory and catch eventual differences of consumption. Cities were: Milan, Turin and Bologna in the north of Italy, Florence and Rome in the center of Italy, Naples, Bari and Palermo in the south of Italy.

Figure 1. Cities selected for the national study in Italy.

Samples were collected as a 24-h composite sample for 7 consecutive days to cover the weekly profile of use. 0.5 L aliquots were frozen immediately after collection and transferred to our Institute by courier for analysis.

Presence of prescription opioids in urban wastewater

Most of the substances investigated were found in urban wastewater in the different cities investigated. Results in concentration (ng/L) are reported below with sampling dates, daily concentrations, means and standard deviations (SD) calculated for the 7 samples collected in each city. Red values are those detected, but below the limits of quantification.

The highest concentrations were found for tapentadol (range of mean concentrations 100-600 ng/L), cis-tramadol and its metabolite O-desmethyl tramadol (range of mean concentrations 60-270 ng/L). Morphine and codeine were the second most abundant substances with mean concentrantions ranging from 34 to 225 ng/L and from 50 to 257 ng/L, respectively. Oxycodone and its main metabolite noroxycodone were found at a lower range of concentrations between 5 and 30 ng/L in all the cities investigated as well as naloxone that was found in the range 4-21 ng/L. The less abundant compounds were hydromorphone that was found in six cities in the low ng/L range (< 10 ng/L) and in 2 cities was below the limit of quantification (1.6 ng/L), hydrocodone that was found only in Naples at very low concentration (mean 2 ng/L), buprenorphine that was detected only in 3 cities at concentrations below the limit of quantification (5.8 ng/L) and its metabolite norbuprenorphine that was never detected.

Milan	Monday 02/11/20	Tuesday 03/11/20	Wednesday 04/11/20	Thursday 05/11/20	Friday 06/11/20	Saturday 07/11/20	Sunday 08/11/20	Mean	SD
Buprenorphine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Norbuprenorphine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Codeine	85.69	90.08	94.64	95.77	93.85	94.96	82.03	**91.0**	**5.3**
Hydrocodone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Hydromorphone	1.05	1.03	1.11	1.08	1.40	1.35	1.18	**1.2**	**0.1**
Morphine	32.08	38.06	44.70	39.97	54.74	55.44	32.40	**42.5**	**9.6**
Naloxone	5.77	6.27	7.23	5.83	6.67	6.99	5.75	**6.4**	**0.6**
Oxycodone	8.03	7.54	8.00	7.48	7.84	9.80	9.15	**8.3**	**0.9**
Noroxycodone	19.658	19.795	20.847	20.113	17.805	20.373	18.12	**19.5**	**1.1**
cis-tramadol	142.68	139.01	146.62	145.27	153.86	161.17	143.41	**147.4**	**7.6**
O-desmethyltramadol	150.142	147.342	174.762	168.398	174.562	167.593	148.735	**161.6**	**12.4**
Tapentadol	233.12	233.94	236.73	236.26	226.38	219.33	223.22	**229.9**	**6.9**

Turin	Monday 02/11/20	Tuesday 03/11/20	Wednesday 04/11/20	Thursday 05/11/20	Friday 06/11/20	Saturday 07/11/20	Sunday 08/11/20	Mean	SD
Buprenorphine	2.23	2.28	2.77	2.82	2.50	2.52	2.96	**2.6**	**0.3**
Norbuprenorphine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Codeine	257.25	259.18	253.44	278.50	251.27	249.67	247.54	**256.7**	**10.5**
Hydrocodone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Hydromorphone	3.01	3.28	2.93	2.96	2.54	3.06	2.77	**2.9**	**0.2**
Morphine	164.05	176.25	168.56	186.08	179.26	171.35	164.30	**172.8**	**8.2**
Naloxone	19.97	21.44	19.86	23.24	18.94	21.48	19.51	**20.6**	**1.5**
Oxycodone	20.44	19.84	20.53	21.98	19.75	22.32	21.39	**20.9**	**1.0**
Noroxycodone	33.47	35.73	34.84	34.21	34.22	30.30	29.41	**33.2**	**2.4**
cis-tramadol	194.83	189.46	185.93	214.57	204.90	202.72	197.54	**198.6**	**9.8**
O-desmethyltramadol	236.67	204.53	203.27	227.39	214.06	207.65	185.62	**211.3**	**16.8**
Tapentadol	399.03	431.83	397.54	452.61	433.10	431.15	393.71	**419.9**	**22.9**

Bologna	Monday 19/10/20	Tuesday 20/10/20	Wednesday 21/10/20	Thursday 22/10/20	Friday 23/10/20	Saturday 24/10/20	Sunday 25/10/20	Mean	SD
Buprenorphine	2.05	1.80	2.04	2.31	2.65	1.88	2.15	**2.1**	**0.3**
Norbuprenorphine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Codeine	243.07	219.92	232.03	200.99	231.02	232.59	222.13	**226.0**	**13.4**
Hydrocodone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Hydromorphone	5.03	4.24	4.92	3.84	4.44	4.94	4.80	**4.6**	**0.4**
Morphine	231.43	218.27	247.43	191.65	225.64	237.28	226.05	**225.4**	**17.6**
Naloxone	19.90	18.39	17.95	16.41	18.09	19.55	18.95	**18.5**	**1.2**
Oxycodone	16.12	15.15	15.55	13.95	17.38	17.01	16.84	**16.0**	**1.2**
Noroxycodone	35.41	30.64	33.27	25.30	35.30	30.48	28.17	**31.2**	**3.7**
cis-tramadol	317.23	272.67	284.18	214.27	274.44	271.29	246.84	**268.7**	**31.9**
O-desmethyltramadol	270.54	229.68	268.45	206.70	208.09	251.95	277.18	**244.7**	**29.9**
Tapentadol	592.61	551.33	572.57	471.63	520.71	556.42	533.64	**542.7**	**39.3**

Florence	Monday 19/10/20	Tuesday 20/10/20	Wednesday 21/10/20	Thursday 22/10/20	Friday 23/10/20	Saturday 24/10/20	Sunday 25/10/20	Mean	SD
Buprenorphine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Norbuprenorphine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Codeine	75.94	70.91	72.21	75.11	73.44	57.50	75.78	**71.6**	**6.5**
Hydrocodone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Hydromorphone	5.24	5.22	5.79	6.72	5.34	4.18	5.37	**5.4**	**0.8**
Morphine	128.32	131.84	118.77	309.48	130.64	85.95	113.08	**145.4**	**74.1**
Naloxone	14.08	12.35	12.04	12.04	18.59	10.07	13.43	**13.2**	**2.7**
Oxycodone	10.33	20.46	12.07	7.47	7.16	5.29	6.45	**9.9**	**5.2**
Noroxycodone	12.98	10.61	10.63	11.96	11.44	8.63	10.43	**11.0**	**1.4**
cis-tramadol	116.25	119.19	117.20	114.45	119.13	100.12	122.97	**115.6**	**7.3**
O-desmethyltramadol	157.43	164.87	164.22	199.44	191.81	166.95	211.34	**179.4**	**21.0**
Tapentadol	314.90	298.97	311.77	320.09	326.17	258.21	341.24	**310.2**	**26.4**

Rome	Monday 19/10/20	Tuesday 20/10/20	Wednesday 21/10/20	Thursday 22/10/20	Friday 23/10/20	Saturday 24/10/20	Sunday 25/10/20	Mean	SD
Buprenorphine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Norbuprenorphine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Codeine	47.60	52.63	53.75	53.67	59.37	46.74	43.14	**51.0**	**5.5**
Hydrocodone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Hydromorphone	1.08	1.10	1.00	1.15	1.32	1.01	1.15	**1.1**	**0.1**
Morphine	45.44	61.33	56.33	57.82	59.33	44.79	35.27	**51.5**	**9.7**
Naloxone	4.10	4.76	4.25	4.83	4.48	3.20	3.60	**4.2**	**0.6**
Oxycodone	4.52	4.63	5.02	4.55	5.05	4.39	4.31	**4.6**	**0.3**
Noroxycodone	6.69	7.94	8.53	7.92	8.37	7.02	6.46	**7.6**	**0.8**
cis-tramadol	55.92	65.69	67.01	64.86	63.86	51.55	46.54	**59.3**	**8.0**
O-desmethyltramadol	61.78	75.84	77.58	72.82	79.62	60.33	60.42	**69.8**	**8.6**
Tapentadol	84.74	106.75	101.36	110.59	111.08	83.76	78.41	**96.7**	**13.9**

Naples	Monday 19/10/20	Tuesday 20/10/20	Wednesday 21/10/20	Thursday 22/10/20	Friday 23/10/20	Saturday 24/10/20	Sunday 25/10/20	Mean	SD
Buprenorphine	1.89	1.92	1.74	1.64	2.25	1.33	0.97	**1.7**	**0.4**
Norbuprenorphine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Codeine	110.03	125.23	114.81	121.03	87.68	55.65	53.57	**95.4**	**30.4**
Hydrocodone	1.81	0.90	1.94	1.82	2.44	2.43	3.01	**2.0**	**0.7**
Hydromorphone	2.63	2.61	2.48	3.20	3.51	2.54	2.95	**2.8**	**0.4**
Morphine	41.10	42.02	44.06	41.87	33.87	18.08	13.67	**33.5**	**12.5**
Naloxone	7.77	9.06	8.36	6.34	5.27	3.54	4.81	**6.5**	**2.0**
Oxycodone	7.89	7.51	6.42	6.77	5.54	3.39	4.19	**6.0**	**1.7**
Noroxycodone	14.37	14.11	12.27	15.16	12.94	9.16	10.65	**12.7**	**2.2**
cis-tramadol	100.25	100.53	94.33	97.21	96.99	67.69	75.29	**90.3**	**13.2**
O-desmethyltramadol	133.81	142.44	134.51	141.83	117.01	73.16	97.46	**120.0**	**26.1**
Tapentadol	268.41	289.28	283.68	272.60	247.23	162.66	162.00	**240.8**	**55.3**

Bari	Monday 19/10/20	Tuesday 20/10/20	Wednesday 21/10/20	Thursday 22/10/20	Friday 23/10/20	Saturday 24/10/20	Sunday 25/10/20	Mean	SD
Buprenorphine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Norbuprenorphine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Codeine	82.00	128.84	115.33	67.60	112.51	71.54	105.71	**97.6**	**23.8**
Hydrocodone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Hydromorphone	1.93	1.65	1.99	0.80	1.62	0.80	2.40	**1.6**	**0.6**
Morphine	93.44	108.18	118.04	95.71	76.75	75.08	153.37	**102.9**	**27.1**
Naloxone	9.65	13.75	14.11	8.69	17.00	9.69	13.11	**12.3**	**3.0**
Oxycodone	9.62	16.86	8.76	7.31	10.94	7.39	8.91	**10.0**	**3.3**
Noroxycodone	23.91	35.65	22.08	14.75	18.78	14.62	19.20	**21.3**	**7.2**
cis-tramadol	159.66	141.03	128.65	143.92	116.70	140.20	176.70	**143.8**	**19.7**
O-desmethyltramadol	178.33	199.91	174.27	178.44	151.02	127.98	226.09	**176.6**	**31.7**
Tapentadol	471.43	696.08	742.73	435.52	669.07	406.64	744.05	**595.1**	**150.5**

Palermo	Monday 19/10/20	Tuesday 20/10/20	Wednesday 21/10/20	Thursday 22/10/20	Friday 23/10/20	Saturday 24/10/20	Sunday 25/10/20	Mean	SD
Buprenorphine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Norbuprenorphine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Codeine	136.20	137.45	152.69	148.98	143.06	165.49	158.56	**148.9**	**10.9**
Hydrocodone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0**	**0.0**
Hydromorphone	3.28	2.95	3.15	3.40	3.15	3.73	3.86	**3.4**	**0.3**
Morphine	68.58	77.22	78.00	79.56	81.71	76.50	79.75	**77.3**	**4.2**
Naloxone	9.97	9.91	10.21	9.78	9.91	8.47	9.84	**9.7**	**0.6**
Oxycodone	7.71	8.41	8.53	9.05	8.45	7.79	8.13	**8.3**	**0.5**
Noroxycodone	12.59	14.84	13.53	14.49	15.32	15.25	13.88	**14.3**	**1.0**
cis-tramadol	87.64	96.29	89.39	94.02	109.19	99.26	98.85	**96.4**	**7.2**
O-desmethyltramadol	96.04	90.80	113.98	89.35	99.57	94.71	75.41	**94.3**	**11.6**
Tapentadol	375.57	402.57	439.66	424.98	393.51	431.95	449.30	**416.8**	**26.8**

Mass loads of prescription opioids in the 8 cities investigated

Concentrations (ng/L) were converted in mass loads (mg/day/1000 inhabitants), considering the daily flow rates (m^3 day^{-1}) of urban wastewater for each day of sampling and the population served by each wastewater treatment plant. The normalisation of mass loads allowed the comparison of use of the different substances in the 8 cities investigated. Results are showed in Figures 2 and 3.

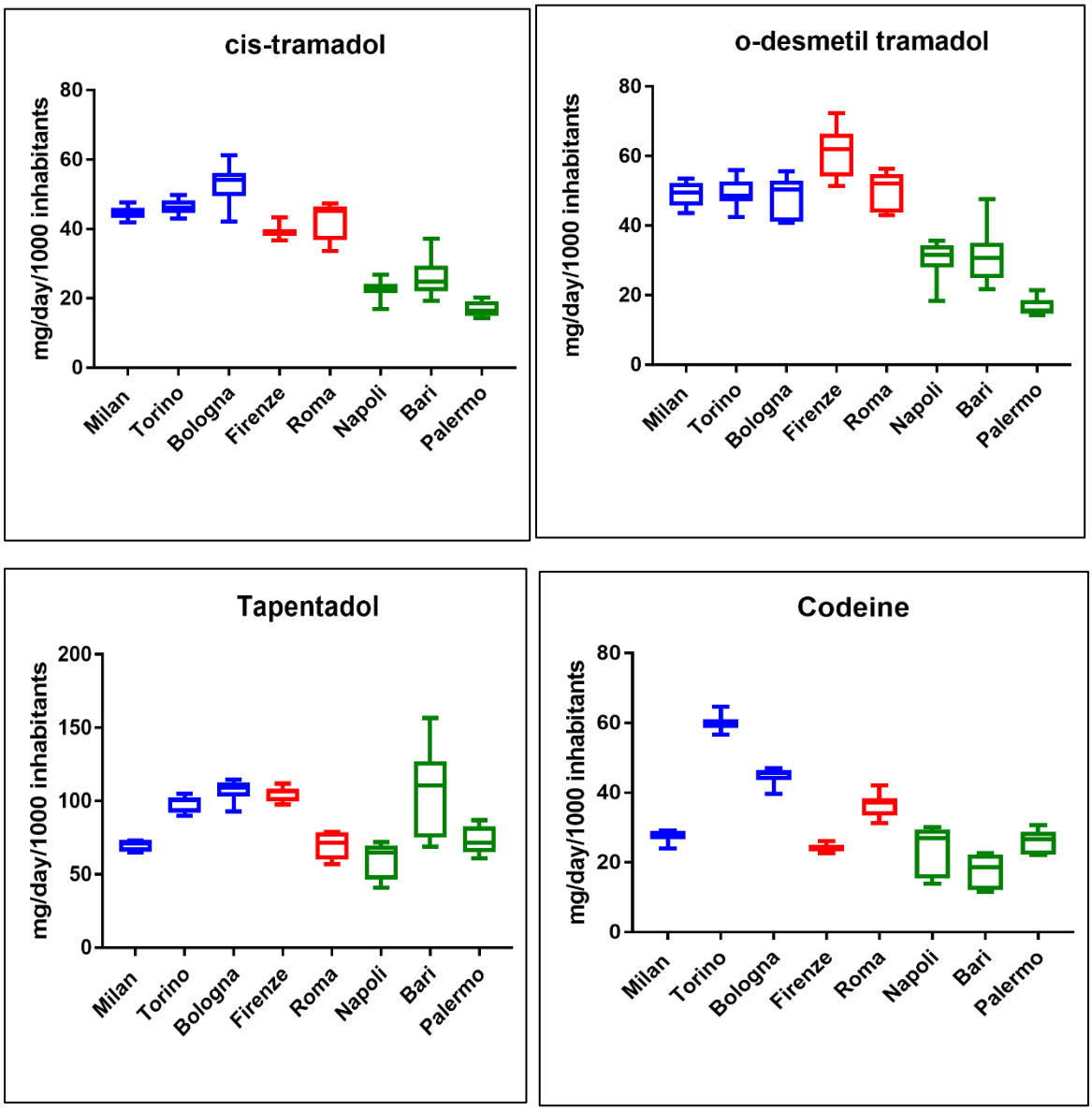

Figure 2. Mass loads normalized to population (mg/day/1000 inhabitants) for the most abundant biomarkers.

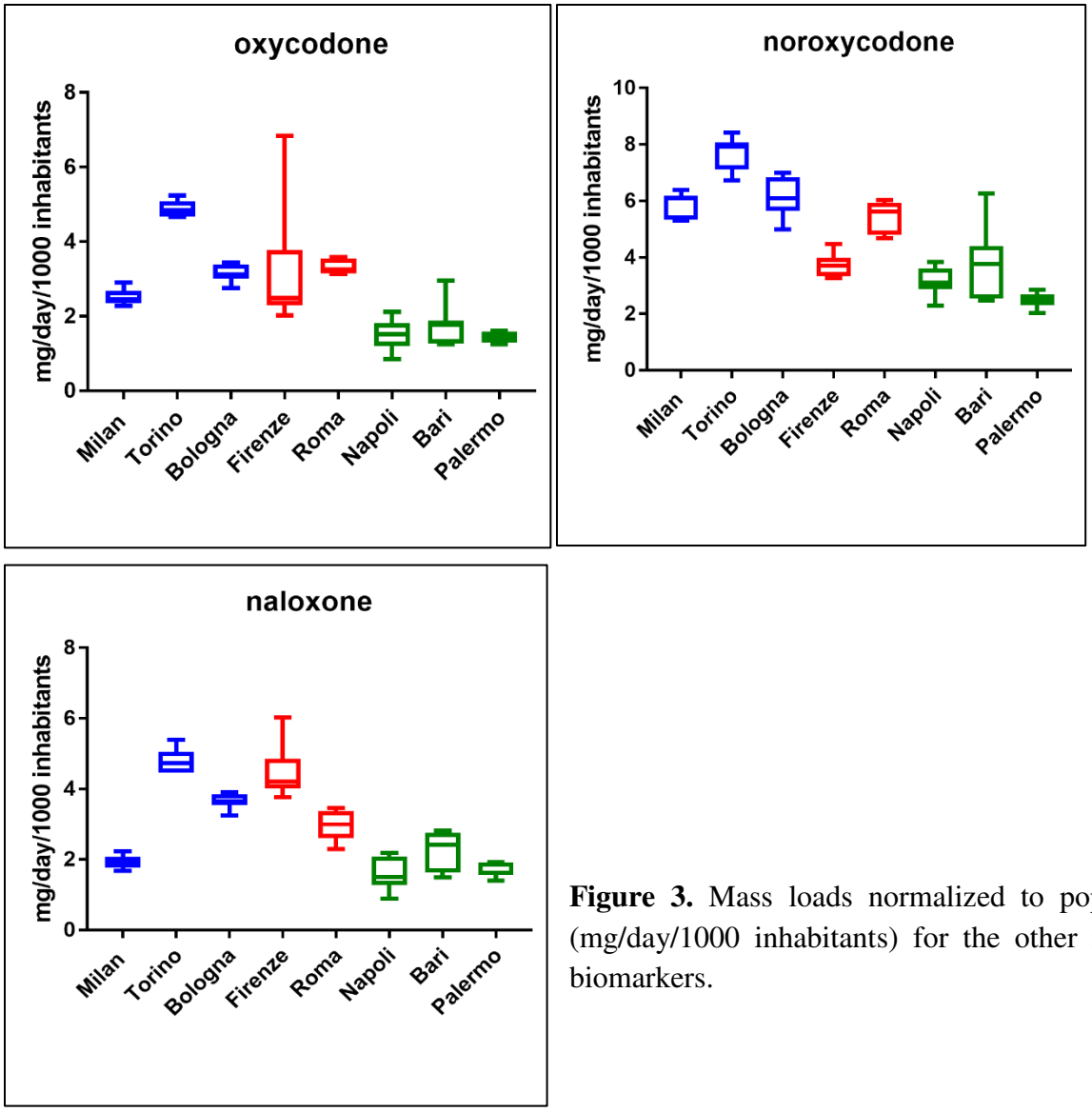

Figure 3. Mass loads normalized to population (mg/day/1000 inhabitants) for the other relevant biomarkers.

A different pattern of use was observed for tramadol and oxycodone with higher consumption in the north and center of Italy compared to the south of Italy. For the other substances there are differences among the single cities, but not ascribable to a specific area.

Metabolism of prescription opioids and development of correction factors

In order to back-calculate prescription opioids consumption, mass loads need to be corrected for specific correction factors that take into account the excretion rate of the biomarkers investigated. This was proposed initially for illicit drugs (Zuccato e al, 2008) and further work have been done for refining correction factors for the main illicit drugs (Castiglioni et al., 2013; Gracia-Lor et al., 2016). The methodology developed in these previous investigations were used in the present project to develop new correction factors for prescription opioids. Briefly, all accessible pharmacokinetic studies in the literature reporting data about human urinary excretion of the selected biomarkers after the different routes of administration were reviewed and collected. The mean percentages of excretion of the parent drugs and their metabolites were calculated for the main route of administration by weighting the mean excretion of each study by the number of subjects included. Finally, these values were weighted considering the most frequent route of administration (when available) and new CFs were obtained. The total uncertainty related to the back-calculation procedure was calculated as the standard deviation (SD) of the mean percentage of excretion.

The average excretion factors obtained from the available literature and the correction factors developed within the present study are reported in Table 2.

Table 2. Average excretion rates of the investigated biomarkers with the respective correction factors.

Biomarkers investigated	*Average Excretion Factors*	*Correction factors*
Buprenorphine	2.8 ± 0.4	35.7
Norbuprenorphine	8.5 ± 3.5	13.3
Hydromorphone	35.1 ± 9.0	2.85
Naloxone	38.9 ±10.4	2.57
Hydrocodone	-	-
Oxycodone	6.9 ±2.1	14.5
Noroxycodone	21.4 ± 5.5	4.89
Tapentadol	58*	1.72
Cis-tramadol	12.4 ± 4.8	8.06
o-desmethytramadol	14.9 ± 5.5	7.08
Codeine	50[§]	2[§]
Morphine	42[§]	3.08[§]

*Few data were available with no variability provided; [§] from previous study (Zuccato et al., 2008)

Estimation of prescription opiods use

The developed correction factors were used to back-calculate the use of the different substances in a first preliminary investigation. There are some datasets that unfortunately are far from being complete (few pharmacokinetic study are available, e.g. tapentadol) and this may generate some uncertainty in the final results. In other cases, when the biomarker is excreted also in the glucuronide form, the sum of excretion rates (parent compounds and glucuronide metabolites) was considered in view of results from previous studies demonstrating that glucuronide in urban wastewater are completely reverted to the parent compound (Castiglioni et al., 2006; Castiglioni et al., 2015). Finally, back-calculation was possible only for those substances found in consistent amounts in wastewater (codeine, naloxone, oxycodone, tramadol, tapentadol). Oxycodone and tramadol were estimated considering both the parent compounds and their specific metabolites in order to compare results and select the most suitable biomarker for estimation of consumption. Morphine estimation was omitted from this study because morphine is also a metabolite of heroin

and this amount should be subtracted from the mass loads. Since it is not possible to have reliable figures of heroin use at the city level we need to further elaborate data in order to get reliable estimates.

Back-calculated estimates of consumption for codeine, naloxone, oxycodone, tramadol, tapentadol and some metabolites (noroxycodone and O-desmethyltramadol) are reported in Table 3. Estimation from parent compounds and metabolites for oxycodone and tramadol were in the same range demonstrating that both the biomarkers can be used for back-calculation of consumption.

Table 3. Average weekly back-calculated use (mg/day/1000 inhabitants) in the 8 investigated cities.

Substances	Milan	Turin	Bologna	Florence	Rome	Naples	Bari	Palermo
Codeine	55.1±3.5	119.9±5.1	89.4±5.0	48.5±2.3	72.7±7.3	47.3±13.7	34.9±9.1	51.9±6.7
Naloxone	5.0±0.5	12.4±0.9	9.4±0.6	11.5±1.9	7.6±1.1	4.1±1.2	5.6±1.4	4.4±0.5
Oxycodone	36.2±3.2	70.7±3.2	45.9±3.7	48.3±24.5	48.0±2.7	21.6±6.0	25.7±8.4	20.9±2.1
Noroxycodone	29.0±2.1	37.9±2.9	30.2±3.4	18.2±2.0	26.4±2.7	15.5±2.5	18.6±6.4	12.1±1.3
cis-tramadol	359.8±15.1	373.7±18.6	428.0±47.7	316.8±17.1	341.0 ±43.8	182.0±24.3	208.6±47.2	135.3±17.9
O-desmethyltramadol	346.6±26.2	349.6±30.6	342.5±42.0	432.6±54.7	352.2±41.0	212.1±42.2	225.3±59.0	116.1±18.7
Tapentadol	119.8±6.1	168.7±9.9	184.5±12.7	181.0±8.4	118.5±16.4	103.0±20.6	183.6±54.5	124.9±16.2

The estimates reported in Table 3 can be compared with prescription data of opiods that are reported in Deliverable 9 (data triangulation). Considering all the uncertainty factors related to both wastewater and national prescription estimates, most of the results were in a quite good agreement. For instance, tapentadol national use from prescriptions is 200 mg/day/1000 inhabitants that is fitting quite well with several of the reported estimates from wastewater, in the same way oxycodone national use from prescriptions that is 30 mg/day/1000 inhabitants, is fitting well wastewater estimates. Tramadol national use from prescriptions is reported as 180-210 mg/day/1000 inhabitants and it is a bit lower than estimates from wastewater found in the north and center of Italy, while it is in line with estimates found in the south of Italy. Finally, naloxone national use from prescriptions is reported as 30 mg/day/1000 inhabitants and results higher than the estimates from wastewater that ranged 4-13 mg/day/1000 inhabitants. We should consider that we are comparing national estimates with results from eight cities that may not fully represent national use. Further research will be performed to reduce the uncertainty factors and refine data triangulation that is already very interesting. The final goal is to build a method able to eventually identify illegal use of prescription opioids.

REFERENCES

Castiglioni, S.; Zuccato, E.; Crisci, E.; Chiabrando, C.; Fanelli, R.; Bagnati, R. (2006) Identification and Measurement of Illicit Drugs and Their Metabolites in Urban Wastewater by Liquid Chromatography-Tandem Mass Spectrometry. Anal. Chem. 78, 8421-8429.

Castiglioni S, Bijlsma L, Covaci A, Emke E, Hernández F, Reid M, Ort C, Thomas K, van Nuijs AL, de Voogt P, Zuccato E. (2013) Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. Environ Sci Technol. 47: 1452-1460.

Castiglioni S, Senta I, Borsotti A, Davoli E, Zuccato E. (2015) A novel approach for monitoring tobacco use in local communities by wastewater analysis. Tob Control 24: 38-42

Gracia-Lor E, Zuccato E, Castiglioni S. (2016) Refining correction factors for back-calculation of illicit drug use. Sci Total Environ. 573:1648-1659.

Zuccato, E.; Chiabrando, C.; Castiglioni, S.; Bagnati, R.; Fanelli, R. (2008) Estimating community drug abuse by wastewater analysis. Environmental Health Perspectives. 116, 1027-1032.