Wastewater-Based Epidemiology for the assessment of population exposure to chemicals: the need for integration with Human Biomonitoring for global One Health actions

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Abstract

WBE has now become a complimentary tool in SARS-CoV-2 surveillance. This was preceded by the established application of WBE to assess the consumption of illicit drugs in communities. It is now timely to build on this and take the opportunity to expand WBE to enable comprehensive assessment of community exposure to chemical stressors and their mixtures. The goal of WBE is to quantify community exposure, discover exposure-outcome associations, and trigger policy, technological or societal intervention strategies with the overarching aim of exposure prevention and public health promotion. To achieve WBE's full potential, the following key aspects require further action: (1) Integration of WBE-HBM (human biomonitoring) initiatives that provide comprehensive community-individual multichemical exposure assessment. (2) Global WBE monitoring campaigns to provide much needed data on exposure in low- and middle-income countries (LMICs) and fill in the gaps in knowledge especially in the underrepresented highly urbanised as well as rural settings in LMICs. (3) Combining WBE with One Health actions to enable effective interventions. (4) Advancements in new analytical tools and methodologies for WBE progression to enable biomarker selection for exposure studies, and to provide sensitive and selective multiresidue analysis for trace multi-biomarker quantification in a complex wastewater matrix. Most of all, further developments of WBE needs to be undertaken by co-design with key stakeholder groups: government organisations, health authorities and private sector.

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1. Introduction

Wastewater-based epidemiology (WBE) has recently facilitated global SARS-CoV-2 surveillance (Bivins, North et al. 2020, Lundy, Fatta-Kassinos et al. 2021). This was preceded by the already established, harmonized, and coordinated application of WBE to evaluate illicit drug use in communities by the SCORE group (Gonzalez-Marino, Baz-Lomba et al. 2020) as well as large scoping initiatives including wider group of lifestyle chemicals (Lopez-Garcia, Perez-Lopez et al. 2020, Montes, Rodil et al. 2020). There is now a timely opportunity to build on this infrastructure and these two successful applications, and further expand WBE to assess public exposure to chemical stressors. While a strong potential of WBE utilisation as an epidemiology tool for public health assessment in the context of communicable disease (pathogens) has been discussed in recent reviews and perspective papers (Sims and Kasprzyk-Hordern 2020, Tiwari, Adhikari et al. 2023), there is little published in the context of WBE application to estimate public exposure to chemicals (Daughton 2018, Gracia-Lor, Rousis et al. 2018, Pico and Barcelo 2021).

2. WBE – the concept

Wastewater typically contains a variety of biological and chemical entities including a wide range of human excretion products of endogenous and exogenous origin resulting from exposure to xenobiotics (e.g., illicit drugs, pharmaceuticals, food or environmental toxicants, chemicals in personal care products), infectious agents (e.g. RNA and DNA markers) and internal processes (e.g., specific disease or exposure-linked proteins, genes, and metabolites). WBE postulates that the measurement of these specific biomarkers pooled by the sewerage system can provide valuable evidence of the quantity and type of xenobiotic chemical, biological or physical agents to which the served population was exposed. Therefore, WBE can provide anonymised, comprehensive, and objective information on the exposure status of urban dwellers and the receiving surrounding environments in (near) real-time.

For biomarkers that are stable in urban wastewater and efficiently conveyed to the wastewater treatment plant (WWTP), the collective amount excreted by humans in each time interval is reflected in the mass load reaching the WWTP. To obtain representative samples and to maintain low uncertainty of measurements, 24h-composite samples are collected and monitored over extended periods of time (weeks, months, years). Concentrations of biomarkers in wastewater obtained with sensitive and selective *state-of-the-art* analytical techniques (e.g., chromatography coupled with mass spectrometry, polymerase chain reaction) are used to back-calculate to mass loads (amount/day) or gene loads, after considering wastewater flow rate. Chemical mass loads can then be used to estimate public exposure to chemicals individually, or as a mixture, and inform spatial and temporal trends (e.g., demography, season, location or incident-driven increase in levels of certain chemicals), as well as monitor their prevalence in the surveyed catchment. Similarly, gene loads can be used to estimate prevalence of infection in a community as well as to identify emerging trends. Furthermore, by dividing daily exposure loads by the size of the population served by the WWTP, results can be normalized to population (mg/day/1000 inhabitants), allowing an estimated per capita intake calculation (after considering human metabolism) as well as external exposure to these stressors.

3. Assessing public exposure to chemical stressors – the need for a new integrated WBE – HBM approach

Rapid assessment of public exposure to chemical stressors is required for the prevention, control, or mitigation of risks. There is growing evidence of cause-effect association between man-made chemicals present in industrial and household products, often leaching into the environment, and the adverse public health outcomes. The effects of exposure to endocrine-disrupting chemicals include neurodevelopmental changes associated with language delay in mother-child and pregnancy cohorts (Caporale, Leemans et al. 2022), poor air quality linked with higher prevalence of asthma, and other diseases in urban populations such as diabetes, infertility and hormone-sensitive cancers in women and men (Yilmaz, Terekeci et al. 2020) (Kortenkamp, Scholze et al. 2022). Strategies to control and regulate chemicals are limited due to gaps in characterisation of exposure resulting from limited risk assessment methods. New approaches are needed to identify cause-effect linkages between the environment and human health. Studies on environmental stressor levels and associated health effects are currently facilitated using HBM (Fuller, Landrigan et al. 2022). However, a typical limitation of HBM, due to logistical difficulties, is the limited size of study groups, only spot (often singular) sampling regime (although pooled urine is also utilised), as well as the requirement of substantial resources and the time required to analyse samples from many individuals. Therefore, there is a need for an evidence-based exposure diagnostics and risk prediction system, which will collate long-term comprehensive, spatiotemporal datasets on multi-chemical, aggregate exposure status, and trigger rapid response from regulatory and public health sectors with the aim of preventing disease and promoting public health.

This tool, if operated in (near) real-time and if linked with timely response systems, could allow exposure threats to be rapidly identified, at low cost, and instantly dealt with, reducing the global burden on public health. Similarly, the effectiveness of measures introduced to reduce emissions and exposure to these chemicals can easily be evaluated. WBE has the potential to provide a significant contribution to epidemiological research by becoming a new tool in the current epidemiology toolkit. It can provide anonymous but comprehensive data on multi-chemical exposure by millions of urban dwellers (Gracia-Lor, Rousis et al. 2018), (Gracia-Lor, Castiglioni et al. 2017). Its limitations are concomitant with the inability to identify individuals or unravel linkages between exposure and health outcome within various demographic and societal groups. It is also focussed on urinary/faecal biomarkers, not dealing with other matrices of HBM. Another limitation is that many developing countries do not have wastewater infrastructure and so it is difficult to estimate the per capita exposure and intake calculations. Other sources of exposure biomarkers (metabolites) also need to be considered. Hence, we promote WBE's integration with ongoing human biomonitoring campaigns and current public health surveillance systems. The latter can be only facilitated by engagement with governments, public health agencies, environmental agencies and regulators, and water utilities. One future opportunity lies within large interdisciplinary projects such as the new European Initiative PARC (European Partnership for the Assessment of Risks from Chemicals) that will facilitate close cooperation between WBE specialists and HBM experts.

4. Future work – the need to fill knowledge gaps on chemical exposure globally

The WBE concept is relatively simple and results can be rapidly obtained as demonstrated by the global application of SARS-CoV-2 surveillance and smaller scale applications focussed on assessing chemical exposure in Europe and Australia: plastic additives (Tang, He et al. 2020, Tang, He et al. 2020, Gonzalez-Marino, Ares et al. 2021, Kasprzyk-Hordern, Proctor et al. 2021, Kumar, Adhikari et al. 2022), flame retardants (O'Brien, Thai et al. 2015, Been, Bastiaensen et al. 2018), pesticides (Rousis, Gracia-Lor et al. 2017) (Campos-Mañas, Fabregat-Safont et al. 2022) and mycotoxins (Gracia-Lor, Zuccato et al. 2020) (Table 1). Several factors influencing WBE reliability and applicability need to be understood to obtain quantitative measurements. These are primarily: selection of specific human metabolic biomarkers, characteristics of urban water cycle including sewer systems (population size and mobility in a WWTP catchment, flow rate), and understanding the fate of biomarkers (stability, biotransformation, sorption). Novel screening approaches for human biomonitoring of a multitude of contaminant metabolites in urine based on high-throughput *in vitro* incubation, suspect and non-target Liquid Chromatography and High Resolution Mass Spectrometry (LC-HRMS) screening involving *in silico* deconjugation and automated data evaluation (Huber, Müller et al. 2021, Huber, Krauss et al. 2022) create new opportunities to discover new relevant biomarkers providing insights to so far unknown exposures.

Table 1. Examples of chemical exposures investigated by WBE in recent studies

Biomarkers investigated	**Area of Study**	**Reference**
Pesticides -pyrethroids -triazines and organophosphates	 Europe (8 countries, 5 M inhabitants) Norway (4 cities, 0.9 M inhabitants) Europe (4 countries, 1.6M inhabitants)	 (Rousis, Gracia-Lor et al. 2017) (Rousis, Gracia-Lor et al. 2020) (Rousis, Gracia-Lor et al. 2021)
Flame retardants	Europe (4 countries) Australia	(Been, Bastiaensen et al. 2018) (O'Brien, Thai et al. 2015)
Bisphenols	UK (5 cities, >1.2M inhabitants) Australia	(Lopardo, Petrie et al. 2019, Kasprzyk-Hordern, Proctor et al. 2021) (Tang, He et al. 2020)
Mycotoxins	Europe (2 countries, 1.8 M inhabitants) Latvia	(Gracia-Lor, Zuccato et al. 2020) (Berzina, Pavlenko et al. 2022)
Antibiotics and ARGs (Antibiotic Resistance Genes)	Europe (7 countries, >4M inhabitants)	(Castrignano, Yang et al. 2020)
Phthalate plasticizers	Spain (13 cities) Australia	(Gonzalez-Marino, Ares et al. 2021) (Tang, He et al. 2020)

WBE clearly has the potential to comprehensively assess chemical exposure at the community level since urban wastewater can be considered as a diagnostic matrix for the exposure status of a sewer shed. It provides excellent opportunities to develop a wide range of innovative solutions to rapidly and quantitatively assess patterns and factors related to chemical exposure within populations, while also providing a means of collecting complementary data for epidemiological and socio-economic studies to undertake comprehensive evaluations of the implications of the chemical exposure to public health. To achieve its full potential, two key developments need to be achieved:

1. Integrated WBE-HBM initiatives that provide comprehensive community-individual multichemical exposure assessment. The benefit is twofold: HBM informed biomarker selection, and evaluation of the potential of community WBE monitoring vs individual testing to establish a tiered approach towards large-scale exposure studies.
2. Global WBE monitoring campaigns. WBE, as a comprehensive and inclusive tool, has a unique opportunity and potential to provide much needed data on exposure in LMICs and fill knowledge gaps in underrepresented highly urbanised as well as rural settings in LMICs (Kasprzyk-Hordern, Adams et al. 2022). WBE is indeed well-suited for LMICs where often socio-economic impacts are much harsher (Shrestha et al. 2021). It provides a more cost-effective alternative for mass surveillance and reduces the economic and manpower burden of clinical testing especially during pandemics when resources are strained, and testing capacity is overwhelmed (Aarestrup and Woolhouse 2020). It can also provide a useful measure of the integration of modern medicine and traditional remedies. There are concerns about the ability to implement WBE in LMICs where centralised wastewater management systems are non-existent, inadequate or of low quality. However, with careful planning, representative samples for WBE can be collected from other sewage collection sites such as pit latrines and septic tanks. In addition, untreated sewage wastewater can also be collected from strategically designated locations (Fallati, Castiglioni et al. 2020). Admittedly, sampling then becomes more labour-intensive and there are possible ethical ramifications, but randomised sampling of these sites can overcome some of these limitations.

5. Exploring WBE as an enabler of One Health - key research needs

One Health is a cross sectoral and multidisciplinary effort aimed at understanding and management of public and environmental health. Embedding WBE in One Health ethos has wide-ranging benefits, especially in the context of chemical exposure studies (Kasprzyk-Hordern, Proctor et al. 2022). WBE provides a unique opportunity to enable research within the One Health domain: (i) holistic evaluation of public and environmental health status, (ii) informing One Health actions (i.e., policy, technological or social interventions), and (iii) assessment of the effectiveness of mitigation strategies. Combining WBE with One Health actions can provide multiple benefits: from cost savings to more targeted and effective interventions leading to decreased environmental and public health burdens from hazardous chemicals. The One Health model incorporates a dynamic set of bio-physicochemical and socioeconomic indicators that are difficult to unravel. WBE is ideally set to provide high-resolution spatiotemporal evidence to support the One Health agenda. One successful application of One Health is its adoption in the antimicrobial resistance (AMR) challenge as it is multifaceted with human and animal health impacts, as well as food security and safety. The first AMR-WBE applications have been recently published (Hendriksen, Munk et al. 2019, Castrignanò, Yang et al. 2020). However, much more effort is required to allow integration of the One Health tool as well as intersectoral cooperation between environmental and public health, private and public sectors. One key development includes incorporating testing the wider receiving environment in a catchment area rather than wastewater itself. This is important to account for other than human sources, e.g. runoff from agricultural sites, animal farms.

One of the key benefits of WBE in the context of One Health is an opportunity for interrogation of WBE data alongside other socioeconomic and sociodemographic indices, to for example, understand influences and disparities in chemical consumption in communities (Choi, Tscharke et al. 2019). Further research is required to develop mathematical models allowing for data triangulation. Furthermore, the scientific assessment and control of risk posed by a specific hazard using WBE is made possible with the use of Internet of things (IoT) technologies. The outcome of this will help the government and policy makers in the management processes of pollution monitoring, environmental data analysis, decision making and risk control.

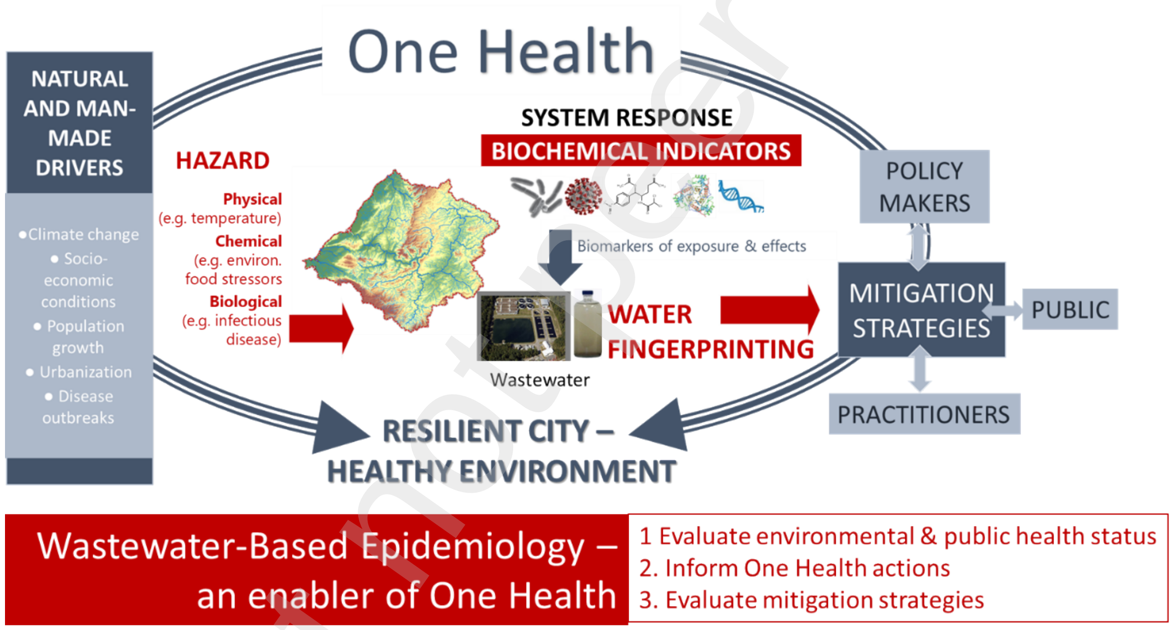

Figure 1. WBE as an enabler of One Health

6. A critical need for new analytical approaches for comprehensive water profiling

Sensitive and selective methods that allow for trace analysis of multiple biomarker targets are critical to the success of WBE. Chromatography and mass spectrometry are techniques of choice to deliver on both selectivity and sensitivity of measurement as well as simultaneous analysis of multiple chemical targets. While targeted analysis with triple quadrupole mass analysers delivers the best sensitivity and fully quantitative measurements, high-resolution mass spectrometry (HRMS) unlocks new opportunities to detect, monitor and potentially identify new biomarkers by investigation of chemically related compounds (Hernandez, Castiglioni et al. 2016).

Current and future developments are focussed on machine learning *in silico* prediction tools and (semi-) quantitative analysis taking advantage of the technical and creative improvements in non-target screening workflows and strategies (Bijlsma, Berntssen et al. 2019, Aalizadeh, Nikolopoulou et al. 2022).

The ultimate goal for future WBE analytics is the development of autonomous sampling and sensing devices (Kasprzyk-Hordern, Adams et al. 2022) for real-time output delivery. Their utility is critical in early warning and for sensing in remote locations. Existing electrochemical and biosensing platforms developed for healthcare applications struggle with complex matrix, lack of required selectivity and sensitivity that mass spectrometry delivers, but it is expected that future advances in sample preparation modules enriching the signal and simplifying the matrix will provide required boost for much needed expansion of WBE sensing capability (Yang, Castrignanò et al. 2016).

7. WBE frameworks need to be developed by co-design

WBE innovation is intrinsically interdisciplinary and cross-sectoral. Its unique position at the research-policy interface provides opportunities for rapid knowledge translation. As an example, WBE has recently been recognised by the EU in their recent Proposal for a Directive of the European Parliament and of the Council concerning urban wastewater treatment COM(2022) 541, Article 17 where several public health parameters are recommended for monitoring in wastewater: viruses, pathogens, contaminants of emerging concern and any other public health parameters that are considered relevant. Future applications must therefore include the development of WBE systems for chemical exposure and associated risks, both in terms of cutting-edge research, training and implementation via co-design with key stakeholders (governments, private sector and the public) to support (inter)national legislation and to provide surveillance systems for public health protection.

8. Responsible innovation

WBE, due to its nature, can provide extended, comprehensive datasets of potential importance to various sectors. Data management, also in the context of FAIR (Findability, Accessibility, Interoperability, and Reusability) is in need of systematisation while WBE develops to maximise its future impact.

WBE provides information on communities, hence, it does not identify individuals, but, as WBE progresses, and sub-catchment, near source applications are being considered, ethical guidelines need to be developed and implemented to protect vulnerable groups from stigmatisation.

Conclusions

Wastewater-based epidemiology (WBE) has enabled global SARS-CoV-2 surveillance as well as community-wide illicit drug use assessment. Newly established infrastructure provides the opportunity for the expansion of WBE to enable comprehensive assessment of public exposure to chemical stressors and their mixtures. To achieve its full potential, the following key aspects require further action:

1. Integrated WBE-HBM initiatives that provide comprehensive community-individual multichemical exposure assessment. The benefit is twofold: HBM informed biomarker selection, evaluation of the potential of community WBE monitoring vs individual testing to establish a tiered approach towards large-scale exposure studies.
2. Global WBE monitoring campaigns. WBE, as a comprehensive and inclusive tool, has a unique opportunity and potential to provide much needed data on exposure in LMICs and fill in the gaps in knowledge especially in the underrepresented highly urbanised as well as rural settings in LMICs.
3. Combining WBE with One Health actions can provide multiple benefits: from cost savings to more targeted and effective interventions leading to decreased environmental and public health burdens from hazardous chemicals.
4. Advancements in new mass spectrometry-based analytical tools and methodologies are required for WBE progression to: (1) enable biomarker selection for exposure studies, and (2) provide sensitive and selective multiresidue analysis for trace multi-biomarker quantification in a complex wastewater matrix.
5. Advances in sample preparation modules enriching the signal and simplifying the matrix are critically needed to provide required boost for much needed expansion of WBE sensing capability and repurposing of sensor technology developed for healthcare applications.
6. The development of WBE systems for chemical exposure and associated risks, both in terms of cutting-edge research, training and implementation needs to progress via co-design with key stakeholders (governments, private sector and the public) to support (inter)national legislation and to provide fit-for-purpose surveillance systems for public health protection.
7. WBE data management is in need of systematisation while WBE develops to maximise its future impact.

Acknowledgments

The authors would like to acknowledge support from the EU Interwaste project 'Synergising International Research Studies into the Environmental Fate and Behaviour of Toxic Organic Chemicals in the Waste Stream' (Grant agreement ID: 734522). L. Bijlsma acknowledges that the project that gave rise to these results received the support of a fellowship from "la Caixa" Foundation (ID 10 0 010434). The fellowship code is LCF/BQ/PR21/11840012. He also acknowledges grant RYC2020-028936-I funded by MCIN/AEI/ 10.13039/501100011033 and by "ESF Investing in your future". Support from Engineering and Physical Sciences research Council (EP/V028499/1) is also appreciated.

References

Aalizadeh, R., V. Nikolopoulou, N. Alygizakis, J. Slobodnik and N. S. Thomaidis (2022). "A novel workflow for semi-quantification of emerging contaminants in environmental samples analyzed by LC-HRMS." Anal Bioanal Chem.

Aarestrup, F. M. and M. E. J. Woolhouse (2020). "Using sewage for surveillance of antimicrobial resistance." Science **367**(6478): 630-632.

Been, F., M. Bastiaensen, F. Y. Lai, K. Libousi, N. S. Thomaidis, L. Benaglia, P. Esseiva, O. Delémont, A. L. N. van Nuijs and A. Covaci (2018). "Mining the Chemical Information on Urban Wastewater: Monitoring Human Exposure to Phosphorus Flame Retardants and Plasticizers." Environ Sci Technol **52**(12): 6996-7005.

Berzina, Z., R. Pavlenko, M. Jansons, E. Bartkiene, R. Neilands, I. Pugajeva and V. Bartkevics (2022). "Application of Wastewater-Based Epidemiology for Tracking Human Exposure to Deoxynivalenol and Enniatins." Toxins (Basel) **14**(2).

Bijlsma, L., M. H. G. Berntssen and S. Merel (2019). "A Refined Nontarget Workflow for the Investigation of Metabolites through the Prioritization by in Silico Prediction Tools." Anal Chem **91**(9): 6321-6328.

Bivins, A., D. North, A. Ahmad, W. Ahmed, E. Alm, F. Been, P. Bhattacharya, L. Bijlsma, A. B. Boehm, J. Brown, G. Buttiglieri, V. Calabro, A. Carducci, S. Castiglioni, Z. C. Gurol, S. Chakraborty, F. Costa, S. Curcio, F. L. de los Reyes, J. D. Vela, K. Farkas, X. Fernandez-Casi, C. Gerba, D. Gerrity, R. Girones, R. Gonzalez, E. Haramoto, A. Harris, P. A. Holden, M. T. Islam, D. L. Jones, B. Kasprzyk-Hordern, M. Kitajima, N. Kotlarz, M. Kumar, K. Kuroda, G. La Rosa, F. Malpei, M. Mautus, S. L. McLellan, G. Medema, J. S. Meschke, J. Mueller, R. J. Newton, D. Nilsson, R. T. Noble, A. van Nuijs, J. Peccia, T. A. Perkins, A. J. Pickering, J. Rose, G. Sanchez, A. Smith, L. Stadler, C. Stauber, K. Thomas, T. van der Voorn, K. Wigginton, K. Zhu and K. Bibby (2020). "Wastewater-Based Epidemiology: Global Collaborative to Maximize Contributions in the Fight Against COVID-19." Environmental Science & Technology **54**(13): 7754-7757.

Campos-Mañas, M., D. Fabregat-Safont, F. Hernández, E. de Rijke, P. de Voogt, A. van Wezel and L. Bijlsma (2022). "Analytical research of pesticide biomarkers in wastewater with application to study spatial differences in human exposure." Chemosphere **307**(Pt 1): 135684.

Caporale, N., M. Leemans, L. Birgersson, P.-L. Germain, C. Cheroni, G. Borbély, E. Engdahl, C. Lindh, R. B. Bressan, F. Cavallo, N. E. Chorev, G. A. D'Agostino, S. M. Pollard, M. T. Rigoli, E. Tenderini, A. L. Tobon, S. Trattaro, F. Troglio, M. Zanella, Å. Bergman, P. Damdimopoulou, M. Jönsson, W. Kiess, E. Kitraki, H. Kiviranta, E. Nånberg, M. Öberg, P. Rantakokko, C. Rudén, O. Söder, C.-G. Bornehag, B. Demeneix, J.-B. Fini, C. Gennings, J. Rüegg, J. Sturve and G. Testa (2022). "From cohorts to molecules: Adverse impacts of endocrine disrupting mixtures." Science **375**(6582): eabe8244.

Castrignano, E., Z. E. Yang, E. J. Feil, R. Bade, S. Castiglioni, A. Causanilles, E. Gracia-Lor, F. Hernandez, B. G. Plosz, P. Ramin, N. I. Rousis, Y. Ryu, K. V. Thomas, P. de Voogt, E. Zuccato and B. Kasprzyk-Hordern (2020). "Enantiomeric profiling of quinolones and quinolones resistance gene qnrS in European wastewaters." Water Research **175**.

Castrignanò, E., Z. Yang, E. J. Feil, R. Bade, S. Castiglioni, A. Causanilles, E. Gracia-Lor, F. Hernandez, B. G. Plósz, P. Ramin, N. I. Rousis, Y. Ryu, K. V. Thomas, P. de Voogt, E. Zuccato and B. Kasprzyk-Hordern (2020). "Enantiomeric profiling of quinolones and quinolones resistance gene qnrS in European wastewaters." Water Res **175**: 115653.

Choi, P. M., B. Tscharke, S. Samanipour, W. D. Hall, C. E. Gartner, J. F. Mueller, K. V. Thomas and J. W. O'Brien (2019). "Social, demographic, and economic correlates of food and chemical consumption measured by wastewater-based epidemiology." Proceedings of the National Academy of Sciences of the United States of America **116**(43): 21864-21873.

Daughton, C. G. (2018). "Monitoring wastewater for assessing community health: Sewage Chemical-Information Mining (SCIM)." Sci Total Environ **619-620**: 748-764.

Fallati, L., S. Castiglioni, P. Galli, F. Riva, E. Gracia-Lor, I. González-Mariño, N. I. Rousis, M. Shifah, M. C. Messa, M. G. Strepparava, M. Vai and E. Zuccato (2020). "Use of legal and illegal substances in Malé (Republic of Maldives) assessed by wastewater analysis." Sci Total Environ **698**: 134207.

Fuller, R., P. J. Landrigan, K. Balakrishnan, G. Bathan, S. Bose-O'Reilly, M. Brauer, J. Caravanos, T. Chiles, A. Cohen, L. Corra, M. Cropper, G. Ferraro, J. Hanna, D. Hanrahan, H. Hu, D. Hunter, G. Janata, R. Kupka, B. Lanphear, M. Lichtveld, K. Martin, A. Mustapha, E. Sanchez-Triana, K. Sandilya, L. Schaefli, J. Shaw, J. Seddon, W. Suk, M. M. Téllez-Rojo and C. Yan (2022). "Pollution and health: a progress update." Lancet Planet Health.

Gonzalez-Marino, I., L. Ares, R. Montes, R. Rodil, R. Cela, E. Lopez-Garcia, C. Postigo, M. L. de Alda, E. Pocurull, R. M. Marce, L. Bijlsma, F. Hernandez, Y. Pico, V. Andreu, A. Rico, Y. Valcarcel, M. Miro, N. Etxebarria and J. B. Quintana (2021). "Assessing population exposure to phthalate plasticizers in thirteen Spanish cities through the analysis of wastewater." Journal of Hazardous Materials **401**.

Gonzalez-Marino, I., J. A. Baz-Lomba, N. A. Alygizakis, M. J. Andres-Costa, R. Bade, A. Bannwarth, L. P. Barron, F. Been, L. Benaglia, J. D. Berset, L. Bijlsma, I. Bodik, A. Brenner, A. L. Brock, D. A. Burgard, E. Castrignano, A. Celma, C. E. Christophoridis, A. Covaci, O. Delemont, P. de Voogt, D. A. Devault, M. J. Dias, E. Emke, P. Esseiva, D. Fatta-Kassinos, G. Fedorova, K. Fytianos, C. Gerber, R. Grabic, E. Gracia-Lor, S. Gruner, T. Gunnar, E. Hapeshi, E. Heath, B. Helm, F. Hernandez, A. Kankaanpaa, S. Karolak, B. Kasprzyk-Hordern, I. Krizman-Matasic, F. Y. Lai, W. Lechowicz, A. Lopes, M. L. de Alda, E. Lopez-Garcia, A. S. C. Love, N. Mastroianni, G. L. McEneff, R. Montes, K. Munro, T. Nefau, H. Oberacher, J. W. O'Brien, R. Oertel, K. Olafsdottir, Y. Pico, B. G. Plosz, F. Polesel, C. Postigo, J. B. Quintana, P. Ramin, M. J. Reid, J. Rice, R. Rodil, N. Salgueiro-Gonzalez, S. Schubert, I. Senta, S. M. Simoes, M. M. Sremacki, K. Styszko, S. Terzic, N. S. Thomaidis, K. V. Thomas, B. Tscharke, R. Udrisard, A. L. N. van Nuijs, V. Yargeau, E. Zuccato, S. Castiglioni and C. Ort (2020). "Spatio-temporal assessment of illicit drug use at large scale: evidence from 7 years of international wastewater monitoring." Addiction **115**(1): 109-120.

Gracia-Lor, E., S. Castiglioni, R. Bade, F. Been, E. Castrignano, A. Covaci, I. Gonzalez-Marino, E. Hapeshi, B. Kasprzyk-Hordern, J. Kinyua, F. Y. Lai, T. Letzel, L. Lopardo, M. R. Meyer, J. O'Brien, P. Ramin, N. I. Rousis, A. Rydevik, Y. Ryu, M. M. Santos, I. Senta, N. S. Thomaidis, S. Veloutsou, Z. G. Yang, E. Zuccato and L. Bijlsma (2017). "Measuring biomarkers in wastewater as a new source of epidemiological information: Current state and future perspectives." Environment International **99**: 131-150.

Gracia-Lor, E., N. I. Rousis, F. Hernandez, E. Zuccato and S. Castiglioni (2018). "Wastewater-Based Epidemiology as a Novel Biomonitoring Tool to Evaluate Human Exposure To Pollutants." Environmental Science & Technology **52**(18): 10224-10226.

Gracia-Lor, E., E. Zuccato, F. Hernandez and S. Castiglioni (2020). "Wastewater-based epidemiology for tracking human exposure to mycotoxins." Journal of Hazardous Materials **382**.

Hendriksen, R. S., P. Munk, P. Njage, B. van Bunnik, L. McNally, O. Lukjancenko, T. Roder, D. Nieuwenhuijse, S. K. Pedersen, J. Kjeldgaard, R. S. Kaas, P. Clausen, J. K. Vogt, P. Leekitcharoenphon, M. G. M. van de Schans, T. Zuidema, A. M. D. Husman, S. Rasmussen, B. Petersen, C. Amid, G. Cochrane, T. Sicheritz-Ponten, H. Schmitt, J. R. M. Alvarez, A. Aidara-Kane, S. J. Pamp, O. Lund, T. Hald, M. Woolhouse, M. P. Koopmans, H. Vigre, T. N. Petersen, F. M. Aarestrup, A. Bego, C. Rees, S. Cassar, K. Coventry, P. Collignon, F. Allerberger, T. O. Rahube, G. Oliveira, I. Ivanov, Y. Vuthy, T. Sopheak, C. K. Yost, C. W. Ke, H. Y. Zheng, B. S. Li, X. Y. Jiao, P. Donado-Godoy, K. J. Coulibaly, M. Jergovic, J. Hrenovic, R. Karpiskova, J. E. Villacis, M. Legesse, T. Eguale, A. Heikinheimo, L. Malania, A. Nitsche, A. Brinkmann, C. K. S. Saba, B. Kocsis, N. Solymosi, T. R. Thorsteinsdottir, A. M. Hatha, M. Alebouyeh, D. Morris, M. Cormican, L. O'Connor, J. Moran-Gilad, P. Alba, A. Battisti, Z. Shakenova, C. Kiiyukia, E. Ng'eno, L. Raka, J. Avsejenko, A. Berzins, V. Bartkevics, C. Penny, H. Rajandas, S. Parimannan, M. V. Haber, P. Pal, G. J. Jeunen, N. Gemmell, K. Fashae, R. Holmstad, R. Hasan, S. Shakoor, M. L. Z. Rojas, D. Wasyl, G. Bosevska, M. Kochubovski, C. Radu, A. Gassama, V. Radosavljevic, S. Wuertz, R. Zuniga-Montanez, M. Y. F. Tay, D. Gavacova, K. Pastuchova, P. Truska, M. Trkov, K. Esterhuyse, K. Keddy, M. Cerda-Cuellar, S. Pathirage, L. Norrgren, S. Orn, D. G. J. Larsson, T. Van der Heijden, H. H. Kumburu, B. Sanneh, P. Bidjada, B. M. Njanpop-Lafourcade, S. C. Nikiema-Pessinaba, B. Levent, J. S. Meschke, N. K. Beck, C. D. Van, N. D. Phuc, D. M. N. Tran, G. Kwenda, D. A. Tabo, A. L. Wester, S. Cuadros-Orellana and S. Global Sewage (2019). "Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage." Nature Communications **10**.

Hernandez, F., S. Castiglioni, A. Covaci, P. de Voogt, E. Emke, B. Kasprzyk-Hordern, C. Ort, M. Reid, J. V. Sancho, K. V. Thomas, A. L. N. van Nuijs, E. Zuccato and L. Bijlsma (2016). "Mass spectrometric strategies for the investigation of biomarkers of illicit drug use in wastewater." Mass spectrometry reviews.

Huber, C., M. Krauss, V. Reinstadler, S. Denicolo, G. Mayer, T. Schulze, W. Brack and H. Oberacher (2022). "In silico deconjugation of glucuronide conjugates enhances tandem mass spectra library annotation of human samples." Analytical and Bioanalytical Chemistry **414**(8): 2629-2640.

Huber, C., E. Müller, T. Schulze, W. Brack and M. Krauss (2021). "Improving the Screening Analysis of Pesticide Metabolites in Human Biomonitoring by Combining High-Throughput In Vitro Incubation and Automated LC–HRMS Data Processing." Analytical Chemistry.

Kasprzyk-Hordern, B., B. Adams, I. D. Adewale, F. O. Agunbiade, M. I. Akinyemi, E. Archer, F. A. Badru, J. Barnett, I. J. Bishop, M. Di Lorenzo, P. Estrela, J. Faraway, M. J. Fasona, S. A. Fayomi, E. J. Feil, L. J. Hyatt, A. T. Irewale, T. Kjeldsen, A. K. S. Lasisi, S. Loiselle, T. M. Louw, B. Metcalfe, S. A. Nmormah, T. O. Oluseyi, T. R. Smith, M. C. Snyman, T. O. Sogbanmu, D. Stanton-Fraser, S. Surujlal-Naicker, P. R. Wilson, G. Wolfaardt and C. O. Yinka-Banjo (2022). "Wastewater-based epidemiology in hazard forecasting and early-warning systems for global health risks." Environment International **161**.

Kasprzyk-Hordern, B., K. Proctor, K. Jagadeesan, F. Edler, R. Standerwick and R. Barden (2022). "Human population as a key driver of biochemical burden in an inter-city system: Implications for One Health concept." Journal of Hazardous Materials **429**.

Kasprzyk-Hordern, B., K. Proctor, K. Jagadeesan, L. Lopardo, K. J. O'Daly, R. Standerwick and R. Barden (2021). "Estimation of community-wide multi-chemical exposure via water-based chemical mining: Key research gaps drawn from a comprehensive multi-biomarker multi-city dataset." Environment International **147**.

Kasprzyk-Hordern, B., K. Proctor, K. Jagadeesan, L. Lopardo, K. J. O'Daly, R. Standerwick and R. Barden (2021). "Estimation of community-wide multi-chemical exposure via water-based chemical mining: Key research gaps drawn from a comprehensive multi-biomarker multi-city dataset." Environment International **147**: 106331.

Kortenkamp, A., M. Scholze, S. Ermler, L. Priskorn, N. Jørgensen, A. M. Andersson and H. Frederiksen (2022). "Combined exposures to bisphenols, polychlorinated dioxins, paracetamol, and phthalates as drivers of deteriorating semen quality." Environ Int: 107322.

Kumar, R., S. Adhikari, E. Driver, J. Zevitz and R. U. Halden (2022). "Application of wastewater-based epidemiology for estimating population-wide human exposure to phthalate esters, bisphenols, and terephthalic acid." Sci Total Environ **847**: 157616.

Lopardo, L., B. Petrie, K. Proctor, J. Youdan, R. Barden and B. Kasprzyk-Hordern (2019). "Estimation of community-wide exposure to bisphenol A via water fingerprinting." Environ Int **125**: 1-8.

Lopez-Garcia, E., C. Perez-Lopez, C. Postigo, V. Andreu, L. Bijlsma, I. Gonzalez-Marino, F. Hernandez, R. M. Marce, R. Montes, Y. Pico, E. Pocurull, A. Rico, R. Rodil, M. Rosende, Y. Valcarcel, O. Zuloaga, J. B. Quintana and M. L. de Alda (2020). "Assessing alcohol consumption through wastewater-based epidemiology: Spain as a case study." Drug and Alcohol Dependence **215**.

Lundy, L., D. Fatta-Kassinos, J. Slobodnik, P. Karaolia, L. Cirka, N. Kreuzinger, S. Castiglioni, L. Bijlsma, V. Dulio, G. Deviller, F. Y. Lai, N. Alygizakis, M. Barneo, J. A. Baz-Lomba, F. Béen, M. Cíchová, K. Conde-Pérez, A. Covaci, E. Donner, A. Ficek, F. Hassard, A. Hedström, F. Hernandez, V. Janská, K. Jellison, J. Hofman, K. Hill, P. Y. Hong, B. Kasprzyk-Hordern, S. Kolarević, J. Krahulec, D. Lambropoulou, R. de Llanos, T. Mackuľak, L. Martinez-García, F. Martínez, G. Medema, A. Micsinai, M. Myrmel, M. Nasser, H. Niederstätter, L. Nozal, H. Oberacher, V. Očenášková, L. Ogorzaly, D. Papadopoulos, B. Peinado, T. Pitkänen, M. Poza, S. Rumbo-Feal, M. B. Sánchez, A. J. Székely, A. Soltysova, N. S. Thomaidis, J. Vallejo, A. van Nuijs, V. Ware and M. Viklander (2021). "Making Waves: Collaboration in the time of SARS-CoV-2 - rapid development of an international co-operation and wastewater surveillance database to support public health decision-making." Water Res **199**: 117167.

Montes, R., R. Rodil, A. Rico, R. Cela, I. Gonzalez-Marino, F. Hernandez, L. Bijlsma, A. Celma, Y. Pico, V. Andreu, M. L. de Alda, E. Lopez-Garcia, C. Postigo, E. Pocurull, R. M. Marce, M. Rosende, M. Olivares, Y. Valcarcel and J. B. Quintana (2020). "First nation-wide estimation of tobacco consumption in Spain using wastewater-based epidemiology." Science of the Total Environment **741**.

O'Brien, J. W., P. K. Thai, S. H. Brandsma, P. E. Leonards, C. Ort and J. F. Mueller (2015). "Wastewater analysis of Census day samples to investigate per capita input of organophosphorus flame retardants and plasticizers into wastewater." Chemosphere **138**: 328-334.

Pico, Y. and D. Barcelo (2021). "Identification of biomarkers in wastewater-based epidemiology: Main approaches and analytical methods." Trac-Trends in Analytical Chemistry **145**.

Rousis, N. I., E. Gracia-Lor, M. J. Reid, J. A. Baz-Lomba, Y. Ryu, E. Zuccato, K. V. Thomas and S. Castiglioni (2020). "Assessment of human exposure to selected pesticides in Norway by wastewater analysis." Sci Total Environ **723**: 138132.

Rousis, N. I., E. Gracia-Lor, E. Zuccato, R. Bade, J. A. Baz-Lomba, E. Castrignano, A. Causanilles, A. Covaci, P. de Voogt, F. Hernandez, B. Kasprzyk-Hordern, J. Kinyua, A. K. McCall, B. G. Plosz, P. Ramin, Y. Ryu, K. V. Thomas,

A. van Nuijs, Z. G. Yang and S. Castiglioni (2017). "Wastewater-based epidemiology to assess pan-European pesticide exposure." Water Research **121**: 270-279.
Sims, N. and B. Kasprzyk-Hordern (2020). "Future perspectives of wastewater-based epidemiology: Monitoring infectious disease spread and resistance to the community level." Environ Int **139**: 105689.
Tang, S., C. He, P. Thai, S. Vijayasarathy, R. Mackie, L. L. Toms, K. Thompson, P. Hobson, B. Tscharke, J. W. O'Brien and J. F. Mueller (2020). "Concentrations of phthalate metabolites in Australian urine samples and their contribution to the per capita loads in wastewater." Environ Int **137**: 105534.
Tang, S. Y., C. He, P. K. Thai, A. Heffernan, S. Vijayasarathy, L. Toms, K. Thompson, P. Hobson, B. J. Tscharke, J. W. O'Brien, K. V. Thomas and J. F. Mueller (2020). "Urinary Concentrations of Bisphenols in the Australian Population and Their Association with the Per Capita Mass Loads in Wastewater." Environmental Science & Technology **54**(16): 10141-10148.
Tiwari, A., S. Adhikari, D. Kaya, M. A. Islam, B. Malla, S. P. Sherchan, A. I. Al-Mustapha, M. Kumar, S. Aggarwal, P. Bhattacharya, K. Bibby, R. U. Halden, A. Bivins, E. Haramoto, S. Oikarinen, A. Heikinheimo and T. Pitkänen (2023). "Monkeypox outbreak: Wastewater and environmental surveillance perspective." Sci Total Environ **856**(Pt 2): 159166.
Yang, Z., E. Castrignanò, P. Estrela, C. G. Frost and B. Kasprzyk-Hordern (2016). "Community Sewage Sensors towards Evaluation of Drug Use Trends: Detection of Cocaine in Wastewater with DNA-Directed Immobilization Aptamer Sensors." Sci Rep **6**: 21024.
Yilmaz, B., H. Terekeci, S. Sandal and F. Kelestimur (2020). "Endocrine disrupting chemicals: exposure, effects on human health, mechanism of action, models for testing and strategies for prevention." Rev Endocr Metab Disord **21**(1): 127-147.