European Monitoring Centre
for Drugs and Drug Addiction

European Syringe Collection and Analysis Enterprise

Generic Protocol

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Contents

Introduction

Monitoring of illicit drug use may be difficult, particularly among people who inject drugs, because of the associated stigma. Some people who inject drugs access harm reduction programmes such as needle exchange programmes, where they can return used injecting material and get new and sterile material. It is a cost-effective intervention that reduces the risk of drug-related infectious diseases. Depending on the country and cities where people who inject drugs live, different types of services may be available to them, including low-threshold facilities, drug treatment services, pharmacies and drug consumption rooms. Information on injecting drug use practices can be obtained from surveys conducted in these structures, often on a self-report basis. However, surveys are not always feasible, and people who inject drugs that do not access these services are not reached. In addition, in the case of new substances emerging locally, drug users might not be aware of the real composition of the drugs.

This protocol documents a new approach that has been developed to monitor substances injected by people who inject drugs through analytically confirmed data at the local level: the analysis of residual content of used syringes (Néfau et al., 2015). Used syringes contain traces of drugs that can be analysed to inform public health professionals about injecting drug use, and to contribute to the understanding of drug addiction among people who inject drugs. Used syringes can be collected from low-threshold services where needle exchange programmes are in place. They can also be collected from automatic injection kit dispensers (AIKD) combined with street bins (where people who inject drugs can obtain their new sterile injection kit in return of a used syringe), thereby obtaining injecting material from populations that might not be in contact with health and social services.

This method was first implemented by research teams and low-threshold services in France (Paris) (Néfau et al., 2015), Hungary (Budapest) (Péterfi et al., 2018) and Switzerland (Lausanne) (Lefrançois et al., 2016). In 2017, the EMCDDA supported its French focal point (Observatoire Français des Drogues et des Toxicomanies) in developing a partnership with European peers involved in this research in order to set up a European multi-city study – the European Syringe Collection and Analysis Enterprise (ESCAPE). The aim of this project is to coordinate a yearly collection campaign of used syringes in a sentinel network of European cities, using a common methodology in order to obtain representative and comparable data on injecting drug use. This generic protocol outlines the methodology. It is used by participating study sites to develop their own study protocol, taking into account local specificities while guaranteeing a common European analysis.

Objectives

Primary objective

The primary objective is to provide public health professionals with laboratory-confirmed information on substances injected by people who inject drugs in a sentinel network of European cities, including marginalised people who inject drugs who are not using social and health services, and detect new trends by:

- assessing the frequency and percentage of occurrences of drug detection in syringes, by city, by year;
- assessing the frequency and percentage of occurrences of different drug combinations, by city, by year.

Secondary objectives

In addition, the project will seek to:

- assess the frequency and percentage of occurrences of adulterant detection, by drug, by city, by year;
- assess the extent of syringe reuse.

Ethical considerations

No data on individuals are collected in the ESCAPE project. The unit of analysis is the syringe. The results for each syringe can be traced back to a collection site, but not to any individual user.

Each study team is responsible for getting ethical approval if required at the national level.

The ESCAPE network is committed to following the General Data Protection Regulation of the EU.

Methods

Study methods

Study design

ESCAPE is a multi-city observational study, with multiple data collection sites in each participating city.

Yearly, collection and analyses of a sample of used syringes is conducted in each participating city.

Study population

While the unit of analysis is the syringe, the study population is people who inject drugs who use services or street bins provided at the sites where samples are collected.

This population may include people who are not in contact with drug services, if samples are collected from street bins of automatic injection kit dispensers.

The study population may differ by demographic and socio-economic characteristics across collection sites and cities.

Study period

One collection campaign is conducted every year. The collection campaign lasts for one month. The month of syringe collection is agreed by the network at the beginning of each year.

Outcomes

Identification of substances which are contained in used syringes (see List of substances in Annex).

Identification of combinations of substances which are contained in used syringes.

If possible: macroscopic and microscopic observations (blood traces, damaged needles, etc.).

Sampling

Sample size

In order to maximise representativeness, a minimum of 150 syringes should be collected in each participating city.

Sampling sites

Location of sampling sites
If possible, five sites should be selected within each participating city. Sites should be located in distinct neighbourhoods, preferably with various demographic and socio-economic characteristics. The geographical distribution of sites should offer a good coverage of the city.

Depending on the number of sites per city, the syringe collection campaigns should follow the following minimum sample size distribution:

- 1 site: a minimum of 150 syringes,
- 2 sites: a minimum of 75 syringes per site,
- 3 sites: a minimum of 50 syringes per site,
- 4 sites: a minimum of 38 syringes per site,
- 5 sites: a minimum of 30 syringes per site.

Types of sampling sites
The choice of the type of sites where syringes are collected depends on their availability in the participating city. Research teams should provide a description of the sites where collection takes place (type of structure, description of the area) as well as contextual information (city population size, people who inject drugs population size estimate for the city, number of clean syringes distributed per year in the city, etc.). There are three main types of sites where data collection can take place.

- **Bins of street automatic injection kit dispensers (AIKD):** people who avail of AIKD are not well known by field workers. Syringes collected from AIKD can therefore provide drug use information on a population that might not be in contact with social and health services.
- **Low-threshold facilities or drop-in structures with needle and syringe programmes:** collection may be conducted in low-threshold services where needle exchange programmes are implemented. In this case, people who exchange their used syringes are usually in contact with social and/or health workers. While not within the scope of this protocol, a small-scale survey can be conducted during syringe collection in order to obtain complementary information on injection practices (reuse, sharing).
- **Drug consumption rooms (DCR):** similarly, syringe collection may be conducted in DCR, where people can inject drugs under the supervision of medically trained staff. Syringe collection should not interfere with the operation of the DCR. Information on the drugs injected can be useful to the DCR staff, allowing them to confirm the main drugs injected and to detect emerging ones and thereby tailor harm reduction strategies accordingly.

Sampled material

Inclusion criteria
Used syringes from specific street bins or from dedicated facilities (see section on the types of sampling sites) are included provided that have been disposed of not more than one month before the collection campaign.

In bins of street automatic injection kit dispensers:

- only 1 cc syringes should be collected (usually AIKD bins are designed to collect 1 cc syringes only).

In dedicated facilities, the volume of syringes can vary:

- collecting 1 cc syringes is preferable;
- syringes of other sizes can be collected depending on the injecting practices in the city.

The volume of each analysed syringe should be recorded on the data collection tool.

Exclusion criteria
Excluded are syringes:

- that have been disposed of more than one month before the collection campaign or for which it is not possible to determine the maximum time since disposal;
- with a broken barrel;

- that cannot be pumped (as this can happen when they are clogged with blood, it is important to collect more syringes than the minimum requested).

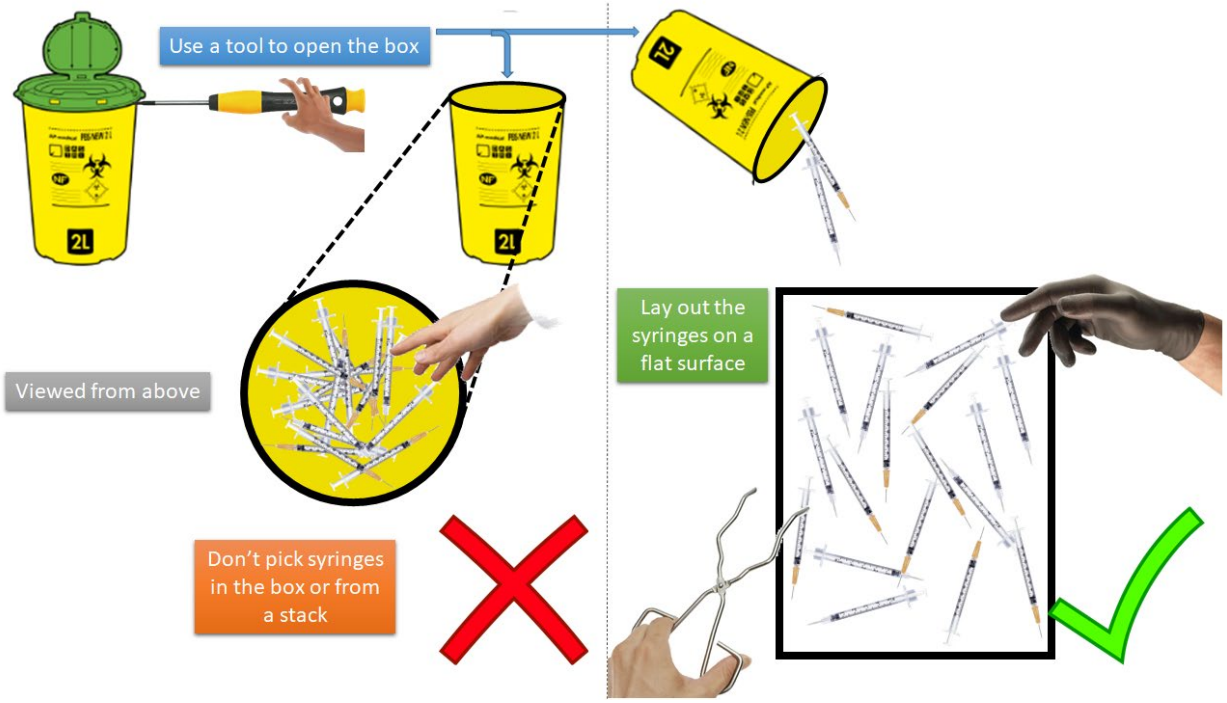
Randomisation and selection

Manipulating syringes is hazardous because of the risk of accidental blood exposure and potential exposure to blood-borne viruses. It is crucial to respect safety measures and to use specific equipment (see Annex 2) to reduce the risks.

- All syringes must be collected in a sharps container. The sharps container should be large enough to be shaken in order to mix all syringes collected. Mixing the syringes will reduce the chances of including too many syringes from the same user.
- The sharps box should then be opened and the syringes tipped from it onto a flat surface – taking care not to stack them on top of one another – before being removed for analysis using a laboratory tongs or by hand wearing protective gloves (see Figure 1).
- If it is not possible to shake the box before laying the syringes on a flat surface, syringes should be selected randomly on the flat surface.
- If syringes are collected in low-threshold services or DCRs, boxes brought by the different users are emptied in a larger box which is then shaken in order to mix all collected syringes. Then, the same procedures described in Figure 1 should be applied to select the syringes.

FIGURE 1

Safety measures to collect and select syringes



Macroscopic and microscopic observations

Different characteristics of the collected syringes can be observed. These characteristics can be useful when interpreting the results and should be recorded.

Blood traces. The detection of a drug in a syringe indicates that the syringe was used to inject the drug. In some cases, an alternative explanation is that the drug (or its metabolite) may come from traces of blood drawn into the syringe during an injection. In such a case, the user would have consumed the drug prior to the injection, possibly through other modes of administration (e.g. smoking, snorting). Recording the presence of visible blood traces in syringes could help assess the extent of this measurement bias.

Attrition marks. The presence of wear marks (e.g. erased graduations) on the syringe can indicate several manipulations and consequently can be an indicator of reuse.

Distinctive signs. If the same signs (e.g. coloured syringe plunger) are observed on several syringes, it can indicate that these syringes have been brought by the same user and help assess the extent of selection bias.

Damaged needle (microscopic observation). Needles presenting attrition might have been used more than once and can therefore be used as an indicator of reuse (unless the needles have been intentionally broken).

Laboratory analysis

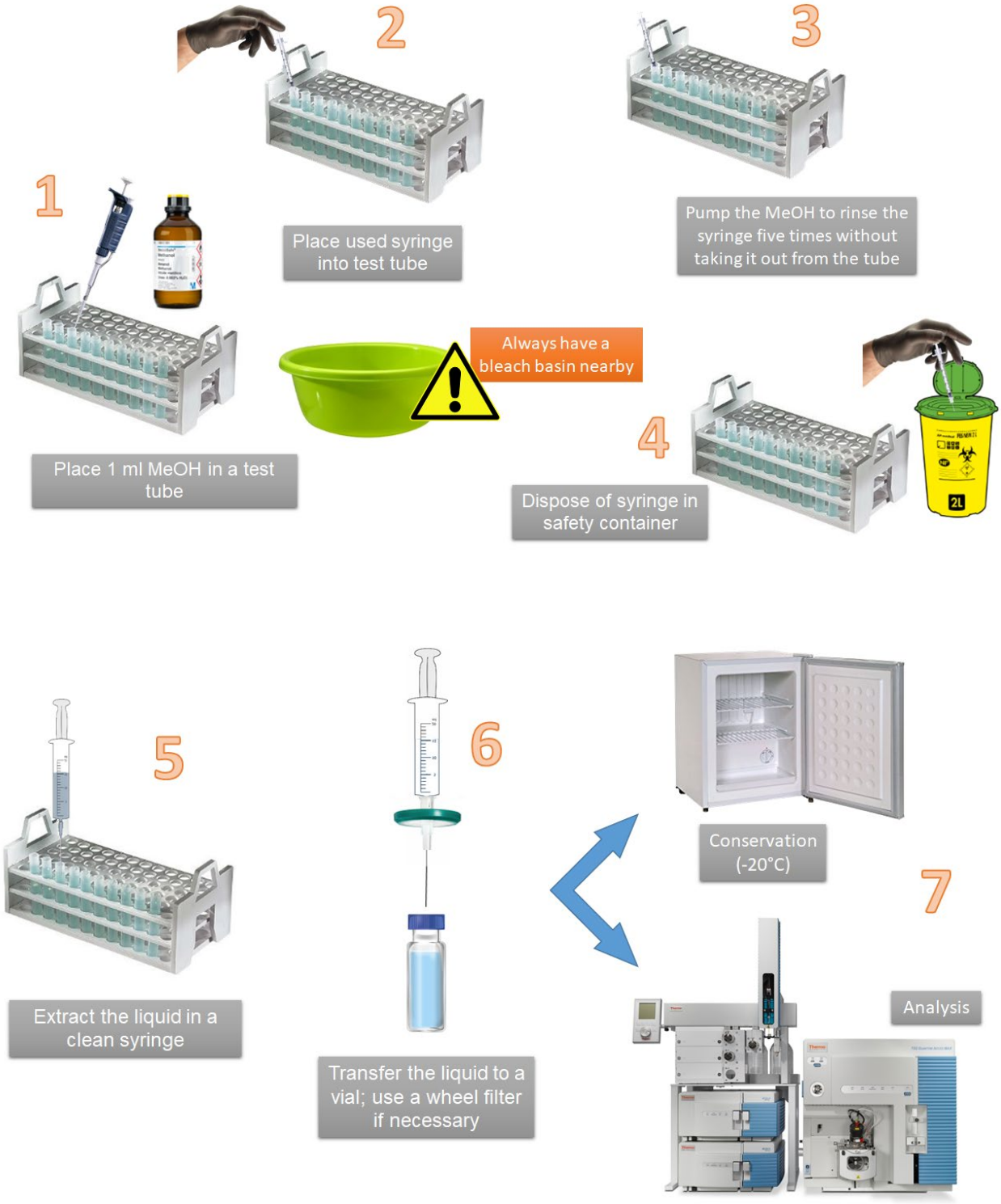
Sample preparation

Syringes should be rinsed up to five times with methanol (MeOH). The maximum volume of MeOH generally used is 1 ml. An alternative to filtration is centrifugation of the samples (2000 rev/min for 5 minutes).

To reduce blood exposure risks, the sample preparation method illustrated in Figure 2 is recommended.

In the event of needle injury with possible blood exposure, put your hand (or the injured part of your body) into a basin with bleach and press the wound to evacuate blood. After 2 minutes, put a swab on the wound and go to hospital to report the incident, where doctors can prescribe post-exposure prophylaxis against HIV.

FIGURE 2
Recommended sample preparation steps



Analytical methods

Different analytical methods can be used (gas chromatography (GC), ultra-high- or high-performance liquid chromatography (UHPLC or HPLC) coupled with mono or tandem mass spectrometry (MS or MS/MS) but it is recommended to use a screening method. Screening methods allow a wider range of substances to be detected (including new substances). If a target method is used, a required minimum list of substances has been established, including classical drugs, new psychoactive substances (NPS) and medicines which are known to be injected in participating cities (Annex 3). Several cutting agents, degradation products and metabolites are also included in that list. The minimum set of substances is reviewed each year by the network.

The required minimum list of substances is complemented by any other substances that the participating research teams wish to investigate, depending on local specificities or on the analytical methods used (Annex 3).

Methods used by some of the ESCAPE study teams are documented in Annex 4 and have also been described in the literature (Gjerde et al., 2020; Lefrançois et al., 2016; Néfau et al., 2015; Péterfi et al., 2018). They can be reproduced and used by other laboratories. Depending on the analytical equipment available, it is possible to develop and use other methods. The aim is to be able to detect at least the substances from the required minimum set of substances, and as many other substances as possible (Annex 3).

No blood analyses (e.g. rhesus or DNA analyses) will be conducted.

Data management and analysis

Data collection

Codebook

Participating sites report data on each syringe analysed using a standardised spreadsheet with the variables listed in the codebook (Table 1). The list of variables is reviewed each year and the trade-off between information and workload of study teams is assessed.

TABLE 1
ESCAPE codebook

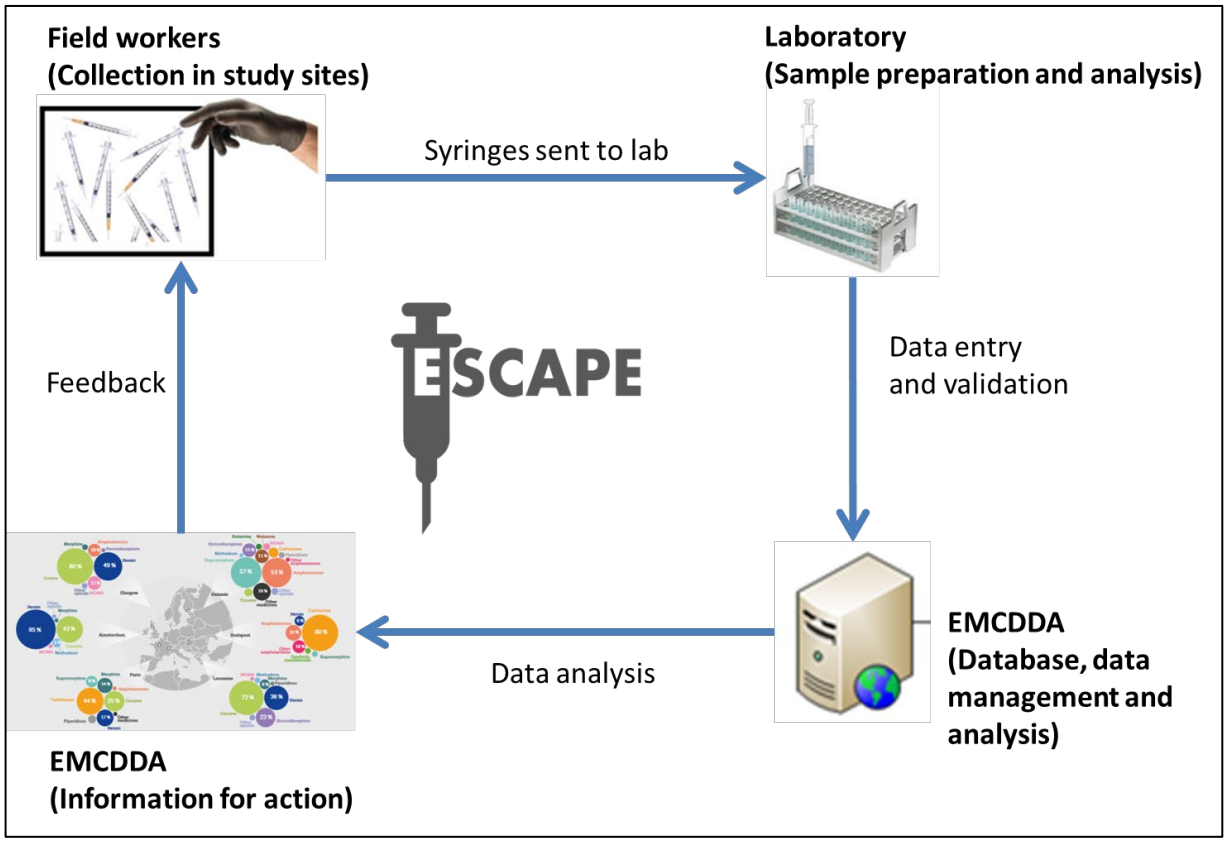
Variable name	Description	Value
IDUNIQUE	IDUNIQUE is a unique identifier for each syringe within a particular study year	=CONCATENATE(COL3;"_"; COL5; "_";COL4)
COUNTABR	2-Letter or 3-letter country codes for participating countries	FIN, FRA, DEU, HUN,…
ID	Code given to each syringe by the laboratory	S1, S2, S3…
DATECOL	Date when the syringe was collected	DD/MM/YYYY
LOC	Name or code of the location where the syringe was conducted. Several sites (5 max) for each city	L1, L2, L…
CITY	City code of participating cities	AMS, BUD, COL, GLA, HEL, LAU, PAR…

Variable name	Description	Value
STRTYPE	Type of structure where the collection is performed: automatic injection kit dispensers/low-threshold facilities/supervised injecting centre (or injecting room)	AIKD, LT, SSIR
X_WGS84	World Geodetic System 1984 variable X (longitude)	
Y_WGS85	World Geodetic System 1984 variable Y (latitude)	
Unmade extraction	Unmade extraction	Yes/No
Blood	Presence of blood traces	Yes/No
Broken needle	Broken needle	Yes/No
Attritions	Attrition marks	Yes/No
Signs	Distinctive signs	Yes/No
Difficult to rinse	Problems rinsing the syringe	Yes/No
DATEANAL	Date of syringe analysis	DD/MM/YYYY
Substance	List of substances detected	See Annex 3

Data flow

The standardised spreadsheets are then validated and included in a centralised European database at the EMCDDA where further data management and data analysis are performed (Figure 3).

FIGURE 3
ESCAPE data flow



Data analysis

Primary indicators

The main geographical unit of analysis is the city.

- Counts of syringes analysed, by city, by year
- Counts and proportion of syringes testing positive for at least one drug category, by city, by year
- Counts and proportion of syringes testing positive, by drug category, by city, by year
    - The denominator for the proportion is all syringes testing positive for at least one drug category
- Counts and proportion of syringes testing positive for more than one drug category, by combination of drug categories, by city, by year
    - The denominator for the proportion is all syringes testing positive for at least one drug category

Other indicators

- Counts and proportion of syringes testing positive for at least one drug, by city, by year
- Counts and proportion of syringes testing positive, by drug, by city, by year
    - The denominator for the proportion is all syringes testing positive for at least one drug category
- Counts and proportion of syringes testing positive for more than one drug, by combination of drug, by city, by year
    - The denominator for the proportion is all syringes testing positive for at least one drug category
- Counts and proportion of syringes testing positive for adulterants, by drug, by city, by year
    - The denominator for the proportion is syringes testing positive for the drug of interest

Other geographical units of interest might include sites (within cities) and overall results (pooling of European results).

References

Gjerde, H., Bretteville-Jensen, A. L., Furuhaugen, H., Bache-Andreassen, L., Bergh, M. S. and Vindenes, V. (2020), 'Determination of drug residues in used syringe needles', *Drug Testing and Analysis* 12(3), pp. 410-16. (available at https://onlinelibrary.wiley.com/doi/10.1002/dta.2759).

Lefrançois, E., Esseiva, P., Gervasoni, J.-P., Lucia, S., Zobel, F. and Augsburger, M. (2016), 'Analysis of residual content of used syringes collected from low threshold facilities in Lausanne, Switzerland', *Forensic Science International* 266, pp. 534-40. (available at https://linkinghub.elsevier.com/retrieve/pii/S0379073816303255).

Néfau, T., Charpentier, E., Elyasmino, N., Duplessy-Garson, C., Levi, Y. and Karolak, S. (2015), 'Drug analysis of residual content of used syringes: A new approach for improving knowledge of injected drugs and drug user practices', *International Journal of Drug Policy* 26(4), pp. 412-19. (available at https://linkinghub.elsevier.com/retrieve/pii/S095539591400276X).

Péterfi, A., Csorba, J., Figeczki, T., Kiss, J., Medgyesi-Frank, K., Posta, J. and Gyarmathy, V. A. (2018), 'Drug residues in syringes and other injecting paraphernalia in Hungary', *Drug Testing and Analysis* 10(2), pp. 357-64. (available at http://doi.wiley.com/10.1002/dta.2217).

Annexes

Annex 1 **Definitions**

- **Adulterant**: A pharmacologically active compound that dealers mix with drugs to increase the volume of the product in order to maximise profits. For instance, levamisole – originally an anthelmintic medication, which has some antidepressant properties – is a common adulterant of cocaine. Pharmacologically inert diluents (such as sugar) were not screened for in this study.
- **By-product of production**: Some drugs may be the result of the production process of another drug. For instance, codeine traces might be found in heroin.
- **Degradation product**: A compound resulting from the natural breakdown of a drug over time. The degradation of a drug can occur in the syringe. For instance, heroin will naturally degrade into 6-MAM (6-monoacetylmorphine) and morphine. In the analysis, any syringe testing positive for 6-MAM in the presence of morphine, codeine or meconin was assumed to have once contained heroin and was reclassified as a 'heroin syringe'.
- **Drug**: A psychoactive substance consumed with the aim of altering the user's mood and perception, through its effect on the central nervous system.
- **Drug category**: In order to simplify the presentation of results for the large number of substances covered in this study, drugs were grouped into 17 drug categories according to their public health relevance and on the basis of their shared characteristics. The categories may thus combine chemical, pharmacological and use perspectives. For example, heroin and methadone are reported separately from 'other opioids' and 'other medications', respectively. Some drug categories (e.g., cocaine) include a single drug, while others (e.g., synthetic cathinones) include several drugs
- **Metabolite**: Metabolites are residues of a drug after it is broken down in the body. They can be found in the blood, urine or faeces of users after consumption of the drug regardless of the route of administration. Blood containing metabolites can enter a syringe during injection. In this study, tests were carried out for metabolites of heroin (6-MAM), cocaine (benzoylecgonine) and benzodiazepines (7-aminoclonazepam). Some metabolites, for instance 6-MAM, can also result from degradation. Syringes testing positive only for metabolites were excluded from the analysis.
- **Syringe** will refer to a needle and/or a barrel, depending on the ability of the partners on the field to collect each of those parts. In some cities, people who inject drugs visit their needle and syringe programme to get new needles (or a 'puck' of 42 sterile needles) as they give back their used ones. In these cities, field workers are therefore much more likely to gather needles than barrels.

Annex 2 **Safety equipment and supplies**

Manipulating syringes is hazardous because of the risk of accidental blood exposure and potential exposure to blood-borne viruses. It is crucial to respect safety measures and to use specific equipment to reduce the risks. In the context of the COVID-19 pandemic, we added protective masks to the list of safety equipment. Disinfection of protective equipment should be done thoroughly. Each study team must conduct its collection campaign and analysis in accordance with their national health and safety recommendations.

Minimum set of required safety equipment

Laboratory coat

Safety glasses

Concentrated bleach solution

Needle resistant gloves

Laboratory tongs

Protective mask

Annex 3 **List of drugs, adulterants and metabolites tested for, by city**

Drug category, metabolite or adulterant	Drug/substance (In bold: required minimum list of substances for laboratory analysis)	Amsterdam	Budapest	Cologne	Helsinki	Lausanne	Oslo	Paris	Vilnius
Amphetamines	**Amphetamine**	x	x	x	x	x	x	x	x
	Methamphetamine	x	x	x	x	x	x	x	x
Cocaine	**Cocaine**	x	x	x	x	x	x	x	x
Heroin	**Heroin**	x	x	x	x	x	x	x	x
Morphine	**Morphine**	x	x	x	x	x	x	x	x
Buprenorphine	**Buprenorphine**	x	x	x	x	x	x	x	x
Naloxone	Naloxone	x	x	x	x	x	x	x	x
Methadone	**Methadone**	x	x	x	x	x	x	x	x
Fentanyl and derivatives	3-methylfentanyl	x	x	x	x	x			x
	4-Chloro-isobutyrfentanyl	x	x	x	x	x	x		x
	4-Fluoro-isobutyryl fentanyl	x	x	x	x	x	x		x
	4-Methoxy-butyryl fentanyl	x	x	x	x	x	x		x
	Acetylfentanyl	x	x	x	x	x	x	x	x
	Acrylfentanyl	x	x	x	x	x	x		x
	Alfentanil	x	x	x	x	x	x		x
	Butyrylfentanyl	x	x	x	x	x	x		x
	Carfentanil	x	x	x	x	x	x	x	x
	Cyclopentylfentanyl	x	x	x	x	x			x
	Cyclopropylfentanyl	x	x		x	x	x		x
	Despropionylfentanyl	x	x		x	x			x
	Fentanyl	x	x	x	x	x	x	x	x
	Furanyl fentanyl	x	x	x	x	x	x	x	x
	Ocfentanyl	x	x	x	x	x	x	x	x
	ortho-Fluorofentanyl	x	x	x	x	x			x
	Valerylfentanyl	x	x	x	x	x	x		x
Other opioids	AH-7921	x	x	x	x	x			x
	Codeine	x	x	x	x	x	x	x	x
	Dihydrocodeine	x	x	x	x	x			x
	Hydrocodone	x	x	x	x	x			x
	Isotonitazene		x		x				x
	Oxycodone	x	x	x	x	x			x
	Tramadol	x	x	x	x	x	x	x	x
	U-47,700	x	x	x	x	x		x	x
Cathinones	**3-MMC**	x	x	x	x	x	x	x	x
	3,4-DMMC	x	x	x	x	x			x
	4-Chloro-alpha-PVP	x	x	x	x	x			x
	4-Chloroethcathinone	x	x	x	x	x			x
	4-Chloromethcathinone	x	x	x	x	x			x
	4-Cl-alpha-PPP		x	x	x				x
	4-Cl-Pentedrone		x		x				x
	4-Fluoro-alpha-PVP	x	x	x	x	x			x
	4-MEC	x	x	x	x	x	x	x	x
	Alpha-PBP		x		x				x
	alpha-PEP (PV8)	x	x	x	x	x			x
	alpha-PHP	x	x	x	x	x			x
	alpha-PHPp	x	x	x	x	x			x
	alpha-PVP	x	x	x	x	x	x	x	x
	Alpha-PVT		x	x	x				x
	bk-MDDMA	x	x	x	x	x			x
	Buphedrone (MABP)	x	x	x	x	x			x
	Butylone (bk-MDMB)	x	x	x	x	x			x
	Dipentylone		x		x				x
	Ephylone (bk-EBDB)		x	x	x				x
	Ethylone (bk-MDEA)	x	x	x	x	x			x
	F-alpha-PHP	x	x		x	x			x
	MDPBP	x	x	x	x	x			x
	MDPPP		x	x	x				x
	MDPV	x	x	x	x	x	x	x	x
	Mephedrone (4-MMC)	x	x	x	x	x	x	x	x

Drug category, metabolite or adulterant	Drug/substance (In bold: required minimum list of substances for laboratory analysis)	Amsterdam	Budapest	Cologne	Helsinki	Lausanne	Oslo	Paris	Vilnius
	Methedrone (bk-PMMA)	x	x	x	x	x			x
	Methylone	x	x	x	x	x	x	x	x
	Mexedrone	x	x	x	x	x			x
	N-acetyl mephedrone		x		x				x
	N-ethyl-pentedrone		x		x				x
	Naphyrone	x	x	x	x	x			x
	N-ethylhexedrone	x	x	x	x	x			x
	N-ethylnorpentedrone		x		x				x
	Pentedrone	x	x	x	x	x	x	x	x
Synthetic cannabinoids	4CN-Cumyl-BINACA		x		x				x
	5F-APINACA	x	x		x	x	x		x
	5F-MDMB-PINACA	x	x		x	x			x
	5F-PB-22	x	x		x	x	x		x
	AB-CHMINACA	x	x		x	x			x
	AB-FUBINACA	x	x		x	x			x
	AMB-FUBINACA	x	x		x	x			x
	MMB-CHMINACA		x		x				x
Benzodiazepines	3OH-Phenazepam	x	x	x	x	x			x
	Alprazolam	x	x	x	x	x	x	x	x
	Bromazepam	x	x	x	x	x			x
	Chlordiazepoxide	x	x	x	x	x			x
	Clobazam	x	x	x	x	x			x
	Clonazepam	x	x	x	x	x	x	x	x
	Clonazolam	x	x	x	x	x			x
	Delorazepam	x	x	x	x	x			x
	Deschloroetizolam	x	x	x	x	x			x
	Desmethyldiazepam	x	x	x	x	x	x		x
	Diazepam	x	x	x	x	x	x	x	x
	Diclazepam	x	x	x	x	x	x		x
	Etizolam	x	x	x	x	x	x	x	x
	Flubromazepam	x	x	x	x	x	x		x
	Flubromazolam	x	x	x	x	x	x		x
	Flunitrazepam	x	x	x	x	x	x	x	x
	Lorazepam	x	x	x	x	x			x
	Lormetazepam	x	x	x	x	x			x
	Meclonazepam	x	x	x	x	x			x
	Metizolam	x	x	x	x	x			x
	Midazolam	x	x	x	x	x	x	x	x
	Nifoxipam	x	x	x	x	x			x
	Nitrazepam	x	x	x	x	x	x		x
	Oxazepam	x	x	x	x	x	x	x	x
	Phenazepam	x	x	x	x	x	x		x
	Pyrazolam	x	x	x	x	x			x
	Temazepam	x	x	x	x	x		x	x
Piperidines	2-DPMP		x	x	x				x
	3,4-CTMP		x		x				x
	4-Fluoro-methylphenidate	x	x	x	x	x			x
	Ethylphenidate	x	x	x	x	x	x	x	x
	Methylphenidate	x	x	x	x	x	x	x	x
MDMA	MDA	x	x	x	x	x		x	x
	MDEA	x	x	x	x	x		x	x
	MDMA	x	x	x	x	x	x	x	x
Ketamine	**Ketamine**	x	x	x	x	x	x	x	x
Other medicines	Bupropion	x	x	x	x	x			x
	Carbamazepine	x	x	x	x	x			x
	Doxepin		x	x	x				x
	Etorecoxib		x		x				x
	Gabapentin	x	x	x	x	x			x
	Methiopropamine	x	x	x	x	x	x	x	x
	Methotrexate	x	x	x	x	x		x	x
	Piracetam		x	x	x				x

Drug category, metabolite or adulterant	Drug/substance (In bold: required minimum list of substances for laboratory analysis)	Amsterdam	Budapest	Cologne	Helsinki	Lausanne	Oslo	Paris	Vilnius
	Pregabalin	x	x	x	x	x			x
	Propranolol		x	x	x				x
	Quetiapine	x	x	x	x	x			x
	Sertraline		x	x	x				x
	Tiapride	x	x	x	x	x			x
	Tizanidine	x	x	x	x	x			x
	Zolpidem	x	x	x	x	x	x	x	x
	Zopiclone	x	x	x	x	x	x	x	x
Other amphetamines	3-Fluoromethamphetamine	x	x		x	x			x
	4-Fluoro-amphetamine	x	x	x	x	x		x	x
	F-ethamphetamine		x		x				x
	N-acetylamphetamine		x		x				x
	N-propylamphetamine	x	x		x	x			x
	PMA	x	x	x	x	x			x
	PMMA	x	x		x	x			x
Other drugs	5-EAPB	x	x	x	x	x		x	x
	Amisulpride		x	x	x				x
	Mephtetramine	x	x	x	x	x			x
	THC	x	x	x	x	x	x		x
Metabolites and degradation products	**6-monoacetylmorphine (heroin)**	x	x	x	x	x	x	x	x
	7-Aminoclonazepam (clonazepam)	x	x	x	x	x	x		x
	7-Aminoflunitrazepam (flunitrazepam)	x	x	x	x	x	x		x
	7-Aminonitrazepam (nitrazepam)		x	x	x		x		x
	10-monohydroxycarbamazepine (carbamazepine)	x	x		x	x			x
	α-hydroxy-alprazolam (alprazolam)	x	x	x	x	x			x
	α-hydroxy-midazolam (midazolam)	x	x	x	x	x			x
	Acetylcodeine (heroin)	x	x	x	x	x			x
	Amphetamine AC		x						x
	Benzoylecgonine (cocaine)	x	x	x	x	x	x	x	x
	Ecgonine methyl ester		x	x				x	x
	EDDP (methadone)	x	x	x	x	x			x
	HMMA (MDMA)	x	x		x	x			x
	Hydrocotarnine		x						x
	Meconin (opiate)	x	x	x					x
	Metamizole breakdown		x	x					x
	N-[2-(3,4-methylenedioxyphenyl)-1-methylvinyl]-N,N-dimethylamine		x						x
	Nicotine		x	x					x
	Norbuprenorphine (buprenorphine)	x	x	x	x	x			x
	Norcocaine		x	x					x
	Norcodeine		x	x					x
	Normorphine		x	x					x
	Noscapine		x	x					x
	O-desmethyltramadol (tramadol)	x	x	x	x	x			x
	Ritalinic acid		x	x	x				x
	Thebromine		x	x					x
	Theophylline		x	x					x
Adulterants	Caffeine	x	x	x		x			x
	Dextromethorphan	x	x	x	x	x	x	x	x
	Dibutylhydroxytoluene		x						x
	Dimethylsulfone		x						x
	Diphenhydramine		x	x	x				x
	Griseofulvine	x	x	x		x			x
	Hydroxyzine	x	x	x	x	x			x
	Levamisole	x	x	x	x	x	x	x	x
	Lidocaine	x	x	x		x			x
	Papaverin		x	x					x
	Paracetamol	x	x	x		x			x
	Phenacetin	x	x	x		x			x
	Procaine		x	x					x

Annex 4 **Analytical methods used by laboratories (first two campaigns)**

		Amsterdam/Lausanne	Budapest	Cologne	Glasgow	Helsinki		Oslo	Paris
Laboratory		Unit of Forensic Toxicology and Chemistry, University Center of Legal Medicine, Lausanne-Geneva	Toxicology Laboratory of the Institute of Forensic Medicine of the University of Debrecen	Institute of Forensic Medicine, Medical Centre, University of Freiburg	Forensic Medicine and Science (FMS), University of Glasgow	Forensic Toxicology Unit at National Institute for Health and Welfare		Departement of forensic Sciences, Oslo University Hospital	Laboratory of Public health and Environment Paris Sud University
Separation method						**I**	**II**		
	Method	GC	GC	HPLC	HPLC	UHPLC	UHPLC	UHPLC	UHPLC
	Brand / model	Agilent / 6890N Network	Agilent / 7890A	Dionex / Ultima 3000	Agilent / 1200 Series HPLC	Agilent / 1290 Infinity II	Waters Acquity	Agilent 1290 Infinity LC System	Thermo Scientific / Accela Pump
	Column	DB-XLB capillary column (30 m length, 0.25 mm in diameter and 0.25 µm film thickness)	HP-35ms UI capillary column (30 m length, 0.25 mm in diameter and 0.25 µm film thickness)	Acclaim® RSLC 120 C18 2.2 µm 120A 2.1x100 mm	Phenomenex Gemini C18 (150 x 2mm, 5µm)	Waters Acquity CSH C18 75 mm, 2.1 mm i.d., 1.7 µm particle + 5 mm precolumn	Waters Acquity HSS T3 C18 150 mm, 2.1 mm i.d., 1.7 µm particle + 5 mm precolumn	Acquity HSS T3-column (2.1 x 100 mm, 1.8 µm; Waters Corporation)	Waters Acquity UPLC BEH Phenyl 1.7 µm, 2.1x100 mm
	Gas or Eluant	Helium	Helium	Eluent A: Water, 2 mM ammonium formate, 0.1% formic acid, 1% acetonitrile Eluent B: Acetonitrile, 2 mM ammonium formate, 0.1% formic acid, 1% water	Eluant A: Deionised Water Eluant B: Methanol A&B : supplemented to contain 2mM Ammonium Acetate and 0.1% Formic Acid	Eluent A: 5mM ammonium formate-0.05% formic acid Eluent B: 100% acetonitrile	Eluent A: 5mM ammonium acetate-0.1% formic acid (aqueous); Eluent B: 100% methanol (organic),	Eluent A: 5mM ammonium formate pH 3.1; Eluent B: Methanol.	Eluent A: 5mM formic acid /ammonium formate buffer Eluent B: 100% acetonitrile
	Flow and Gradient	constant flow mode 1.2 mL/min	constant flow mode 1.2 mL/min	constant flow mode 0.5 mL/min 0.0 – 1.0 min: 1 % B 1.0 – 8.0 min: 1 % B to 95 % B 8.0 – 9.0 min: 95 % B 9.0 – 9.1 min: 95 % B to 1 % B 9.1 – 11.0 min: 1 % B	Flow Rate: 0.3 mL/min Elution: Gradient and Isocractic	Gradient elution 0.5 ml/min flow	Gradient elution, 0.3 ml/min flow	flow rate : 0.4 mL/min 0.0 – 0.15 min: 5 % B 0.15 – 0.3 min: 30 % B 0.3 – 2.7 min: 50 % B 2.7 – 3.8 min: 90 % B 3.8 – 4.6: 98 % B 4.6 – 6.0: 5% B	flow rate : 0.4 mL/min 0.0 – 1.0 min: 2 % B 1.0 – 7.0 min: 98 % B 7.0 – 12.0 min: 98 % B 12.0 – 14.0 min: 2 % B 14.0 – 15.1 min: 2 % B
	Temperature	70 °C was held for 1 min, then increased to 200 °C (at 15 °C/min) and to 300 °C (at 10 °C/min). 300°C was held for 7 min and then increased to 320 °C (30 °C/min), at which it was finally held for 3.67 min.	80 °C was held for 1 min, then increased to 300 °C (at 15 °C/min) and the program was held for 21 min.	40 °C	40°C	6 °C (autosampler) 40 °C (UHPLC column)	10 °C (autosampler) 60 °C (UHPLC column)	65°C	40 °C
	Runtime	31 min	37 min	11 min	Variable	11.2 min + equilibration	18 min + equilibration	9 min	9 min + 6 min equilibration
Detection method						**I**	**II**		
	Method	Mass detection	Mass detection	Mass detection	Mass detection	Tandem mass spectrometry (MS/MS)	Time-of-Flight Mass Spectrometry	Tandem mass spectrometry (MS/MS)	Tandem mass spectrometry (MS/MS)
	Brand / model	Agilent / 5973 Network	Agilent / 5975C	Bruker / amaZon speed	AB Sciex / 3200 QTRAP	Agilent / 6495 QqQ	Bruker Daltonics MicroTOF Q II	Agilent 6490 Triple Quadrupole	Thermo Scientific / TSQ Quantum Access Max
	Temperature	230 °C for the ion source and 150 °C for the quadrupole	230 °C for the ion source and 150 °C for the quadrupole, EI mode	320 °C for the ESI ion source	Ionisation: Turbo Ion Spray (Electrospray, ESI) held 350°C	350 °C (sheath gas)	200 °C (dry gas)	Ion source at 300 °C	Ion source at 300 °C
	Scan mode	Full	Full	Full Scan, MS^2, MS^3	MRM	Dynamic multiple reaction monitoring (dMRM)	Full Scan MS and bbCID	Multiple reaction monitoring (MRM)	Multiple reaction monitoring (MRM)
	Details	10–400 m/z mass range for the first 7 min then 30–550 m/z mass range, with a sampling rate of 2 scans/s	30–650 m/z mass range with a sampling rate of 2 scans/s	Scan range: 70 - 800 m/z Scan speed: 32000 m/z*s-1 Data dependent acquisition of $MS^2$and MS^3 spectra Spectral Library containing approx. 1050 compounds	MS operated in QQQ mode not QTRAP mode	electrospray ionisation at positive mode (ESI+); multiwash and separate in-house developed injection program for UHPLC separations	electrospray ionisation at positive mode (ESI+); automated compound ID and reporting based on a reverse search against an exact mass database with approx. 1200 compounds	electrospray ionisation at positive mode (ESI+)	electrospray ionisation at positive mode (ESI+)

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