State-of-the-art analytical approaches and strategies to assess disposal of drugs for wastewater-based epidemiology

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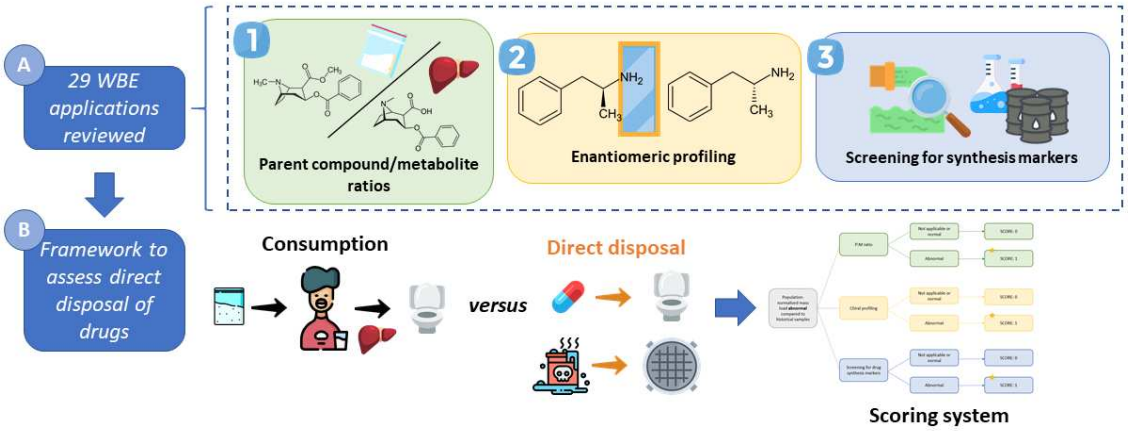
Abstract

Not all residues of drugs found in influent wastewater are the result of consumption. Identifying intentional or accidental disposal is crucial in wastewater-based epidemiology to ensure the accuracy of observed spatio-temporal trends in consumption patterns. So far, only a limited number of studies provided analytical evidence for the direct disposal of illicit drugs or pharmaceuticals. Additionally, only minimal standardization in the workflow is employed to distinguish direct disposal from consumption. PubMed, SCOPUS, and Web of Science databases were searched using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, 2020) guidelines. The search focused on wastewater-based epidemiology publications in which the dumping event was strongly suspected or identified through i) parent compound-metabolite ratios, ii) enantiomeric profiling, and iii) non-target and suspect screening. In total, 29 studies were included in this systematic literature review. This study aims to review existing approaches to assess direct disposal of drugs in influent wastewater, review literature for potential dumping events, and proposes a simple evidence-based scoring system for the identification of direct disposal of drugs in influent wastewater, based on available analytical evidence. This framework is a first effort to standardize dumping/disposal assessment, while more research is needed to further refine the decision criteria and analytical techniques used within the proposed strategy.

Graphic/Visual abstract:



Keywords: Wastewater-based epidemiology, direct disposal, drugs, parent-metabolite ratio, enantiomeric profiling, non-target and suspect screening

1 Introduction

Over the past decade, wastewater-based epidemiology (WBE) has evolved into a valuable complementary epidemiological information source to gather community-wide health information on the exposure to different xenobiotics. This approach measures concentrations of human biomarkers in influent wastewater (IWW) and converts these to population-normalized mass loads (PNML), expressed in mg/day/1000 inhabitants, by multiplying with the daily wastewater flow rate and dividing by the population served by the wastewater treatment plant (WWTP) (Baker et al., 2014; Zuccato et al., 2008). Trace concentrations (ng/L) of the target analytes can be quantified by employing accurate and precise analytical methods based on solid-phase extraction (SPE) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (Andres-Costa et al., 2017; Baker & Kasprzyk-Hordern, 2011; Fatta et al., 2007; van Nuijs et al., 2011). Although the majority of WBE studies has focused on lifestyle-related biomarkers (e.g., illegal drugs, alcohol, and tobacco) (Gonzalez-Marino et al., 2020; van Wel et al., 2016), the number of investigations focusing on public health biomarkers (e.g., pharmaceuticals, environmental contaminants, pathogens, disease markers) has been increasing (Ahmed et al., 2020; Been et al., 2018; Boogaerts, Quireyns, et al., 2021; Choi et al., 2019; Daughton, 2018; Gracia-Lor et al., 2017). A major advantage of WBE is that it can be applied to monitor consumption trends at high spatio-temporal resolution, and that it can provide data in near-real time at the population level. However, WBE cannot provide details on individual consumption patterns and socio-demographic information of the users. In this sense, WBE is not able to tell anything about the administration form, co-consumption, dose frequency, dose purity, individual compliance, and individual drug use preferences (Castiglioni, 2016). For this reason, it is important to combine epidemiological data from multiple information sources to obtain a more accurate picture on the exposure to different drugs.

Biomarkers suitable for WBE purposes should meet the following criteria: they must be i) excreted in sufficient amounts, ii) specific for human metabolism, and iii) stable in influent wastewater. In this light, human metabolites have been favored over parent compounds since the measurement of parent drugs could be influenced by direct disposal in the sewer systems (Castiglioni et al., 2013). However, metabolic candidates that fulfill the abovementioned criteria cannot always be found, and parent compounds have been used in multiple WBE applications (Gonzalez-Marino et al., 2020; Xu et al., 2017). The measurement of parent compound is of interest (e.g., consumption, identifying dumping), but results must be critically evaluated.

Different solutions have been proposed to distinguish direct disposal from actual consumption and subsequent excretion of the parent drugs in the sewage system. Direct disposal in the sewer can be

deliberate, such as criminals attempting to avoid police detection, or patients disposing of unused medication rather than through recommended take-back programs (Depaolini et al., 2016; Emke et al., 2018; Vazquez-Roig et al., 2014). In some cases, flushing of unused or unwanted medication is recommended to reduce risk of fatal ingestion (e.g., fentanyl patches) (*Drug Disposal: FDA's Flush List for Certain Medicines*, 2020). Incidental disposal may occur through handwashing, transport, sweat, wiping of residual drugs (e.g. cocaine) into the toilet/sink (Castiglioni et al., 2013; Verovsek et al., 2021).

An overview of state-of-the-art analytical methods to assess direct disposal of parent drugs in the wastewater system is given in Figure 1. Current analytical approaches employed for the identification of dumping events mainly consist in 1) the measurement of parent drug-metabolite ratios (P:M), 2) enantiomeric profiling of parent compound, and 3) utilizing non-target and suspect screening workflows to search markers representative for waste from illegal drug production (e.g., drug precursors, intermediates, impurities, and final parent compound). These methods have been previously applied successfully to identify dumping events (Bijlsma et al., 2012; Boogaerts, Jurgelaitiene, et al., 2021; Emke et al., 2014, 2018), to characterize drug trafficking routes (Boogaerts, Jurgelaitiene, et al., 2021; Castrignano et al., 2018; Emke et al., 2018; Reymond et al., 2022), and to distinguish between therapeutic use and illegal use (Bijlsma et al., 2012; Kasprzyk-Hordern & Baker, 2012a).

Figure 1. Schematic overview of wastewater-based epidemiology and the current analytical approaches to assess direct disposal of parent compound in the sewer system.

The European Union Agency for Law Enforcement Cooperation (EUROPOL) indicated that the illegal dumping of waste is a growing concern, reflected by the large-scale domestic production of illegal substances in Europe (e.g., Belgium, Netherlands) (Europol, 2019, 2020). This was also illustrated by the different monitoring campaigns of the Sewage CORe group Europe (SCORE), with increasing reports of synthetic drug dumping in Western Europe (González-Mariño et al., 2020). In this sense, early identification of specific chemical waste profiles might be useful to highlight disposal in the sewer of drug production processes within the wastewater catchment area, and for refining WBE back-estimations.

In this study, we reviewed the current situation of applying complementary analytical approaches (e.g., P:M ratio, enantiomeric profiling of drugs, non-target screening of synthesis markers) to WBE data towards a better understanding of the fate of illicit drugs and pharmaceuticals, hereafter referred to as drugs, present in the sewer system. Our goal was to evaluate state-of-the-art strategies used to evaluate direct disposal of parent compounds, and to give insight in the future research that is needed to fill the current knowledge gaps. Additionally, we will provide a new framework on how these

analytical strategies can be employed to assess dumping of drugs, to further complement and improve the reliability of WBE investigations. For more information on the WBE methodology and its uncertainties, readers are referred to the studies by Castiglioni et al., Baker et al. and Choi et al (Baker et al., 2014; Baker & Kasprzyk-Hordern, 2011; Castiglioni et al., 2013; Choi et al., 2018).

2 Materials and methods

This review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyse (PRISMA) from 2020 (Page et al., 2021). PRISMA offers an evidence-based approach for reporting in systematic reviews and meta-analyses and was used to improve the transparency of the literature search.

2.1 Literature search

Several electronic search platforms were utilized: PubMed, Web of Science, and SCOPUS. Search strategies combined variations of subject terms or keywords regarding i) wastewater or wastewater-based epidemiology, ii) dumping, and iii) illicit drugs or pharmaceuticals. Search results were limited to manuscript published after the pioneering application of WBE in 2005 (Zuccato et al., 2005). Full search strategies for each search platform are reported in the Supplementary Information (S.1). The initial screening based on abstract and title subjects was manually performed by three investigators, prior to a full-text screening for inclusion and exclusion criteria. Conflicts with eligibility conditions were removed after mutual consent. Furthermore, references of the included articles were manually reviewed for relevant studies. In addition, the bibliography of the primary author was reviewed to track down additional references.

2.2 Inclusion- and exclusion criteria

Only WBE applications in which the dumping event were either suspected or identified through i) parent compound-metabolite ratios, ii) enantiomeric profiling, and iii) non-target and suspect screening were included. Additionally, only English publications were included. We excluded applications that only reported aberrant population-normalized mass loads without further explanation, since it is also possible that these loads originate from ‘special events’ with associated high consumption. Additionally, studies that focused exclusively on method development and validation, applications that evaluated removal efficiencies during wastewater treatment, or studies that investigated environmental contamination in aquatic environments other than influent wastewater were not included in this review.

2.3 Search results

An initial search was performed on 27 Nov 2021 and updated on 02 Feb 2022 with newly found records. A total of 5 349 citations were retrieved after performing executing the search strategies (S.1). All citations were imported as a reference into Mendeley, and duplicates were identified (1 431) and manually checked using the deduplication tool (*Mendeley Desktop*, 2021). After deduplication, 1 495 studies were screened on title and abstract content, from which another 1 077 records were excluded. After full-text screening, the remaining 29 studies were included in this systematic literature review. In Figure 2, the search results are summarized as a PRISMA flow diagram.

Figure 2. PRISMA flow diagram

3 State-of-the-art analytical approaches to assess direct disposal of parent compound

In this section, we discuss state-of-the-art analytical methods to assess disposal of drugs in the sewer system. A deviation in the historical levels of PNML of a certain compound is often the first indication of a dumping event. For example, a Dutch WBE study discovered that the PNML of amphetamine (AMP) and 3,4-methylenedioxymethamphetamine (MDMA) in 2011 were >10-fold higher compared to 2010 (Emke et al., 2014). When considering excretion profiles and average dose of AMP and MDMA, this resulted in an estimated prevalence of use in the catchment that raised suspicions about the origin of these high PNML. To further investigate this, the authors applied enantiomeric profiling of these synthetic drugs in the wastewater samples (see 3.2 Enantiomeric profiling, Table 2). Similar strategies were also applied in other studies (Boogaerts, Jurgelaitiene, et al., 2021; Emke et al., 2014; Kasprzyk-Hordern & Baker, 2012a; Lai et al., 2018). In the upcoming subsections, we discuss the different approaches that could be employed in case of unexpectedly high PNML levels that are not in line with historical data to determine the origin of this increase, being either increased consumption or direct disposal.

3.1 Parent-metabolite ratios

A total of 21 out of 29 studies (72%) confirmed, or strongly suspected, direct disposal of a pharmaceutical or illicit drug using parent-metabolite (P:M) ratios (Table 1).

Using this approach, the ratio of two measured biomarkers, the parent compound, and its metabolite(s), in influent wastewater is calculated. This allows for differentiation between actual human consumption and direct disposal, since direct disposal will contribute to the load of parent

compound in wastewater, and well-chosen metabolites will only be present in influent wastewater due to human consumption. It should be noted that in-sewer degradation of biomarkers should also be evaluated, as it may skew P:M ratio's (Ahmed et al., 2021; McCall et al., 2016; van Nuijs et al., 2012). Direct disposal of a parent compound will result in a significant increase of the P:M ratio. The P:M ratio is calculated as shown in Equation 1. More information can be found in the publications of *Bijlsma et al.*, *Kasprzyk-Hordern et al*., *Postigo et al.* and *van Nuijs et al.* (Bijlsma et al., 2012; Kasprzyk-Hordern et al., 2009; Postigo et al., 2010; van Nuijs et al., 2009).

$$P{:}M\ ratio = \frac{Concentration\ of\ parent\ compound}{Concentration\ of\ metabolite}$$

Equation 1. Calculation of parent-metabolite ratio.

Based on specific characteristics, such as the excretion profile of unchanged parent drug and formed metabolite, cut-off values have been proposed (e.g., 0.75 for cocaine:benzoylecgonine (COC:BE) ratio (van Nuijs et al., 2009)). A value above this ratio suggests that not all measured drug is the result from human consumption and indicates disposal of non-consumed drug into the sewage system (Bijlsma et al., 2012).

Although the use of cut-off values might be relevant in specific cases, it is associated with some limitations. For example, currently applied cut-off values can be variable, for example *Van Nuijs et al.* proposed a cut-off value for COC:BE of 0.75, while *Postigo et al.* used 0.27 (Postigo et al., 2010; van Nuijs et al., 2009). The thresholds of COC and BE were estimated from urinary excretion rates, which were based on limited pharmacokinetic information (Thai et al., 2016; van Nuijs et al., 2009). The sample size of human pharmacokinetic studies is often small and may not be representative for the average excretion profile in different communities and for the different ways of drug use. Metabolism and excretion of drugs are known to differ between individuals (e.g., due to differences in CYP metabolization), or even within patients given different health conditions (Ahsan et al., 2020; Eusuf & Thomas, 2019).

For this reason, excretion factors estimated from human pharmacokinetic studies need to be further refined to obtain more accurate and representative P:M thresholds for a certain demographic population. Simplifying excretion factors used for the calculation of these P:M baselines to only urinary excretion may not provide fully accurate estimates, as the IWW matrix also contains excretion products from other human matrices (e.g., faeces, blood, saliva, sweat). Variations in excretion factors may also arise from differences in dosage forms, administration routes, and co-consumption of other substances (e.g., alcohol, tobacco, and caffeine). Therefore, more research is needed to further refine P:M thresholds for the identification of direct disposal of drugs in IWW. As an alternative approach, it

is recommended that the P:M ratio corresponding with the IWW sample of the suspected dumping event is compared with the historical mean P:M ratio from the same location. This approach might be more valid for the confirmation of direct disposal of parent drug compared to the use of thresholds based on pharmacokinetic data. In the literature, P:M ratios were determined for different parent compounds and metabolites. Studies, that consider P:M ratios when verifying a possible dumping event, are included in Table 1.

Table 1. Overview of included parent:metabolite ratio studies in review. [a] obtained or calculated from supplementary information, [b] estimated from graphical data, and abbreviations: amphetamine (AMP), benzoylecgonine (BE), cocaine (COC), cotinine (COT), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), fluoxetine (FLUO), levamisole (LEV), methadone (MTD), methamphetamine (METH), nicotine (NIC), norfluoxetine (NORFLUO), not mentioned (N.m.), pholedrine (PHO), and statistical test applied (Stat. test)

In some instances, the use of P:M ratios alone might not be enough for the detection of direct disposal of drugs. For example, the consideration whether AMP or methamphetamine (METH) in wastewater originated from consumption or dumping is more complex in comparison with other compounds listed in Table 1, since these compounds are also metabolites of several pharmaceuticals (e.g., selegiline (Cody, 2002; Xu et al., 2017)). Given AMP is also a metabolite of METH, it is not always possible to verify direct disposal using P:M ratios for these compounds. However, dumped METH would reduce the AMP:METH ratio since dumped METH does not undergo human metabolism (Cody, 2002; Xu et al., 2017). The pharmacokinetics of METH is dependent on the urine pH. Given urine pH of 6-8, about 4-7 % of a METH dose will be excreted as AMP. It should furthermore be noted no chiral inversion takes places, e.g., S-(+)-METH is metabolized to S-(+)-AMP. (Cody, 2002; Schepers et al., 2003)

It is also recommended to evaluate different P:M ratios if multiple metabolites are available for a given parent drug. This could especially be useful when the parent compound is heavily metabolized and/or when there is overlap between multiple metabolic pathways (e.g., benzodiazepines). By combining information from different ratios, it is possible to obtain a more informed view on the cause of the observed P:M shift. A major downside of this approach is that multiple metabolic biomarkers must be identified and validated for WBE purposes, which is not always possible.

In summary, when metabolites can be measured in IWW, P:M ratios can deliver valuable information on the origin of the parent compound in the wastewater system. This approach does not only limit itself to illegal substances but could also be employed for other types of human biomarkers (e.g., pharmaceuticals) for which metabolites have been identified.

3.2 Enantiomeric profiling

A total of 8 out of 29 studies (28%) confirmed, or strongly suspected, direct disposal of a pharmaceutical or illicit drug based on enantiomeric profiling.

A chiral molecule, most commonly due to an asymmetric carbon atom, has at least two enantiomers. Many pharmaceuticals (> 50%) and illicit drugs in use today have at least one chiral centre (Kasprzyk-Hordern & Baker, 2012a; Nguyen et al., 2006). The biological and pharmacological activity can vary considerably between enantiomers. For example, S-(+)-AMP has a two-fold higher stimulant activity than R-(−)-AMP (Kasprzyk-Hordern & Baker, 2012b). Since many human drug targets (e.g., receptors, enzymes) are enantioselective in nature, receptor binding, metabolization and excretion favours one enantiomer, resulting in a change of enantiomeric composition after administration and metabolism (Kasprzyk-Hordern & Baker, 2012b). This enantioselective metabolism provides the basis to discern consumption from direct disposal in influent wastewater. Enantiomeric profiling is especially useful in the context of direct drug disposal when only a parent drug, and no metabolites, can be measured. The enantiomeric fraction (EF) can be calculated in different ways:

$$EF = \frac{E_1}{E_1 + E_2}$$

Equation 2. Calculation of enantiomeric fraction.

In Equation 2, E_1 and E_2 are the internal standard corrected concentration of the first and second enantiomer of a chiral drug, respectively (Kasprzyk-Hordern & Baker, 2012b). An EF equal to 0.5 represents a racemic mixture, and in case of the presence of a single enantiomer the EF equals 0 or 1 (Kasprzyk-Hordern & Baker, 2012a). In literature, E1 and E2 defined based on optical activity (+/-), spatial arrangement (R/S), or elution of enantiomer peaks based on retention time. To compare results between studies, the reader must consider how the EF is defined. Chromatographically resolving individual enantiomers of chiral compounds can be achieved by gas-, or more commonly liquid chromatography. Indeed, all applications included in the present review used liquid chromatography. (Bijlsma et al., 2021) Chiral separation is performed using derivatisation, specific chiral stationary phases, or adding chiral additives to the mobile phase. Separation of isomers may also be achieved using ion mobility. (Bijlsma et al., 2021) For more analytical information, readers are referred to the review by *Evans et al.* and *Langa et al.* (Evans & Kasprzyk-Hordern, 2014; Langa et al., 2021).

Table 2. Overview of included enantiomeric profiling applications in review. Included studies analysed every sample for enantiomeric fractions, with exception of Boogaerts et al. that only analysed the aberrant samples (Boogaerts, Jurgelaitiene, et al., 2021). Here, EF refers to the enantiomeric fraction calculation used in the original study, [a] calculated from supplementary information, [b] estimated from graphical data, and abbreviations: amphetamine (AMP), enantiomeric fraction

(EF), fluoxetine (FLUO), 3,4-Methylenedioxymethamphetamine (MDMA), methamphetamine (METH), and statistical test applied (Stat. test).

Applying enantiomeric profiling requires (i) no other sources contributing to the parent drug, (ii) a known enantiomeric profile of the administered the drug, and (iii) a known excretion enantiomeric profile (Estevez-Danta et al., 2021; Langa et al., 2021; Petrie et al., 2016). If these requirements are not met, enantiomeric profiling might not be appropriate, or assumptions must be made. For example, illicit METH can be synthesised as enantiomerically pure S-(+)-METH or as a racemic mixture depending on the production process applied (Gao et al., 2018; Remberg & Stead, 1999). Upon intake of racemic METH, S-(+)-METH would be enriched in wastewater, whereas consumption and direct disposal cannot be distinguished through consumption of enantiomerically pure S-(+)-METH. In a pan-European study, most locations under investigation leaned towards the enantiopure S-(+)-METH (EF=0.89-1.00). Norway was an exception with an EF of 0.49 ± 0.02, indicating direct disposal racemic METH (Castrignano et al., 2018). METH is also a metabolite of other drugs (e.g., benzphetamine, clobenzorex, selegiline); contribution of these possible other sources to the total load of METH in wastewater makes it difficult to unequivocally state the origin of METH in wastewater. However, the European study highlights geographical differences in illicit drug production and proves the usefulness of enantiomeric profiling to assess this (Castrignano et al., 2018).

With an enantiopure drug formulation, most likely, no discrimination can be made between administration and direct disposal, as the same enantiomer will be excreted and no difference in EF can observed. This is not the situation for all drugs, as sometimes enantiomeric inversion can happen in humans, as is for example the case for some anti-inflammatory drugs (e.g., ibuprofen, ketoprofen, naproxen) (Caballo et al., 2015). Another complication is that the expected enantiomeric ratio can be difficult to determine. Even when pharmacokinetic testing has been done, the pharmacokinetic profile of individual enantiomers is often not available, and wide individual variability exists. This makes it difficult to propose a cut-off to discern direct disposal from administration. Furthermore, the expected EF might not translate well to influent wastewater, e.g., microbial degradation can also be enantioselective. Information should to be gathered regarding the fate of each individual enantiomer in-sewer and in-sample (Evans et al., 2015; Gasser et al., 2012; Vazquez-Roig et al., 2014). An experimental setup to assess stability can be found in *Depaolini et al*. (Depaolini et al., 2016). As with P:M ratios we recommend comparing obtained data with historic data for a first indication of direct disposal.

In conclusion, enantiomeric profiling is a useful analytical approach to assess direct disposal of the parent compound, and in case of illicit drugs also the synthesis route used. Unlike P:M ratios, it can be used even when only the parent compound can be measured in influent wastewater. To apply

enantiomeric profiling, the compound must undergo enantioselective metabolism, or enantiomeric inversion must occur, as the administered enantiomeric fraction must be different from the excreted EF to be able to distinguish both.

3.3 Screening for drug synthesis markers

Three out of 29 studies (10%) investigated the application of liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) to identify markers of chemical waste from the illegal manufacturing of stimulants in influent wastewater (Table 3). These markers of illegal drug synthesis include precursors, intermediates, impurities, and the final product. Within this method, features (i.e., suspect molecules characterized by retention time, *m/z* value, isotopic pattern, and a tentative molecular formula) are screened against different mass libraries, such as *mzCloud*, *mzVault* and *Chemspider* (i.e., non-target screening). Simultaneously, suspect screening was performed against different compiled suspect lists of AMP markers, and followed by the identification and confirmation of features. Although most applications focus on LC-HRMS, limited targeted LC-MS/MS approaches to measure these markers in IWW have been reported (Kasprzyk-Hordern & Baker, 2012a; Vazquez-Roig et al., 2014). A more detailed description of this analytical approach can be found in Reymond et al., Emke et al. and Boogaerts et al (Boogaerts, Jurgelaitiene, et al., 2021; Emke et al., 2018; Reymond et al., 2022).

Table 3. Overview of non-target screening applications for drug synthesis markers included in this review.

Two studies applied a group-based prioritization in which features were attributed to a "dumping" and "consumption" group. This was based on concentrations measured in IWW on the date of a potential dumping event. The included studies were able to discern the presence of specific markers of illegal drug synthesis in IWW in the "dumping" group which could not be identified in the "consumption" group. For this reason, this method cannot only be applied for the confirmation of direct discharge of chemical waste from illegal drug manufacturing, but also for the identification of the actual drug synthesis route used. In other words, the application of this method aims at identifying a specific chemical fingerprint in the wastewater matrix that can be used in a forensic context for policy makers. For example, *Emke et al.* confirmed the presence of benzylmethylketone (BMK) along with BMK-intermediates and impurities in Dutch IWW samples (Emke et al., 2018). BMK is a precursor for the illegal production of AMP through Leuckart reductive amination, yielding racemic AMP.

A major limitation of this group-based prioritization is that the dumping might still take place even though no sudden increase in PNML was observed. For example, due to lower amounts of final product in the disposed chemical waste, or relatively low mass loads of direct dumping compared to consumed mass loads. Additionally, this approach does not consider dispersion and residence times in the

wastewater system, which might lead to the detection of features in the follow-up of the dumping event.

Recently, *Reymond et al.* proposed an alternative approach to prioritize features related to illegal drug synthesis (Reymond et al., 2022). This similarity-based approach assumes that features associated with chemical waste will show similarities with the load of the final product. In other words, it is assumed that not only the final product, but also drug precursors end up in the sewage system during a dumping event. This method assumes that illegal drug waste not only contains by-products of the drug manufacturing process, but also relatively high levels of the final product. This will lead to an increase in both the parent compound and synthesis markers. Although the comparison between group-based prioritization and similarity-based prioritization was only made once, a similarity-based strategy showed better results with, presumably, a higher number of markers of illegal drug production identified. A major advantage compared to group-based prioritization is that this technique accounts for changes in wastewater flow rates and that group splitting is not done based on an arbitrary concentration threshold. However, the untargeted screening of drug synthesis markers in IWW needs to be further optimized and the most appropriate prioritization strategy to be selected. Although this approach is potentially useful for synthetic drugs, its application is not applicable for other substances (e.g., pharmaceuticals) for which consumption, production and disposal are regulated.

Even at this early stage, both approaches show promising results for the detection of illegal waste disposal and can distinguish mass loads originating from a dumping event from those originating from actual drug use. Although suspect and non-target screening of illegal drugs synthesis markers in IWW is more laborious compared to determination of P:M ratios and enantiomeric profiling, it contributes to a better understanding on the extent and impact of illegal drug waste disposal. It can also be applied as an early warning system to detect spatio-temporal changes in drug manufacturing.

4 Future implications and strategies to assess direct disposal of drugs

4.1 Evidence-based framework to assess dumping of drugs

Figure 3 summarizes a general flowchart for the identification of direct disposal of substances in the wastewater system. This framework attributes a combination of selection criteria that result in different confidence levels based on all available analytical evidence provided by the methods described in section 3. We acknowledge that this scoring system might not be applicable in specific situations due to some inherent limitations. However, the main aim of this section is to adopt a simple evidence-based strategy for analytical scientists to assess the origin of suspiciously high PNML. To our knowledge, only Petrie et al. have proposed a workflow for the confirmation of the dumping of drugs in the wastewater system (Petrie et al., 2016). However, that workflow did not include a criterion related to the screening of chemical illegal drug waste.

Figure 3. Framework to assess direct disposal of drugs in the wastewater systems. Points are attributed to each abnormal result (star symbols). Adopted from: (Petrie et al., 2016).

The current review proposes a different identification strategy, based on three confidence levels that could be used in case of a suspected dumping event. Initial reservations regarding the origin may arise from a deviation of historic PNML levels: the suspicious increase in PNML should be flagged as 'suspected event'. This could either be due to a special event (e.g., festival, holiday) resulting in higher consumption, methodological uncertainties with regards to WBE back-calculations (e.g., flow rates, population number) or the direct disposal of drugs in the wastewater system. In each case, the observed increase should be carefully assessed, and these values should be flagged if necessary. It is highly advised to re-analyze the samples, when possible, to make sure that no analytical errors were made. In addition, we recommend the inclusion of replicates to further confirm these elevated drug loads and to exclude errors in sample preparation and data analysis.

We also advise that the analytical approaches in section 3 – P:M ratio, enantiomeric profiling, screening - should be applied in case of a 'flagged' sample to further identify the origin of the increase in PNML. For this purpose, we propose the following scoring system:

- Score = 1: Confirmed by one approach (P:M ratio, enantiomeric profiling, or screening for drug synthesis markers)
- Score = 2: Confirmed by two approaches
- Score = 3: Confirmed by all three approaches

In case of score 1 or 2, confirmation by the remaining approach(es) may not be possible because either (i) the analytical equipment and/or expertise are unavailable, or (ii) the use of the other approaches yields 'normal' and thus conflicting results (i.e., not indicative of disposal).

The application of these different methods is highly compound-specific. For example, screening for synthesis markers will not be relevant for pharmaceuticals that are not produced illegally. It is also possible that none of the approaches are applicable for some compounds and that the abnormal PNML is the only indication for potential dumping. In this case, it is recommended that the PNML in the whole sampling campaign are kept as 'flagged' values and that results are excluded when consumption figures are interpreted.

This framework assumes that each analytical approach contributes equally to the further identification and confirmation of direct disposal of the parent drug. However, it needs to be remarked that some of the abovementioned approaches (e.g., P:M ratios and enantiomeric profiling) are linked to more methodological limitations compared to others, and this aspect is currently not considered with the proposed strategy. Furthermore, dumping of pharmaceuticals by patients, hospitals or pharmacies might be unpredictable and occur at infrequent time intervals during the wastewater sampling campaign. An observed increase in PNML might also be related to the occurrence of a special event in the catchment area (e.g., festival). Information on the presence of such events should also be taken into consideration when applying this framework, as it might be indicative of temporary increased consumption instead of direct disposal of parent compounds in IWW.

Although data triangulation could be applied to further confirm a suspected dumping, this was not included as a confirmation criterium in the current workflow. Obtained data from other data sources may not be robust enough to distinguish between human consumption and dumping of drugs without any other (analytical) confirmation (Figure 1). Differences between WBE data and other data sources may arise from several methodological uncertainties. For example, predicted PNML based on prescription data might deviate from the measured PNML due to potential disposal of pharmaceuticals in the sewer system, but also because not all prescribed pharmaceuticals are used by patients. Additionally, predicted PNML might be lower than measured PNML since not all pharmaceutical use is recorded in sales and prescription data (e.g., illegal trade, imported pharmaceuticals, pharmaceuticals sold and consumed elsewhere, …) (Boogaerts, Ahmed, et al., 2021).

4.2 Further implications of dumping of parent compounds for trend analysis

Even though it is evident that the proposed workflow poses some challenges, more streamlined strategies to assess dumping in influent wastewater are necessary for the correct interpretation of WBE results. At this moment, only a limited number of studies provide analytical evidence for the

occurrence of a dumping event (Table 1-3). In this handful of studies, there is only minimal standardization in the workflow employed to distinguish direct disposal of parent compounds from consumption. For this reason, this study proposes an alternative assessment strategy for the identification and confirmation of the dumping of parent drugs, as presented in Figure 3. This is of importance as the inclusion of increased PNML originating from direct disposal can impact the accuracy of observed spatio-temporal trends in consumption patterns of (il)legal substances based on the WBE approach.

As a cut-off for the exclusion of WBE data for spatio-temporal trend analysis, we propose a score of 1. If this threshold is exceeded, weekly WBE data should be excluded in assessing consumption patterns. We also recommend being cautious with the interpretation of WBE when only limited information is available, for example when none of the abovementioned analytical methods could be applied. Wariness is also required when only an inexplicable high PNML is observed, but none of the approaches are indicative of disposal. We also suggest that a score of 1, that is confirmed by one technique, but contradicted by the others, should be interpreted with caution. In all these cases, it is recommended to keep these PNML as 'flagged' values and to exclude them for assessing consumption. The implementation of this framework could especially be helpful in catchment areas where domestic production of illegal substances has been reported previously.

5 Conclusions

This review shows that multiple approaches are available to assess the direct disposal of drugs in influent wastewater which is of importance in wastewater-based epidemiology. The proposed framework provides a tool to objectively evaluate possible disposal events in a wastewater catchment. However, more research is needed to further refine the different decision criteria and analytical techniques used to identify direct disposal of drugs. More efforts should also be made to investigate if a more statistical approach could be applied to define threshold for abnormal PNML values. At this instance, WBE researchers should judge themselves if the PNML is normal or indicative of dumping based on historical data, which might be regarded as an arbitrary approach. However, the proposed framework could be employed as an early guideline and standardization for future WBE research, even if further refinement of the model is required by others in the field.

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8 Tables

741 Table 1

Compound	Country	Sampled year	Investigated P:M ratio	Baseline/expected P:M ratio (no dumping)	Obtained P:M ratio (possible dumping)	Stat. Test	Note	Reference
AMP, METH	China	2012	METH:AMP	6 (mean, n=30) [b]	250 (n=1) [b]	No		(Khan et al., 2014)
AMP, METH	China	2012-2014	AMP:METH	<0.1	0.13-1.74 (n=5)	No		(Du et al., 2015)
AMP, METH	China	2014-2015	AMP:METH	0.055-0.070	0.017 (n=1)	No		(Xu et al., 2017)
COC	United Kingdom	2007	COC:BE	0.2	0.2-0.8 (n=5)	No		(Kasprzyk-Hordern et al., 2009)
COC	France	2008	COC:BE	0.2	0.32 ± 1.10 (mean, n=18)	No		(Karolak et al., 2010)
COC	The Netherlands	2010	COC:BE	<0.75 (van Nuijs et al., 2009) <0.27 (Postigo et al., 2010)	WWTP A: 0.85 (n=1) WWTP B: 2.20 (n=1)	No		(Bijlsma et al., 2012)
COC	China	2011	COC:BE	0.6 (mean, n=45) [b]	1.05, 1.05 and 1.52 (n=3)	No		(Lai et al., 2013)
COC	Europe-wide	2011	COC:BE	<0.1	0.1-0.7 (n=21)	No	Samples obtained in 21 WWTPs across Europe	(Castiglioni et al., 2013)
COC	Canada	2012	COC:BE	<0.50 (Castiglioni et al., 2006; Gheorghe et al., 2008; Metcalfe et al., 2010; Postigo et al., 2010; Zuccato et al., 2005)	0.49-0.52 (n=10)	No	Samples were collected 24-h, grab and by a POCIS sampler.	(Rodayan et al., 2014)
COC	Greece	2015	COC:BE	N.m.	1.46 (n=1)	No		(Gatidou et al., 2016)
COC	New Zealand	2014	COC:BE	N.m.	N.m.	No	Ratios could not be calculated, COC detected but BE below limit of detection.	(Lai et al., 2017)

COC	South Africa	2017	COC:BE	<0.1 (Castiglioni et al., 2011) <0.75 (van Nuijs et al., 2009)	WWTP A: 0.2-0.6 (range), median 0.3 (n=7) WWTP B: 0.3-0.5 (range), median 0.4 (n=7)	No	Investigators suggest potential co-administration with alcohol, lowering metabolism and leading to cocaine enrichment	(Archer et al., 2018)
COC	United Kingdom	2015	COC:BE	<0.75 (van Nuijs et al., 2009) <0.27 (Postigo et al., 2010)	Campaign 2015: 0.93 ± 1.10 (2 days, n=5) [a]	No	Grab samples collected from portable urinals (i.e., male population) at music festival. Limitation since potentially not representative as BE/COC may have different excretion rate/time.	(Bijlsma et al., 2020)
COC	Turkey	2019	COC:BE	<0.75 (van Nuijs et al., 2009)	0.76-25.83 (mean, n=147)	No		(Kuloglu Genc et al., 2021)
COC	United States	2020	COC:BE	0.27-0.75 (Bijlsma et al., 2012)	0.79-1.84 (n=10)	No		(Montgomery et al., 2021)
FLUO	United Kingdom	2014	FLUO:NORFLUO	WWTP: 0.3-1.9 (predicted based on prescription data)	WWTP, day 1: 2.6 (mean, hourly samples, n=17) WWTP, day 2: 8.3 (mean, hourly samples, n=17)	No	Samples were collected on an hourly basis (between 8:00 and 24:00)	(Petrie et al., 2016)
METH	Australia, New Zealand	2019-2020	METH:PHO	WWTP B: 12.83±4.48 (mean, n=52) [a] WWTP C: 16.89±3.87 (mean, n=54) [a] WWTP D: 19.27±5.64 (mean, n=55) [a] WWTP E: 21.45±2.43 (mean, n=29) [a]	WWTP B: 28.32 (n=1) [a] WWTP C: 30.08, 33.85 (n=2) [a] WWTP D: 53.27 (n=1) [a] WWTP E: 178.90 (n=1) [a]	No		(Bade et al., 2021)
MTD	China	2017	EDDP:MTD	1.07 ± 0.18 (n=10)	0.64 ± 0.23 (mean, n=12)	Yes	An independent sample t-test was used	(Zhang et al., 2019)
MTD	Greece	2020	EDDP:MTD	2 (Du et al., 2019)	WWTP, 2019: 1 (mean, n=7) WWTP, 2020: 1.5 (mean, n=15)	No	Samples 2020 obtained in full lockdown period (COVID-19 restrictions)	(Alygizakis et al., 2021)
NIC	United States	2015-2015	NIC:COT	0.6 (Zheng et al., 2017)	0.6-9.2 (n=33)	No		(Chen et al., 2019)

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743 Table 2

Compound	Country	EF	Expected enantiomer enrichment in WW	Baseline/expected EF (no dumping)	Obtained EF (possible dumping)	Stat. test	Note	Reference
AMP	The Netherlands	$\frac{(+)}{(+)+(-)}$	R-(−)-AMP	0.64 (literature)	WWTP1, 2010: 0.54 ± 0.02 (n=7) WWTP1, 2011: 0.53 ± 0.02 (n=7) WWTP2, 2010: 0.52 ± 0.01 (n=7) WWTP2, 2011: 0.52 ± 0.02 (n=7)	No	Investigators consider presence of racemic AMP due to (1) direct disposal of unused racemic amphetamine and/or (2) illicit use of racemic AMP and enantiopure S-(+)-AMP	(Emke et al., 2014)
AMP	Lithuania	$\frac{(+)}{(+)+(-)}$	R-(−)-AMP	< 0.5 (literature)	0.45-0.55 (n=2) [b]	No	2 different days, 1 WWTP No baseline EF could be determined as concentration was <LLOQ of enantiomeric analysis method on non-dumping days (personal communication)	(Boogaerts, Jurgelaitiene, et al., 2021)
Atenolol	Spain	$\frac{(+)}{(+)+(-)}$	S-(−)-Atenolol	Different WWTP1: 0.46 ± 0.03 (same period, n=15) Different WWTP2: 0.37 ± 0.03 (same period, n=15)	0.5 ± 0.02 (mean, n=15) [a]	No	15 different days, 1 WWTP	(Vazquez-Roig et al., 2014)
FLUO	United Kingdom	$\frac{(+)}{(+)+(-)}$	S-(+)-FLUO	0.56 - 0.68 (n = 4)	0.48 - 0.51 (n=3)	No	3 different days, 1 WWTP	(Petrie et al., 2016)
MDMA	The Netherlands	$\frac{(-)}{(-)+(+)}$	R-(−)-MDMA	Same WWTP: 0.68 ± 0.04 (previous year, n=7) Different WWTP: 0.69 ± 0.03 (same year, n=7)	0.51 - 0.57 (n=7)	No	7 different days, 1 WWTP	(Emke et al., 2014)

MDMA	United Kingdom	$\frac{(-)}{(-)+(+)}$	R-(−)-MDMA	0.68 (mean, n=35 in duplicate)	0.5 - 0.53 (n=3) [b]	No	3 different days, 3 different WWTP	(Kasprzyk-Hordern & Baker, 2012a)
METH	Norway	$\frac{(+)}{(+)+(-)}$	S-(+)-METH	0.89 - 1 (range, different WWTPs in Europe)	0.49 ± 0.02 (n=7)	No	7 different days, 1 WWTP	(Castrignano et al., 2018)
METH	Australia	$\frac{(+)}{(+)+(-)}$	S-(+)-METH	0.85 - 1 (n=146) [b]	Urban WWTP: 0.49 (n=1) Regional WWTP: 0.54 (n=1)	No	Urban WWTP: potential dumping of R-(−)-METH Regional WWTP: potential dumping of R-(−)-METH and/or racemic METH Additional confirmation through AMP/METH ratio	(Gao et al., 2018)
Salbutamol	Italy	$\frac{(+)}{(+)+(-)}$	S-Salbutamol	0.452 ± 0.018 (mean, n=46)	0.484 ± 0.014 (n=10)	Yes	10 different days, 1 WWTP One-way ANOVA test applied between aberrant concentrations and baseline, and between aberrant concentrations and pharmaceutical preparation was not significant.	(Depaolini et al., 2016)

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745 Table 3. Overview of non-target screening applications for drug synthesis markers included in this review.

Product	Country	Sampling year	Screening method	Identified features	Prioritization	Reference
AMP	Netherlands	2016, 2017	Non-target + suspect screening	1-Phenyl-2-propanone oxime 1-Naphthalenemethylamine N-Formylamphetamine Amphetamine 2-Phenylacetamide APAA (Alpha-PhenylAcetoAcetamide) Keto a-Benzylphenethylamine(dibenzylmethylamine) 3-oxo-N-phenylbutanamide 4-Benzylpyrimidine 5-Fenyl-4-methylpyrimidine Di-(b-phenylisopropyl)amine BMK (BenzylMethylKetone) APAA (Alpha-PhenylAcetoAcetamide)b Enol APAAN (Alpha-PhenylAcetoAcetoNitrile) 4,6-Dimethyl-3,5-diphenylpyridin-2-one 2,3-Diacetyl-2,3-diphenylsuccinonitrile	Not applicable	(Emke et al., 2018)
AMP	Lithuania	2018	Non-target + suspect screening	Amphetamine N-ethylamphetamine N-formylamphetamine (formetorex) N-formylmethamphetamine 4-Benzylpyrimidine N,N-di-(b-phenylisopropyl)amine 1-oxo-1-phenyl-2-(β-phenylisopropylimino)propane N,N-di-(b-phenylisopropyl)formamide	Group-based prioritization	(Boogaerts, Jurgelaitiene, et al., 2021)
AMP **MDMA**	Netherlands	2016-2018	Non-target + suspect screening	4'-(imidazole-1-yl)acetophenone (2Z)-2-acetamido-3-(4-methoxyphenyl)acrylic acid Monoisopropylphosphorylserine MDEA (2Z)-2-acetamido-3-(4-methoxyphenyl)acrylic acid PMK Safrole 1-ethyl-2,3,4,9-tetrahydro-1H-beta-carboline-3-carboxylic acid N-cyclohexylacetamide	Group-based prioritization + similarity-based prioritization	(Reymond et al., 2022)

	Hopantenic acid
	2-butylnorleucine

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