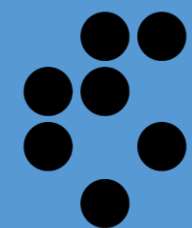


A wastewater-based epidemiological study of licit and illicit drugs among schoolchildren and students in Slovenia

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THE AIM OF THE STUDY

Application of wastewater-based epidemiology (WBE) to different Slovenian educational bodies to:

- Investigate licit and illicit drug consumption trends based on:
 - Institutions' educational level:** primary schools, secondary schools, centres for higher education
 - Geographic location:** seven Slovenian municipalities
 - Level of urbanity:** urban, rural
- Compare results obtained by our study and by traditionally used epidemiological methods (European School Survey Project on Alcohol and Other Drugs (ESPAD), Health Behaviour in School-Aged Children (HBSC) survey)

PARTICIPATION

Wastewater samples (n=42):

- 20 primary schools,
- 15 secondary schools,*
- 12 centres for higher education.
- 37 institutions in urban area,
- 10 institutions in rural area.

* 2 schools offering secondary and higher education

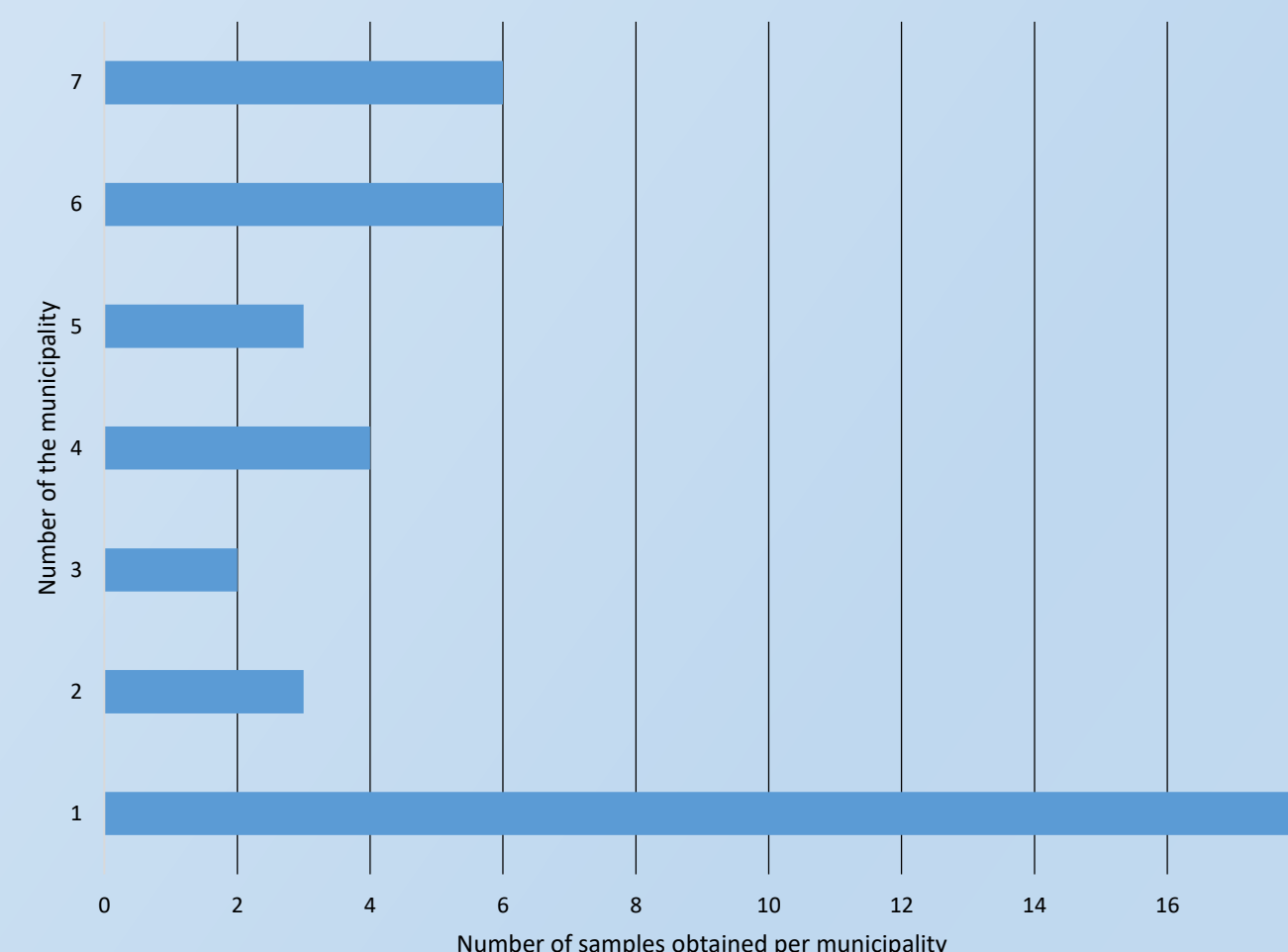


Fig. 1 Number of samples obtained per municipality

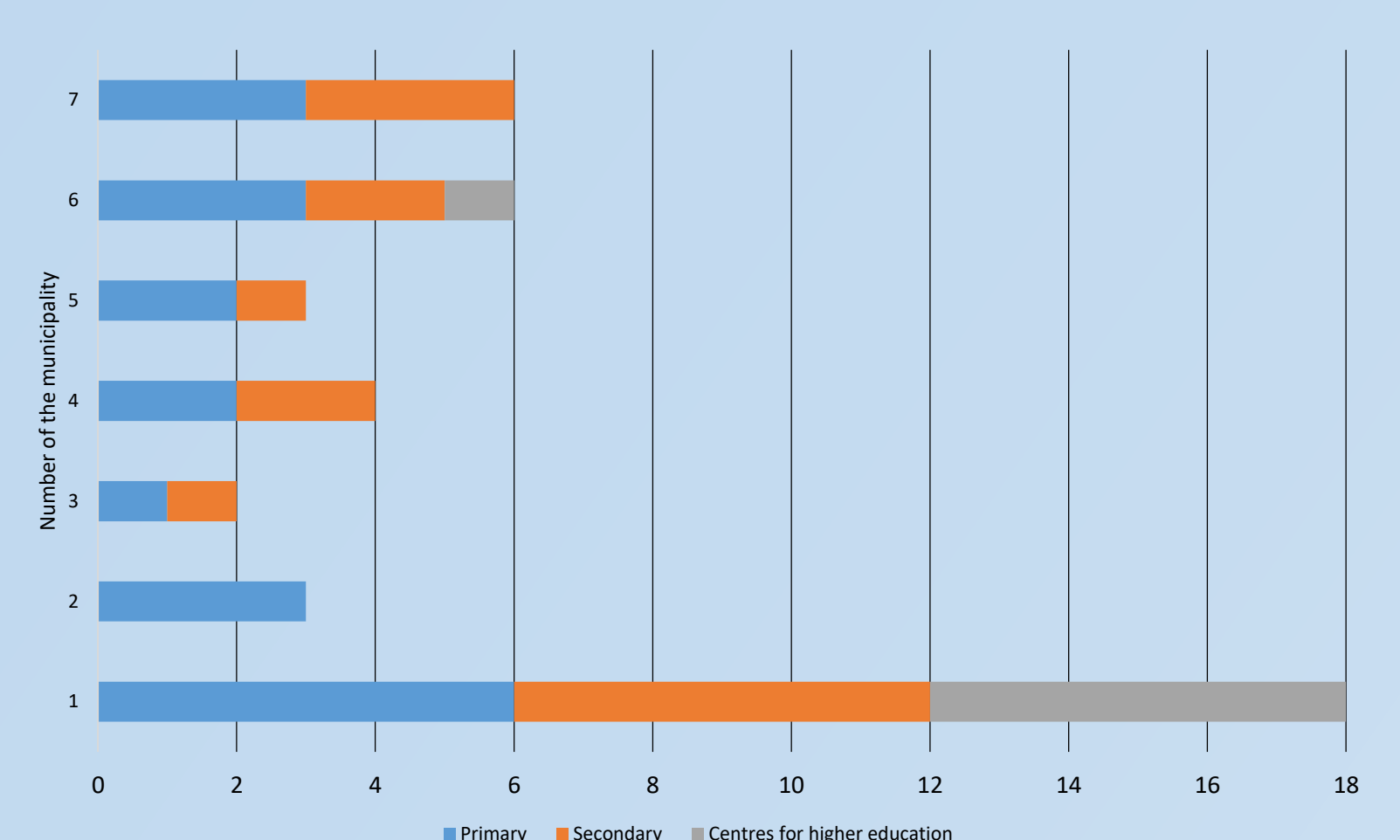


Fig. 2 Number of samples regarding institutions' educational level per municipality

METHOD AND RESULT HIGHLIGHTS

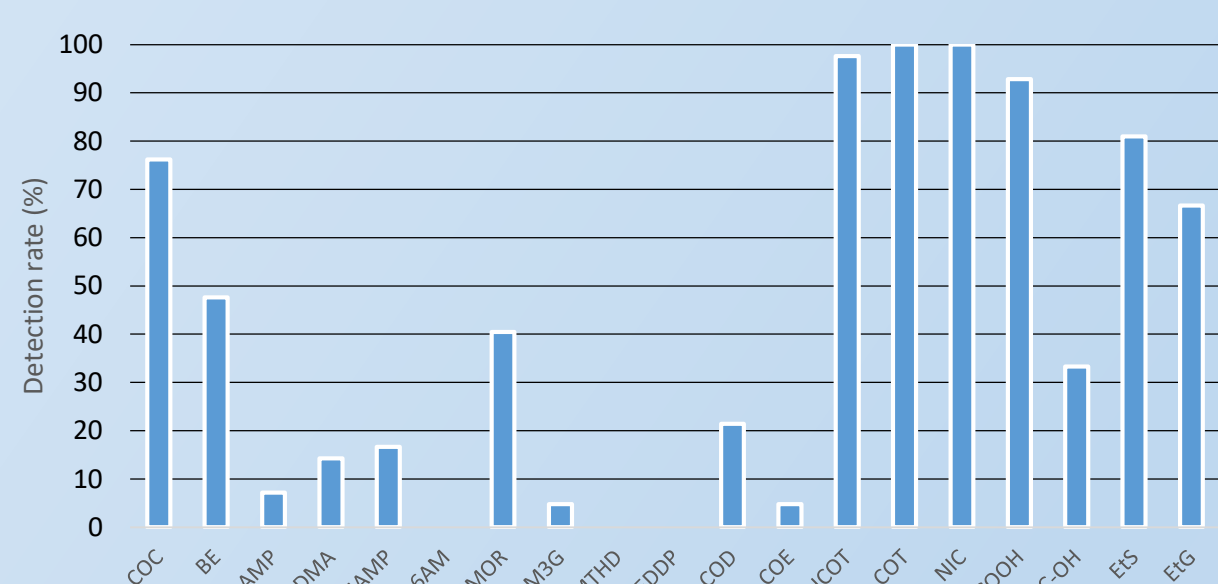
Sampling and wastewater analysis

- Sample selection:** headmasters/deans approval, evaluation of sampling possibility
- Sampling campaign:** Tuesday/Wednesday/Thursday, over class period – one 7-h composite wastewater sample per school
- Sampling difficulties:** Physical boundaries of the sewer - some samples (n=5) contain wastewater from more than one wastewater point-source
- Analysis^{1,2,3}:** filtration, extraction and pre-concentration of analytes (illicit drugs) or direct injection (nicotine and alcohol biomarkers), separation and detection of selected urinary biomarkers (n=19) by LC-MS/MS

Table 1 List of selected licit and illicit drugs and their biomarkers (n=19)

Drug	Selected biomarker(s)
Basic drugs	
Cocaine	Cocaine (COC), benzoylecgonine (BE), cocaethylene (COE)
Amphetamine	Amphetamine (AMP)
Methamphetamine	Methamphetamine (MAMP)
Ecstasy	3,4-methylenedioxymethamphetamine (MDMA)
Heroin	Morphine (MOR), morphine-3-glucuronide (M3G), 6-acetylmorphine (6-AM)
Codeine	Codeine (COD)
Methadone	Methadone (MTHD), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)
Cannabinoids	
THC	11-Nor-9-carboxy-THC (THC-COOH), 1-Hydroxy-THC (THC-OH)
Tobacco	
Nicotine	Nicotine (NIC), cotinine (COT), trans-3'-hydroxycotinine (HCOT)
Alcohol	
Ethanol	Ethyl sulphate (Ets), ethyl glucuronide (EtG)

General findings



- Commonly detected biomarkers:** nicotine, cannabis, alcohol, cocaine (COC and BE)
- None detected:** heroin biomarker (6-AM), methadone biomarkers (MTHD, EDDP)
- Biomarkers per sample:** 4 to 12 (modus: 7)

Fig. 3 Detection rate (%) of biomarkers in obtained samples

RESULTS

Spatial variation

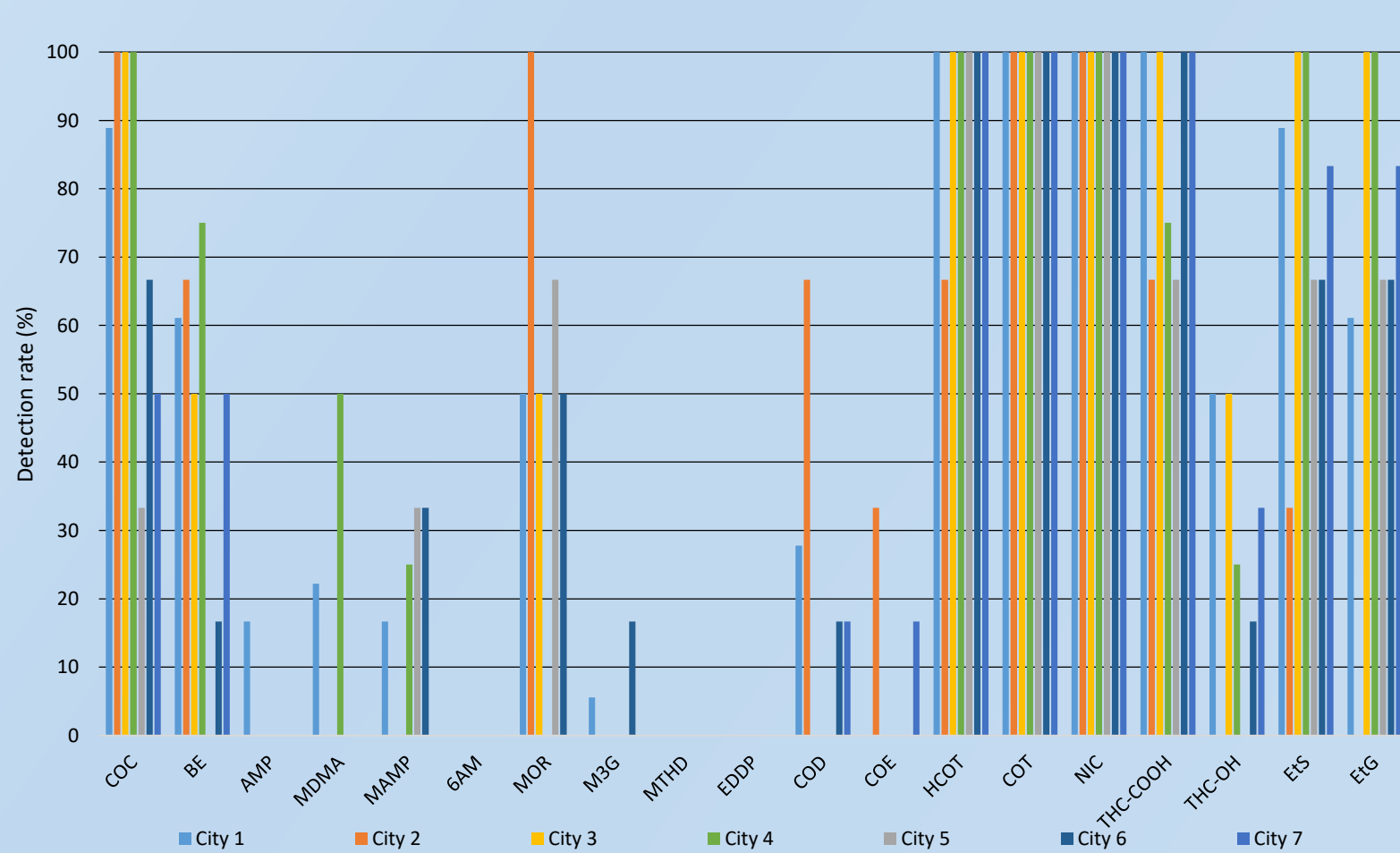


Fig. 4 Detection rate (%) of biomarkers in different municipalities

- Most commonly detected biomarkers:** nicotine, cannabis, alcohol
- Other commonly detected biomarkers:** COC (Cities: 1, 3, 4, 7), MOR (Cities: 2, 5, 6), MDMA (City 4), COD (City 2)
- Stimulants:** AMP (only in City 1), MAMP (in Cities: 1, 4, 5, 6), MDMA (in Cities: 1, 7)
- Biomarkers per sample:** highest in City 1 (modus: 11)

Trends in urban and rural areas

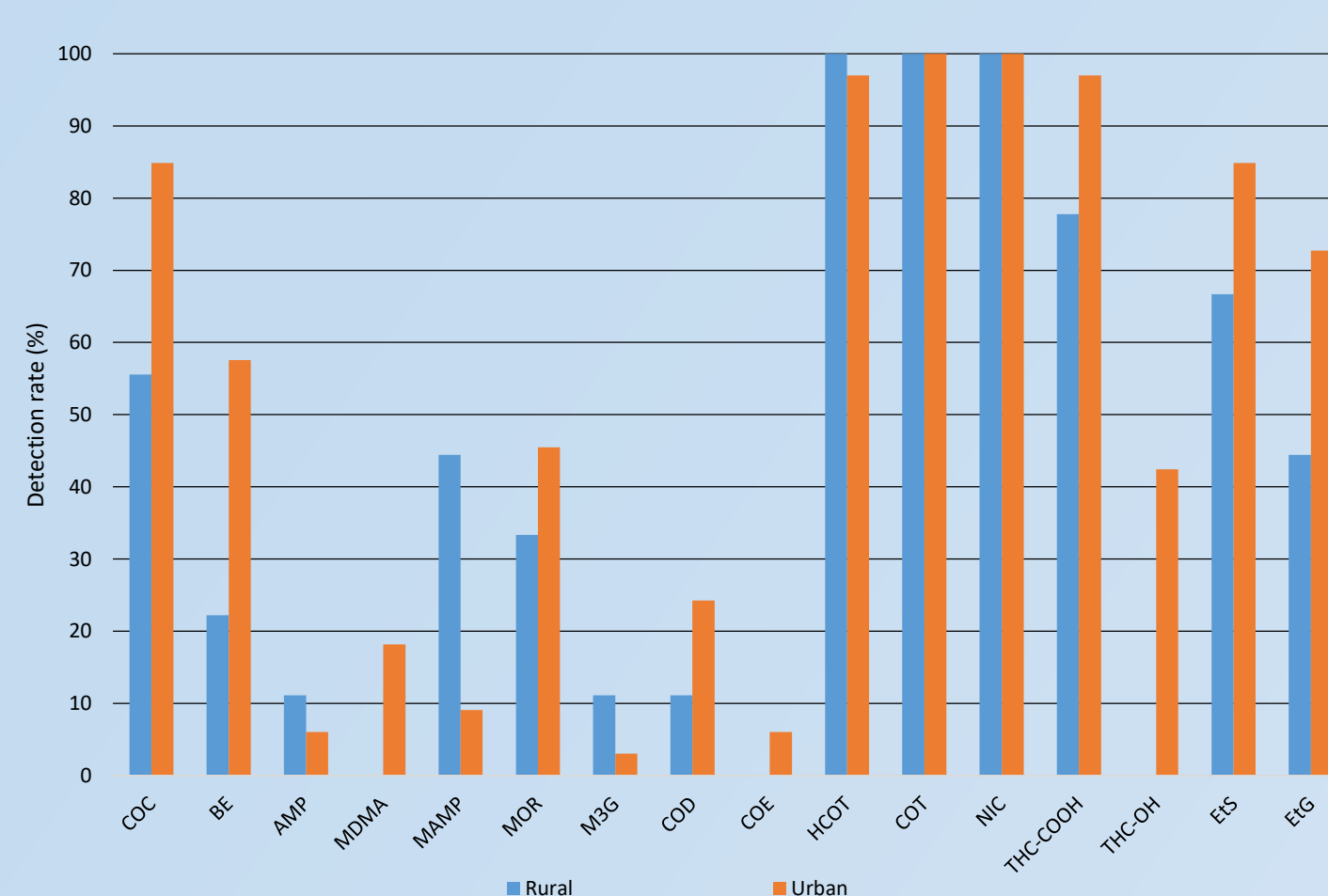


Fig. 5 Detection rate (%) of biomarkers in samples from urban and rural areas

- Urban areas have higher detection rates** (exceptions: MAMP, AMP)
- Biomarkers detected per sample:** similar for urban and rural areas (modus: 7)

Variation based on educational level

- Most commonly detected biomarkers:** nicotine, cannabis, cocaine (COC)
 - Most commonly detected biomarkers in samples of centres for higher education:** BE, MOR
- Detection rates of biomarkers increase with the level of education**
- Lower number of biomarkers detected** in samples from primary schools than in samples from secondary schools and centres for higher education

Comparison of the findings: WBE – traditional epidemiological methods

- Results of WBE agree with the latest traditional epidemiological study - European School Survey Project on Alcohol and Other Drugs (ESPAD) and the Health Behaviour in School-Aged Children (HBSC) project
 - Licit drugs:** the highest consumption was found to be consumption of licit drugs (tobacco, alcohol)
 - Illicit drugs:** cannabis is the most common illicit drug
 - Stimulants:** cocaine the most prevalent

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DECLARATION OF INTERESTS

No conflict of interest was reported by the authors.