Science for Communities

Wastewater-based epidemiology:

A framework to identify and prioritise health determinants for wastewater monitoring

June 2021

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CLIENT REPORT NO: FW21021

ACKNOWLEDGEMENTS

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EXECUTIVE SUMMARY

Wastewater-based epidemiology (WBE) is based on the premise that many of the chemicals or microorganisms we are exposed to in our environment can be absorbed, metabolised, and excreted in urine or faeces, and may be detectable in wastewater. Changes in the levels of these substances in wastewater can then be used to infer exposure. Endogenous markers of human health can also be excreted in urine or faeces, and therefore could also be evaluated by WBE.

In this report the term **biomarker** refers to any human excretion product, chemical or biological, which could function as an indicator of consumption or exposure to environmental hazards, or of the human health state. The range of chemicals people could be potentially exposed to in the environment, compounds which can be consumed (both toxic and non-toxic), and endogenous health factors that could be assessed by wastewater monitoring are collectively referred to as **health determinants**[1] throughout this report.

WBE programmes are currently employed in New Zealand to monitor consumption of illicit substances in conjunction with the New Zealand Police, and for surveillance for SARS-CoV-2 as part of the New Zealand COVID-19 response.

Wastewater-Based Epidemiology has the potential to provide valuable insight into a wide range of other health determinants, including consumption of new psychoactive substances (NPS) and a range of legal substances, including alcohol, tobacco, caffeine, over-the-counter pain relievers and antimicrobials, as well as exposure to environmental contaminants and infectious diseases. It may also have a role in monitoring the rise of antimicrobial resistance (AMR), and to screen communities for endogenous biomarkers of disease.

Not all health determinants will be suitable for wastewater monitoring. The aim of this report is to develop a framework for the evaluation of candidate biomarkers of health determinants. Criteria are formulated based on current knowledge of the requirements for successful monitoring, inclusion of relevant criteria involved in the selection of the New Zealand Environmental Health Indicators (EHIs), and the essential requirement for the health determinant to relate to an issue of significant public health concern in New Zealand.

The framework developed herein consists of two phases: the initial evaluation of candidate health determinants based on criteria similar to those used in selection of EHIs, followed by an assessment of potential biomarkers.

The first criteria to consider is whether the candidate health determinant relates to a significant public health or scientific issue which would benefit from better understanding at a population level. WBE does not need to provide a complete solution but it must contribute at least an additive benefit, that could prompt further action or enable the evaluation of policies or activities. The segmentation of WBE is by its nature restricted by geographical differences defined by the catchments tested. If assessment or actions require targeting to specific population subgroups based on gender, ethnicity, age group, health status etc, WBE is not

[1] https://www.who.int/news-room/q-a-detail/determinants-of-health

able to provide this differentiation. Similarly, if to be meaningful, data needs to be expressed at the individual level or relative to another metric (eg, per kg body weight), a WBE approach is not suitable.

Secondly, it is important to consider if alternative approaches are already being used to monitor the candidate health determinant. The presence of alternative methodologies does not necessarily preclude a WBE approach and may be useful for validation/calibration of wastewater monitoring approaches. However, where alternative methodologies do exist, wastewater monitoring would need to either provide a valuable complementary approach, as seen for COVID-19, or be better than the current approach (ie, faster, more reliable, cheaper).

To be suitable for wastewater-based monitoring, certain background information must be available for both the health determinant and its potential biomarker. It is essential that changes in levels of the biomarker, or detection of a previously absent biomarker, infers changes in prevalence of the associated health determinant. Sufficient knowledge should also exist to allow development of sound detection methodology, which produces easily interpretable results. Ideally this methodology should be consistent with approaches used internationally to allow for comparisons.

The relative importance of some criteria depends on whether wastewater monitoring would be used in a surveillance-based capacity (ie, presence/absence), or for long-term quantitative monitoring (eg, illicit substances). For surveillance approaches it is essential that monitoring provide timely data to allow for rapid public health responses. For long-term monitoring, it is important that the relationship between the health determinant and its chosen biomarker is stable to allow for temporal comparisons.

In the second phase of the framework, a biomarker specific for the candidate health determinant must be identified and evaluated to determine its suitability for wastewater monitoring. Suitable biomarkers must be:

- Excreted in urine or faeces in sufficient levels to be detected in wastewater.
- Sufficiently stable in wastewater, including in-sewer and during collection, transport and storage.
- Specifically present in wastewater due to human excretion. Other sources contributing to the presence of the biomarker in wastewater could confound measurements. For this reason, it may be preferable to monitor for human-specific metabolites of the biomarker of interest, particularly where non-human contribution of the parent compound to wastewater is unavoidable.
- Able to be extracted and quantified from wastewater with sufficient accuracy, reliability, and reproducibility.

An evaluation tree summarising considerations within this framework is presented in Figure 1.

This report presents example framework evaluations for several candidate health determinants, including both suitable and non-suitable candidates to highlight the evaluation process. For example, illicit drug consumption represents an ideal candidate for wastewater monitoring and is a significant public health issue in New Zealand. Other methodologies are currently used for estimating illicit drug use, including surveys, and information on drug seizures and hospital admissions. However, wastewater monitoring is a valuable

complementary approach, providing un-biased, evidence-based, population level results. Five drugs are routinely monitored in New Zealand: methamphetamine, cocaine, fentanyl, heroin and MDMA. For each of these either the drug itself or a human-specific metabolite is used as the biomarker of consumption. These biomarkers are all excreted in urine and are sufficiently stable for monitoring. Well-established methodologies are utilised for extraction and detection of these biomarkers from wastewater, and specific controls are used to ensure results are accurate and reliable. Consumption levels can be quantified and related to the size of the population within the catchment (eg, mg/day/1000 people). Levels can be compared between regions and changes over time can be monitored to indicate consumption trends.

Conversely, several candidate health determinants were deemed not suitable for wastewater monitoring due to a variety of reasons. These include:

- LSD consumption: the active dose of LSD is very low meaning very little is consumed. Unless a large number of people were consuming LSD within the same catchment zone it is unlikely that it would be detectable in wastewater.
- Illicit morphine consumption: morphine can be both a legally prescribed drug and recreationally consumed as an illicit drug. As it would be impossible to distinguish between these two sources of morphine through wastewater monitoring, this approach is unsuitable.
- Persistent organic pollutants (POPs): the WBE framework was used to assess whether wastewater monitoring could replace the biomonitoring surveys currently used for POPs. POPs are monitored as a requirement of the Stockholm Convention, and the World Health Organisation has established protocol guidelines so that monitoring data can be comparable between member countries. Thus, wastewater monitoring should not replace the biomonitoring surveys. Its suitability as a complementary approach was therefore assessed. However, as POPs often persist for long periods in body and excretion rates are generally very low, they will likely be very difficult to monitor in wastewater. Additionally, WBE data is likely not particularly useful for many of these chemicals as effects of exposure are often dosage-dependent and this information would be lost at the population level. Thus, most POPs would likely be unsuitable candidates for wastewater monitoring.

This report highlights the considerable potential that exists for expansion of wastewater monitoring for infectious diseases. The current SARS-CoV-2 wastewater monitoring programme forms an essential part of the national COVID-19 response. This could be expanded into a border health capacity through monitoring of wastewater from inbound international aircraft, and at international airports. Considerable wastewater monitoring infrastructure has been established both in New Zealand and around the world as part of the COVID-19 response. This infrastructure could be adapted to monitor for new emerging infectious diseases, providing an important surveillance role.

Decisions around health determinants that could be implemented into WBE surveillance activities need to be informed by the evaluation framework, perceived benefits to public health in New Zealand, and the limitations of wastewater monitoring outlined in this report. Further, all new WBE programmes will need to carefully consider all potential social and ethical implications, and responsibilities under Te Tiriti o Waitangi.

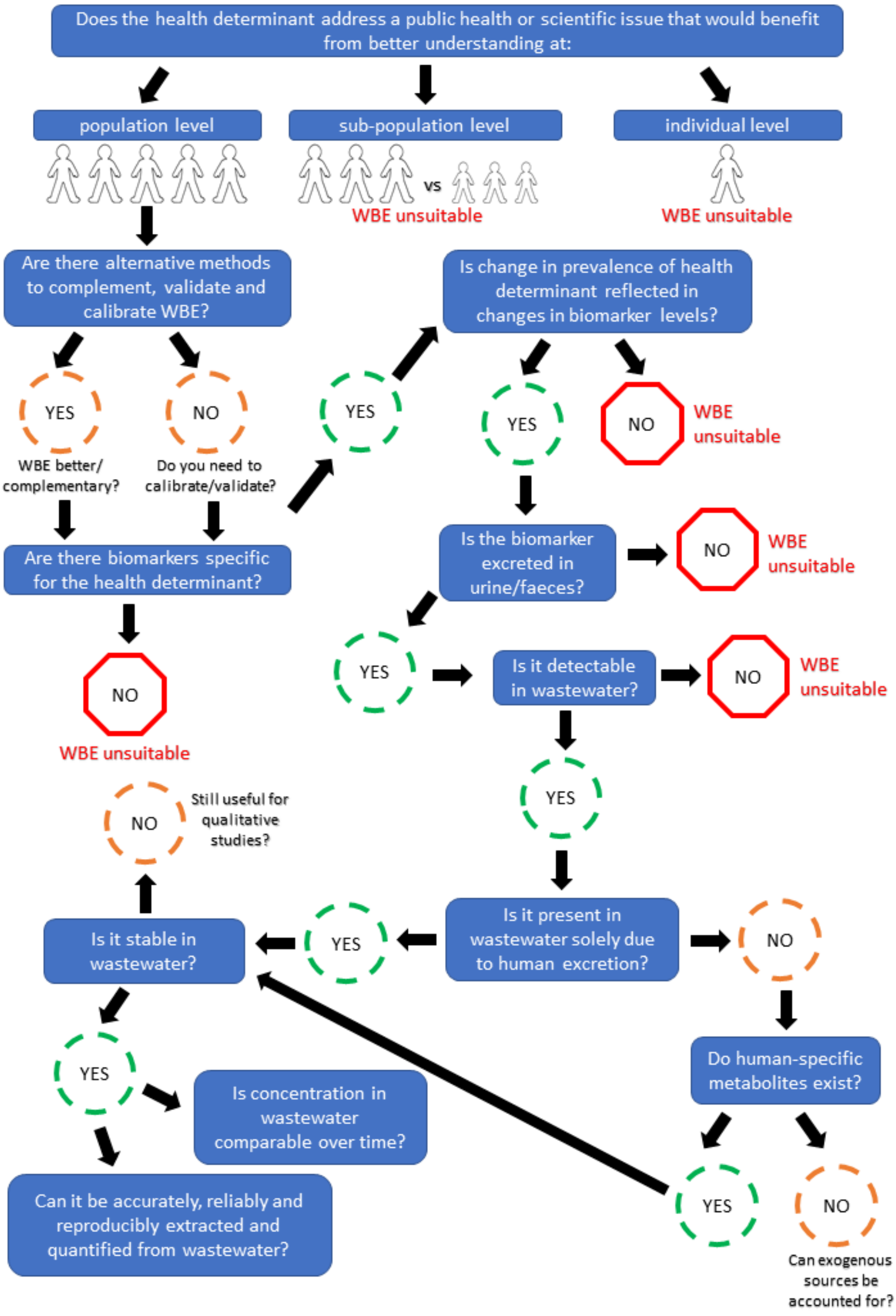

Figure 1 Evaluation tree for the proposed WBE framework

Green and red indicate stop/go criteria for assessment of health determinants and biomarkers. Orange is used to indicate criteria where extra consideration must be given. A WBE approach is not necessarily precluded, as for the red options, but the purpose of the monitoring must be weighed against potential limitations with regards to the criteria. Further detail on the considerations associated with these criteria is provided in the main text.

1. INTRODUCTION

1.1 BACKGROUND

Wastewater contains a wide variety of biological and chemical markers that are excreted in urine and faeces. The analysis of wastewater therefore has the potential to reveal important insights into community exposure to, or consumption of, chemicals, microorganisms and other substances of environmental and public health significance (Vitale et al 2021). The approach is based on the principle that many substances ingested or absorbed by the human body are in turn excreted (either unchanged, or as metabolites) in the urine and/or faeces. As such, the concentrations of these compounds in urban wastewater may reflect the health and/or habits of the community serviced by the wastewater network.

Currently, exposure to hazardous substances or organisms is generally monitored through conventional epidemiological tools, including biomonitoring surveys, reporting data (eg, notifiable diseases, seizures, or arrests in relation to illicit substances) or self-reporting surveys and questionnaires (Bowes and Halden 2019). However, many of these methods are limited by cost and/or scale, meaning they are undertaken infrequently or with small sample sizes. Other methods are subject to reporting bias by participants, or slow turnaround of data.

Wastewater-based epidemiology provides a powerful complementary tool for monitoring overall population health and the spread of disease. It can provide rapid, un-biased monitoring data, allowing prompt public health responses. Using this approach, populations can be monitored en masse meaning potentially millions of people can be screened, a feat impossible through classic clinical screening approaches. Additionally, it has the potential to not only monitor for exposure to hazards but also for endogenous markers of disease, allowing identification of at-risk regions (Lorenzo and Pico 2019).

However, there are limitations to wastewater monitoring which need to be addressed in order to fully optimise this tool. Wastewater is a complex matrix from which extraction and detection of biomarkers can be extremely difficult. Further, each component of the back calculations used to relate the detected analyte with population exposure or behaviour needs assessing. For example, accurately estimating the size of the population within a catchment zone can be challenging, particularly given their dynamic nature with constant movements of people in and out (Rico et al 2017). Thus, each monitoring programme requires rigorous optimisation and validation to ensure accurate and reliable detection. This can be both costly and time-consuming.

The potential of WBE as an important public health tool has been highlighted by its recent use in the global COVID-19 response. However, the full potential of wastewater monitoring has yet to be realised, and future applications are potentially limitless.

1.2 APPROACH AND SCOPE

This report describes a framework that could support the Ministry of Health in identifying and prioritising health determinants that could be monitored through wastewater surveillance. It includes information that has been gathered from published scientific literature, organisational and technical reports, grey literature and discussion with experts in the field of WBE. The report includes:

- A literature review of the use of WBE internationally, including what microorganisms, chemicals, biomarkers or emerging hazards are currently being monitored, or considered for monitoring, through wastewater surveillance. This is intended as a high-level review, rather than an in-depth assessment of all available literature for each potential hazard.
- A framework for assessing potential health determinants for inclusion in wastewater monitoring, which includes priority criteria that are aligned with the Environmental Health Surveillance Programme, including Environmental Health Indicators (EHI) research.
- Comments on environmental hazards that the Ministry of Health and ESR may be considering recommending for inclusion in wastewater surveillance.
- Evaluation of whether it may be appropriate for wastewater monitoring to replace bio-monitoring surveys such as the Persistent Organic Pollutants blood and urine biomonitoring surveys.

Monitoring for exposure to environmental hazards that occurs as a result of workplace exposure is out of scope of this report, as this is a WorkSafe responsibility.

2. WASTEWATER EPIDEMIOLOGY

2.1 WHAT IS WASTEWATER-BASED EPIDEMIOLOGY?

Wastewater-based epidemiology (WBE), also known as wastewater-based surveillance or sewage-based epidemiology is a rapidly developing, multi-disciplinary approach to monitoring the health and wellbeing of people within a wastewater catchment area, by analysing wastewaters for the presence of various chemicals, metabolites, biomarkers and/or microorganisms (Daughton 2018; Lorenzo and Picó 2019; Vitale et al 2021). The technique is based on the principle that any molecule ingested or absorbed by the human body will be excreted in the urine and/or faeces, either unchanged or as metabolites of the parent compound (Fig. 1). Similarly, many microorganisms are shed in urine and/or faeces. The concentrations of these residues or microorganisms in urban sewage can therefore reflect the extent to which the population serviced by the sewerage network is exposed to a hazard (Castiglioni et al 2013). The multidisciplinary approach of WBE incorporates concepts of chemistry, biology, mathematics, economics, engineering, epidemiology, public health, social science, forensic science, law and criminology (Vitale et al 2021). Data provided by WBE may be qualitative and/or quantitative.

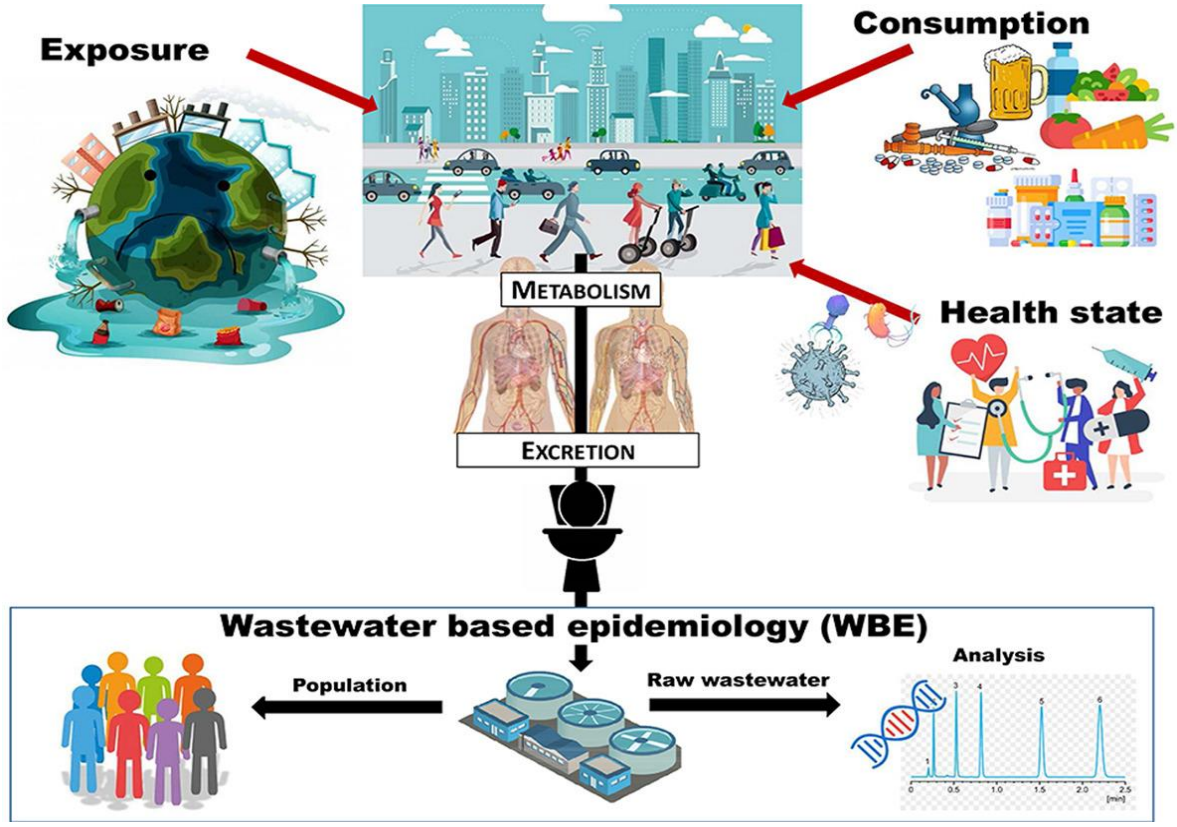

Figure 2 Overview of the concept of wastewater-based epidemiology. Reproduced from Vitale et al (2021).

The most well-developed application for WBE is the analysis of consumption of various legal and illicit drugs by a population (Lorenzo and Picó 2019). However, the potential applications for WBE are rapidly expanding. It is currently being employed as a tool to study consumption of substances such as nicotine, alcohol and caffeine, as well as emerging substances such as new psychoactive substances (NPS); exposure to environmental contaminants such as pesticides; surveillance for microbial pathogens such as poliovirus or SARS-CoV-2; and surveillance for the emergence of antimicrobial resistance (Vitale et al 2021) (Fig. 2). There is also growing interest in the potential to monitor endogenous disease-associated biomarkers to estimate the prevalence of certain diseases within communities.

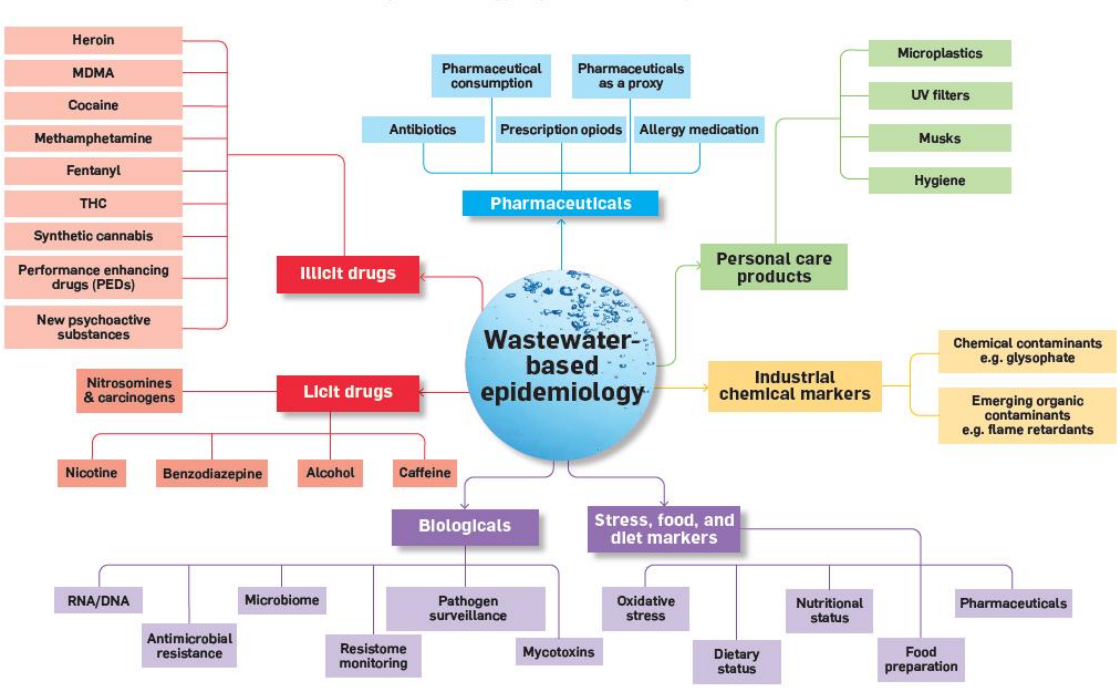

Figure 3 Overview of the potential compounds of interest for wastewater-based epidemiology. Reproduced from Manning and Walton (2020b).

In communities with reticulated sewage systems, samples are collected from the influent to the wastewater treatment plant (WWTP). These samples are representative of the whole community connected to the reticulation and are typically collected as composite samples over 24 hours (Lorenzo and Picó 2019). The wastewater samples are considered a complex matrix, with high concentrations of compounds that can hinder the detection of target compounds, which are often present at trace levels. Sample preparation and analysis will depend on the specific analyte(s) or biomarkers being assessed, but commonly incudes pre-treatment with a combination of filtration or centrifugation and solid-phase extraction to concentrate analytes. Samples are then analysed using techniques such as liquid chromatography and mass spectrometry for chemical analytes, and PCR or culture-based methods for infectious agents (Lorenzo and Picó 2019). If sufficient information is available regarding the volume or flow of wastewater and the population in the catchment, results can be expressed on a per capita basis. Trends in activity (eg, variation in drug consumption through the week or in certain locations) or the impact of specific interventions (eg, policy change) can also be monitored.

2.1.1 Advantages of wastewater-based methods

Wastewater-based epidemiology has several advantages over traditional epidemiological techniques such as retrospective data analysis, surveys and/or direct monitoring of large numbers of individuals. Wastewater-based methods are non-invasive, produce non-biased (objective) data and are anonymous. This avoids problems associated with questionnaire-based research, where with respect to illicit substances in particular, participants may not want to disclose unlawful activities, or indeed may not even know exactly what they are consuming. Data can be obtained much more rapidly than most classic epidemiological methods, and generally covers a much broader range of the population (Castiglioni et al 2013; Lorenzo and Picó 2019; Vitale et al 2021). For example, results from wastewater monitoring for SARS-CoV-2 can be rapidly produced (often within 24-48 hours from receipt of samples), and cases can even be detected in advance of detection by clinical screening (WHO 2020b). Additionally, millions of people can be screened in a relatively short period of time, versus the comparatively small number that can be assessed by clinical screening at any one time. Costs of wastewater monitoring may also be less than for some other study techniques, as once an analysis workflow has been established and validated, continual monitoring may be relatively inexpensive. Additionally, some health determinants, such as illicit substances, can be screened together using the same analysis workflow which can greatly reduce costs.

2.1.2 Limitations of wastewater-based methods

A major limitation of WBE is the uncertainty that can be associated with almost every step of the process (Lorenzo and Pico 2019). Selection of a suitable biomarker for a given health determinant requires considerable pre-existing knowledge around its metabolism and stability in wastewater, which is often lacking. Additionally, some biomarkers tend to adhere to surfaces and can be lost within the sewage system, thereby artificially reducing the perceived presence of that biomarker. In cases where a human-specific metabolite of a biomarker is monitored, back-calculations must be performed to estimate the concentration of the parent compound, which can be difficult to accurately determine. Efficient extraction and detection of biomarkers from wastewater can be very difficult due to the presence of inhibitors, and the potentially low abundance of the biomarker. Monitoring workflows for each new biomarker must go through rigorous optimisation and validation to ensure validity of the results.

Resulting detection values must be corrected based on flow rate and population size. Accurate estimation of population size can be a limitation of WBE approaches (Castiglioni et al 2013; Vitale et al 2021). Traditional methods of estimating population size have included using census data or information from the design capacities of the WWTPs, estimation based on ammonia concentrations, or using hydrochemical parameters (Castiglioni et al 2013). More recently, the notion of identifying an endogenous human biomarker present in the urine of all individuals as a proxy for estimating population size has been explored (Rico et al 2017). The possibility of using mobile phone activity patterns has also been proposed (Thomas et al 2017).

2.1.3 Ethical and social considerations

As WBE is an emerging technology, ethical and public acceptance issues are being actively discussed by scientists, ethicists and policymakers. Although the World Health Organisation (WHO) have issued ethical guidelines for public health surveillance based on principles of common good, equity, respect and good governance (WHO 2017), there is no current ethical blueprint specifically for the use of WBE (Pritchard et al 2016; Daughton et al 2018; Manning and Walton 2020a, 2020b). In general, there tends to be little oversight of WBE technologies by research ethics committees, as the data collected by WBE is not identifiable to individuals and is therefore not considered to raise major ethical concerns (Hall et al 2012; Manning and Walton 2020a). Together with the assumed benefits of WBE (ie, in providing public health data), this lack of 'direct harm' to individuals is used to justify the impingement on participants' autonomy (ie, the inability to gain consent) (Hall et al 2012). Others, including the United States Court of Appeals, have further suggested that there is no reasonable expectation of privacy in wastewater flowing through a public sewer (Gable et al 2020).

The use of WBE within small communities, or specific sub-populations or neighbourhoods does, however, involve ethical risk, as communities may be identified and experience indirect harm (Hall et al 2012; Pritchard et al 2016; Gable et al 2020; Manning and Walton 2020a). This could include creating or exacerbating stigmatisation of residents within the catchment area, but could also extend to policy or judicial responses, or economic impacts that adversely affect the community (Hall et al 2012; Pritchard et al 2016). For example, if a community were found to have 'impaired collective health' this could affect property values, or even health insurances. This raises the question of what would happen if the responsible stressor(s) cannot be identified, or cost-effective mitigation cannot be implemented? Would land use decisions or related regulations be able to be adapted in response to the findings of WBE for a catchment (eg, if exposure to contaminants is revealed)? Some cities have opted out of wastewater-based monitoring for illicit substances for fear of being perceived as 'hot spots' of drug use (Daughton et al, 2018). Gable et al (2020) also raised questions about how the use of wastewater monitoring could impact civil liberties. For example, whilst WBE for SARS-CoV-2 could provide the opportunity to target resources and supporting infrastructure to communities where the virus is detected or has a higher prevalence (eg, increased testing facilities, targeted public health advice), such testing could also trigger actions that directly affect the movement of those communities through localised lockdowns or quarantines. The misrepresentation or miscommunication of WBE data, wilful or otherwise, by media or authorities, could amplify these harms for small communities (Pritchard et al 2016).

As WBE technologies progress and develop into new areas, there needs to be continued consideration given to ethical and public acceptance issues, and how the science and data assets are governed (Manning and Walton 2020a). A recent review by Manning and Walton (2020a) of the ethical and governance issues relating to WBE discussed several common ethical frameworks and how they might be applied to WBE, as well as issues around data governance. The issues were considered in the specific Aotearoa New Zealand context, with Māori rights and interests as a central consideration. Further, an interview-based study by ESR's Social Science Team that explored stakeholder views on ethical approaches to WBE (Manning and Walton 2020b) concluded that a Responsible Research and Innovation (RRI) approach was most appropriate for constructing an ethical framework for WBE, with robust

internal assessment processes that reflect the perspectives of different stakeholders, including Māori as partners under Te Tiriti o Waitangi.

Effective and ethical implementation of public health policy requires a high level of public trust in the motives and actions of those involved, and in the quality and validity of the evidence underpinning policies and decisions (Canadian Water Network 2020; Manning and Walton 2020a). Careful consideration must be given at the outset as to how wastewater monitoring data is likely to be collected, disseminated and used, and the ethical concerns that will inevitably be invoked, particularly for smaller communities (Canadian Water Network 2020). Early community engagement will therefore be key to developing a proactive approach to future ethical issues.

2.2 OVERVIEW OF HEALTH DETERMINANTS THAT ARE CURRENTLY MONITORED OR ARE CANDIDATES FOR WASTEWATER MONITORING

2.2.1 Illicit substances

Illicit or illegal substances are controlled in New Zealand by the Misuse of Drugs Act 1975. They are classified into three classes based on the risk of harm they pose to users[2]. These are class A (very high risk), which includes methamphetamine, magic mushrooms, cocaine, heroin and LSD; class B (high risk), which includes cannabis oil, hashish, morphine, opium, ecstasy and a range of amphetamine-like substances; and class C (moderate risk), which includes cannabis seeds, cannabis plants and codeine. It is an offense against the Misuse of Drugs Act to use, possess, cultivate or traffic these substances. In 2001, Christian Daughton of the US Environmental Protection Agency (EPA) first proposed the idea of screening wastewater for illicit substances (Daughton 2001). He proposed that wastewater monitoring could be an effective, non-intrusive method for assessing usage of these substances at the community level. The first study using this approach was published in 2005 (Zuccato et al 2005). In this study, the presence of cocaine and its metabolite benzoylecgonine was detected in wastewater from four WWTP across Italy. The authors proposed that this methodology could be refined to become an important tool for monitoring the abuse of illicit substances. This approach has since undergone rapid global expansion to monitor a range of illicit substances across the world (Table 1). In 2010, the European Network Sewage Analysis CORe group Europe (SCORE) was established, with the aim of standardising approaches for WBE[3]. In 2012 they published their first study comparing illicit substance use across 19 European cities (Thomas et al 2012). A comprehensive list of WBE studies of illicit drug consumption up until early 2015 can be found in Castiglioni and Gracia-Lor (2016). Results from global wastewater monitoring for illicit substances provides an important reference used by the UNODC (United Nations Office on Drugs and Crime) to monitor global changes in drug consumption (United Nations 2020).

In addition to these large-scale studies, wastewater monitoring can also be used on a small scale. For example, monitoring drug usage within prisons (Postigo et al 2011; Brewer et al 2016) or schools (Panawennage et al 2011); or for monitoring changes in usage during special

[2] https://www.police.govt.nz/advice/drugs-and-alcohol/illicit-drugs-offences-and-penalties
[3] https://score-cost.eu/

events or holidays (Bijlsma et al 2009; van Nuijs et al 2011b). Recently it was also used to assess changes in illicit substance use during COVID-19 lockdown in Innsbruck, Austria (Reinstadler et al 2021).

New Zealand has an active programme monitoring illicit drugs in wastewater, conducted by ESR and funded by the New Zealand Police. Monitoring began in December 2016 in Christchurch and Rosedale, Auckland. In November 2018, this programme was expanded nationwide, with approximately 75% of the population now covered[4]. This programme monitors for the consumption of methamphetamine, MDMA (ecstasy), cocaine, fentanyl and heroin. In addition to these substances, cannabis consumption is also currently being monitored at five sites within New Zealand. However, certain characteristics of cannabis – such as it being lipophilic, not dissolving well in water and its tendency to stick to surfaces such as wastewater pipes – have made analysis in wastewater more difficult. Additionally, due to the considerable chemical differences between cannabis and the other illicit substances being monitored it cannot be added to the same analysis workflow. At this stage there is still too much uncertainty for cannabis measurements to be reliably quantifiable. However, the monitoring data can still be used in trend analyses.

Table 1. Examples of illicit substances that have been investigated using a WBE approach.

Illicit substance	Reference
Cocaine	Zuccato et al 2005; Khan et al 2014; Ort et al 2014; Bijlsma et al 2021; Reinstadler et al 2021
Methamphetamine	Zuccato et al 2011; Khan et al 2014; Ort et al 2014; Bijlsma et al 2021; Reinstadler et al 2021
Cannabis	Zuccato et al 2011; Khan et al 2014; Ort et al 2014; Bijlsma et al 2021
Heroin	Zuccato et al 2011; Khan et al 2014
MDMA (ecstasy)	Khan et al 2014; Ort et al 2014; Bijlsma et al 2021; Reinstadler et al 2021
Amphetamine	Khan et al 2014; Ort et al 2014; Bijlsma et al 2021; Reinstadler et al 2021
LSD	Postigo et al 2011

[4] https://www.police.govt.nz/sites/default/files/publications/wastewater-national-overview-q-1-2020-findings.pdf

2.2.2 New psychoactive substances

In the early 2000s New Zealand began to experience an influx of unregulated new psychoactive substances (NPS) colloquially known as legal highs, party pills, herbal highs, or synthetic cannabis. These drugs were often sold in dairies and liquor stores, and their use frequently led to health and social issues including seizures, psychosis and aggression. In July 2013, to protect the health of New Zealanders, the government introduced the Psychoactive Substances Act. Its aim is to regulate sale, importation and manufacture of NPS. Products proven to be of low risk can be approved for regulated sale by the Psychoactive Substances Regulatory Authority. However, as yet no NPS have been approved[5]. If a NPS is not approved by this authority it is illegal. New psychoactive substances are generally new compounds designed to mimic known illicit drugs; however, they can also include pharmaceuticals which are abused for recreational purposes, such as ketamine and fentanyl (Gent and Paul 2021). New psychoactive substances are commonly produced by manufacturers making minor changes to the chemical structure of existing illicit drugs (Tracy et al 2017). These compounds are often undetectable by routine drug testing methods. The prevalence and variety of NPS is expanding rapidly, with almost 900 NPS monitored by the UNODC (United Nations Office on Drugs and Crime) by 2018 (Shafi et al 2020). These compounds generally fall into four classes:

- Cannabinoids or synthetic cannabinoid receptor agonists (SCRAs):
  Chemicals related to tetrahydrocannabinol (THC), the main psychoactive compound in cannabis. There are more than 150 different SCRAs (commonly known as Spice), including the highly potent JWH-018 (Tracy et al 2017).
- Hallucinogens:
  These are subdivided into psychedelics, which are similar to LSD (lysergic acid dimethylamide) or psilocybin (magic mushrooms); and dissociatives, which are similar to the general anaesthetics ketamine and phencyclidine (PCP) (Tracy et al 2017).
- Stimulants:
  Chemicals related to MDMA (ecstasy), cocaine and amphetamines. A well-known example is mephedrone (bath salts) (Tracy et al 2017; Gent and Paul 2021).
- Depressants:
  These are subdivided into opioids, which are similar to morphine, and include novel fentanyls; and benzodiazepenes, which are similar to diazepam, and include diclazepam and flubromazepam (Tracy et al 2017; Gent and Paul 2021)

Traditional methods for monitoring NPS use mirror those for illicit substances, including surveys, drug seizures, police intelligence, hospital admission data and forensic toxicology, all with known limitations (Bade et al 2019b; 2019c). Over the past five years, wastewater monitoring has emerged as an effective tool to profile the use of NPS (Bade et al 2021; Gent and Paul 2021). New psychoactive substances that have to-date been identified in wastewater monitoring are shown in Table A2. However, there are limitations to wastewater monitoring of NPS. These include the constant emergence of new compounds on the market, structural similarity to existing illicit substances, and lack of mass spectra and standards which makes reliable identification difficult (Bade et al 2019b). Additionally, the often-short lifespan of these substances on the market means development of methods and standards is seldom feasible

[5] https://www.police.govt.nz/advice/drugs-and-alcohol/illicit-drugs-offences-and-penalties

(Bade et al 2019b). This has led to more qualitative suspect-screening approaches; however, these methods are less sensitive than targeted quantitative methods, exacerbating the difficulties associated with detecting the relatively low usage of these chemicals (Bade et al 2019b). Additionally, NPS tend to be extensively metabolised, and lack of pharmacokinetic data limits the ability to concurrently assess different metabolites (Thai et al 2016).

2.2.3 Legal substances

- **Alcohol**

Alcohol is the most used recreational drug in New Zealand (Ministry of Health 2015). Alcohol abuse is linked to more than 200 health conditions and was responsible for more than 3 million deaths worldwide in 2016 alone (WHO 2018a). Consumption has traditionally been monitored through sales statistics and surveys. However, the accuracy of these methods is limited due to inaccurate survey responses and non-inclusion of privately prepared alcohol (eg, home brews) in sales statistics (Gao et al 2020). In recent years, wastewater monitoring has been developed as a complementary approach for assessing alcohol consumption, providing evidence-based data. Wastewater monitoring provides more real-time data than traditional approaches, allowing for investigation of changes in consumption over much shorter time periods than surveys or sales statistics (Andrés-Costa et al 2016). For example, changes in consumption can be monitored on a weekly or even daily basis to gain a clearer picture of alcohol consumption patterns (Daglioglu et al 2020; López-García et al 2020). Wastewater monitoring of alcohol is based on detection of ethyl sulfate (EtS), a metabolite produced by alcohol degradation (Andrés-Costa et al 2016). EtS is excreted in urine and is stable in wastewater over several days (Vitale et al 2021). Recent studies have shown that estimates of alcohol consumption based on wastewater monitoring are comparable to data obtained from survey or sale statistics (Chen et al 2019; Gao et al 2020).

- **Tobacco**

Similar to alcohol, tobacco consumption poses substantial risks to human health. Indeed, tobacco use is the leading cause of preventable death worldwide (WHO 2019). More than 8 million people die from tobacco use each year, with around 1.2 million of those deaths attributable to second-hand smoke (WHO 2019). Around 15% of New Zealand adults smoke tobacco[6]. The New Zealand government has set a goal that by 2025 less than 5% of adults will smoke (Smokefree 2025)[7]. Similar to alcohol, current methods for assessing tobacco consumption rely on sales and survey data. Wastewater monitoring for tobacco was pioneered in 2015 (Castiglioni et al 2015b) and is now used across the globe as a complementary approach to gain more accurate and timely measures of tobacco consumption. Numerous studies have shown good comparability between data from traditional methods and wastewater monitoring, validating the approach (Castiglioni et al 2015b; Chen et al 2019; Mackie et al 2019; Gao et al 2020). The biomarkers generally used in wastewater monitoring for tobacco are the metabolites cotinine and trans-3'-hydroxycotinine produced during

[6] https://www.health.govt.nz/publication/annual-update-key-results-2017-18-new-zealand-health-survey
[7] https://www.smokefree.org.nz/smokefree-in-action/smokefree-aotearoa-2025

metabolism of the addictive component of tobacco, nicotine (Castiglioni et al 2015b). However, it has been suggested that using anabasine or anatabine as the biomarker for nicotine may be more suitable, as they are specific for dried tobacco thereby removing any contribution from nicotine replacement therapies such as patches and gum (Tscharke et al 2016a).

- **Caffeine**

Caffeine is found in a range of substances including coffee, tea, soft drinks, energy drinks and even chocolate. It is estimated that more than 85% of the American population consume at least one caffeinated drink each day (Mitchell et al 2014). Perhaps unsurprisingly then, caffeine is purported to be the most widely used psychoactive substance around the globe (Ferré 2013). Caffeine acts as a psychostimulant in the same way that cocaine and amphetamine do, leading to perceived dependence (Ferré 2013). Excessive caffeine consumption can cause gastrointestinal and cardiorespiratory problems, insomnia and anxiety[8]. Given the wide variety of substances that contain caffeine it is easy to lose track of how much has been consumed. Wastewater monitoring provides an excellent tool for monitoring caffeine consumption at the community level (Gracia-Lor et al 2017a, 2020). In a recent study, wastewater monitoring was even used to show a correlation between tobacco and caffeine consumption in Italy (Gracia-Lor et al 2020).

- **Over-the-counter pain relievers**

Consumption of over-the-counter pain relief medications is extremely common in New Zealand. Paracetamol (known as acetaminophen overseas) is the most used pain relief in New Zealand[9], and arguably the most consumed drug in the country[10]. However, prolonged usage has been linked to dose-dependent increases in cardiovascular, gastrointestinal and renal problems, and increased mortality (Roberts et al 2016). Additionally, paracetamol overdoses are responsible for numerous deaths every year, with 31 deaths in New Zealand between 2007 and 2018. These overdoses are often accidental, with a common mindset that paracetamol is safe, when it can be toxic at doses not much higher than the recommended maximum. Overdoses also represent a huge financial cost for hospitals, with an estimated $3 million spent on paracetamol overdoses in 2012 alone[11]. Paracetamol is also the biggest cause of poisonings in under 5-year-olds[12].

Ibuprofen is a non-steroidal anti-inflammatory that is commonly used in New Zealand as a broad-spectrum pain reliever. However, prolonged usage can lead to kidney and liver damage, gastrointestinal bleeding[13], and increased risk of heart attack (Hernández-Díaz et al 2006).

[8] https://www.medsafe.govt.nz/profs/puarticles/caffeine.htm
[9] https://www.healthnavigator.org.nz/medicines/p/paracetamol/
[10] https://www.rnz.co.nz/news/the-wireless/375268/fransplaining-science-is-paracetamol-doing-more-harm-than-good
[11] https://www.rnz.co.nz/news/the-wireless/375268/fransplaining-science-is-paracetamol-doing-more-harm-than-good
[12] https://www.starship.org.nz/guidelines/paracetamol-poisoning/
[13] https://adf.org.au/drug-facts/ibuprofen/

Additionally, risks are exacerbated for smokers and those individuals with diabetes, high blood pressure, high cholesterol, renal problems or stomach ulcers[14].

Both paracetamol and ibuprofen are available without a prescription and can be bought not only at pharmacies but also at supermarkets. This makes it very difficult to accurately track consumption. For this reason, wastewater monitoring is an excellent tool to assess consumption rates at the community level. Both paracetamol (acetaminophen) and ibuprofen are detected in very high levels in wastewater (up to 500,000 ng/L and 45,000 ng/L respectively) (Roberts and Thomas 2006; Guerra et al 2014; Sims and Kasprzyk-Hordern 2020).

- **Antimicrobials**

A wide range of antimicrobials are used in New Zealand to treat a variety of bacterial infections, and almost all require a prescription[15]. Antibiotic use is high in New Zealand compared to many other developed nations (Williamson et al 2016). This is concerning as overuse of antibiotics is directly linked to the rise of antimicrobial resistance (AMR). There is currently no standard system for monitoring antibiotic consumption in New Zealand other than relying on prescription/dispensation data (Williamson et al 2016). Wastewater monitoring could provide a valuable surveillance tool able to capture differences between levels prescribed and levels consumed, and temporal and spatial patterns in consumption. However, this may be complicated by antibiotics being brought into New Zealand from overseas. A range of antibiotics have been detected in wastewater in international studies (Kasprzyk-Hordern et al 2009; Roberts and Thomas 2009; Guerra et al 2014; Senta et al 2019). These include sulfamethoxazole, azithromycin, clarithromycin, ciprofloxacin, erythromycin, and trimethoprim (Sims and Kasprzyk-Hordern 2020).

2.2.4 Infectious diseases

Infectious diseases, whether caused by bacteria, viruses, fungi or parasites, pose a significant threat to public health. These diseases may be new emerging infections, re-emerging or persistent threats (Bloom and Cadarette 2019). Since the 1970s more than 40 new infectious diseases have been discovered (WHO 2018b), and several old diseases have re-emerged, due to a combination of climate change, drug resistance, poverty and poor sanitation (Sims and Kasprzyk-Hordern 2020). To rapidly respond to new and re-emerging infections, effective monitoring or surveillance systems are required (Sims and Kasprzyk-Hordern 2020). Wastewater monitoring has emerged as a powerful surveillance approach for both routine monitoring of enteric viruses circulating in the community (Lun et al 2018), and for detecting infectious disease outbreaks early in their development (Sims and Kasprzyk-Hordern 2020). For example, norovirus and hepatitis A virus could be detected in wastewater before outbreaks were detected by the health care system (Hellmér et al 2014). More recently, RNA from the COVID-19 virus SARS-CoV-2 has been shown to be detectable in wastewater up to several days before cases are detected through clinical screening (WHO 2020b). This highlights a major advantage of wastewater monitoring, which is that large amounts of virus are often shed

[14] https://www.healthnavigator.org.nz/medicines/i/ibuprofen/
[15] https://www.healthnavigator.org.nz/medicines/a/antibiotics/

before the onset of visible disease symptoms (Hellmér et al 2014). The notion of monitoring wastewater for infectious disease is not new, with surveillance monitoring for poliovirus successfully employed since the 1980s (Huang et al 2005; Hovi et al 2012; Roberts 2013; Asghar et al 2014; Ndiaye et al 2014). This so-called Environmental Poliovirus Surveillance (ENV) is now part of the World Health Organisations Global Polio Eradication Initiative[16] (WHO 2003).

Wastewater monitoring for infectious diseases has risen to prominence due to its recent role in the global COVID-19 response (Ahmed et al 2020a; Medema et al 2020; Randazzo et al 2020; Rimoldi et al 2020; WHO 2020b). As of May 2021, the COVID-19 WBE Collaborative had over 2,200 SARS-CoV-2 monitoring sites across 55 countries registered on its COVID19Poops dashboard[17]. Collaborating groups include the US National Wastewater Surveillance System (NWSS), a partnership between the US Centers for Disease Control and Prevention (CDC), US Department of Health and Human Services (HHS) and federal government[18]; the Australian Collaboration on Sewage Surveillance of SARS-CoV-2 project (ColoSSoS; proposed to transition to POWER – **P**ublic Health **O**utcomes from **W**astewater **E**pidemiology **R**esearch), a co-ordinated effort of over 50 organisations led by Water Research Australia[19]; and the UK National COVID-19 Wastewater Epidemiology Surveillance Programme (N-WESP), comprised of 23 partner organisations and led by the UK Centre for Ecology and Hydrology[20]. In March 2021, the European Commission adopted a recommendation on COVID-19 wastewater monitoring which asks all member states to put wastewater surveillance and reporting systems in place[21]. ESR is a member of the COVID-19 WBE Collaborative and has been monitoring wastewater for SARS-CoV-2 since July 2020, forming an important part of New Zealand's COVID-19 response. Now established, this global wastewater monitoring infrastructure could be readily adapted to other infectious diseases. Indeed, in the USA, utilisation of the capacity established for COVID-19 for other infectious diseases has already been proposed (J. Hewitt, pers com).

A wide range of other infectious microorganisms are known to be detectable in wastewater, including hepatitis A, B and E viruses, noroviruses, rotaviruses, sapoviruses, enteroviruses and zika virus (Vitale et al 2021); the human pathogenic bacterial species *Campylobacter jejuni* and *C. coli* (Waage et al 1999); and the parasitic protozoa *Giardia* (Nasser et al 2012). There are a variety of methods that can be used for detecting infectious microorganisms in wastewater (Corpuz et al 2020). Those methods that may be suitable for wastewater monitoring are discussed in detail below:

[16] https://polioeradication.org/news-post/environmental-surveillance-and-the-polio-eradication-effort/
[17] https://www.covid19wbec.org/covidpoops19
[18] https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance.html
[19] https://www.waterra.com.au/project-details/264
[20] https://nwesp.ceh.ac.uk/
[21] https://ec.europa.eu/jrc/en/news/coronavirus-commission-adopts-common-approach-track-covid-19-through-wastewater-monitoring

- **PCR (Polymerase chain reaction)-based methods**

Most molecular methods for detection of infectious microorganisms rely on a PCR-based approach, such as qPCR (quantitative PCR). For RNA viruses the first step in this process is to convert the viral RNA to complementary DNA (cDNA). Small pieces of DNA (primers) specific for the chosen organism are then attached to the DNA or cDNA template and using an enzyme-based replication process, the microbial DNA is amplified. Products produced by the PCR reaction are labelled with fluorescent dyes or probes, and the fluorescence of the PCR reaction can be monitored over time. The amount of fluorescence can then be used to determine the amount of microbial nucleic acid that was present in the original sample. These methods have been successfully used to quantify astrovirus, human adenovirus, human polyomavirus, norovirus GII, reovirus, enterovirus, rotavirus, sapovirus, and more recently, SARS-CoV-2 in wastewater (Qiu et al 2018; Randazzo et al 2020; Wu et al 2020; Wurtzer et al 2020). This methodology can also be adapted to screen for multiple different viruses at the same time (multiplex qPCR) by using different fluorescent probes for each one. For example, this approach has been used to simultaneously screen wastewater for a range of enteric viruses including rotavirus, norovirus GI and GII, human adenovirus, human polyomavirus and enterovirus (Hamza et al 2014).

An alternative method for quantifying viruses in wastewater is digital PCR (dPCR) which gives absolute quantification of the number of viral genomes present in a sample. One approach is droplet digital PCR, where the sample is fractionated into thousands of droplets within a water-oil emulsion (Jahne et al 2020). Amplification of the template then occurs within each droplet as though each droplet was in a separate tube[22]. This approach has been used to quantify both adenovirus and norovirus within wastewater (Jahne et al 2020). Another approach, which may prove valuable for wastewater monitoring in the future, is chip-based digital PCR where the sample is loaded onto a silicon chip that contains thousands of wells. Amplification then occurs within each well and results are visualised using fluorescence microscopy (Nykel et al 2019).

- **Next generation sequencing**

Next generation sequencing (NGS) can be used to provide a comprehensive, unbiased picture of all microorganisms present within a wastewater sample. This approach provides not only information on the diversity present but can also be used to investigate mutations present within the population. Next generation sequencing has been used to study the diversity of enteroviruses present in wastewater (Tao et al 2020; Lizasoain et al 2021). More recently it has been applied to wastewater monitoring for COVID-19, where it has been used to identify novel mutations within SARS-CoV-2 (Izquierdo-Lara et al 2021).

[22] https://www.bio-rad.com/en-nz/applications-technologies/droplet-digital-pcr-ddpcr-technology

2.2.5 Antimicrobial resistance

Antimicrobial resistance (AMR) refers to the process by which microorganisms, particularly bacteria, change over time such that they are no longer sensitive to antibiotics, making infections more and more difficult to treat. Although these organisms naturally change over time, AMR is mostly driven by overuse and misuse of antibiotics, such as not completing a full course of antibiotics as prescribed. This is often combined with poor sanitation and disease prevention measures. Of particular concern is the rise of so-called 'superbugs' that are resistant to many or all currently available antibiotics, resulting in untreatable infections. The WHO has declared that AMR is one of the top ten threats to global public health[23]. In 2015 they established the Global Antimicrobial Resistance Surveillance System (GLASS) to support global surveillance and research on AMR[24] (WHO 2020a). Antimicrobial resistance is seen as an imminent threat to New Zealand, according to the Prime Minister's Chief Science Advisor[25]. The consumption of antibiotics in New Zealand is high compared with many other developed nations, likely due in part to antimicrobials being prescribed for viral respiratory conditions (Williamson et al 2016). ESR was commissioned by the Ministry of Health to report on antibiotic consumption in New Zealand. The first report released in 2016 revealed that between 2006-2014 consumption had increased by 49% (Williamson et al 2016). To curb rising consumption, PHARMAC launched the 'keep antibiotics working campaign' to educate New Zealanders that antibiotics do not work for colds or flu[26]. Perhaps attributable to the success of this programme, combined with decreased inappropriate prescribing of antibiotics, antibiotic consumption has been declining since 2015 (Thomas et al 2020).

Although antimicrobial resistance is currently thought to be relatively low in New Zealand, its New Zealand prevalence is increasing. The Ministry of Health and Ministry for Primary Industries jointly established the Antimicrobial Resistance Action Planning Group, with the aim to develop a National AMR Action Plan. Their report assessing the current situation and identifying areas for action was released in March 2017 (MoH and MPI 2017a). It identified several priority areas, including increasing efforts to raise awareness of AMR in the community and education on the proper use of antibiotics; further education for health professionals around alternatives to prescribing antibiotics; establishment of a national surveillance program for AMR and antibiotic consumption; and supporting national research into AMR (MoH and MPI 2017a). Based on these identified priority areas an Action Plan was developed (MoH and MPI 2017b). As part of this action plan, ESR has been contracted by the Ministry of Health to manage an AMR surveillance programme[27]. In this programme, antimicrobial susceptibility data from routine laboratory testing, data from periodic surveys of antimicrobial susceptibility of specific organisms sampled from across the country, and monitoring data for rare and emerging resistant bacteria is collated. Wastewater monitoring could be a powerful complementary tool for this surveillance programme. WWTPs have been dubbed 'hotspots' for antimicrobial resistance genes and the mobile genetic elements involved in their transfer between bacterial species (Guo et al 2017; Rodríguez et al 2021). A metagenomic approach

[23] https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
[24] https://www.who.int/glass/en/
[25] https://www.pmcsa.ac.nz/topics/antimicrobial-resistance-and-infectious-disease/antimicrobial-rsistance/
[26] https://pharmac.govt.nz/about/what-we-do/making-best-use-of-medicines/keep-antibiotics-working/
[27] https://surv.esr.cri.nz/antimicrobial/antimicrobial_resistance.php

to AMR surveillance is used by the Global Sewage Surveillance Project[28] (Hendriksen et al 2019), a joint study between the WHO and the National Food Institute, Technical University of Denmark. During the first round of sampling, analysis of untreated wastewater from 60 countries revealed differences in both the prevalence and diversity of AMR genes across these countries, with prevalence strongly influenced by environmental, health, and socio-economic factors (Hendriksen et al 2019). ESR has been collaborating in ongoing sampling related to this project.

In New Zealand, ongoing research at ESR is developing and evaluating the potential of three complementary WBE approaches to understanding the AMR triad (antimicrobials, resistance genes and resistant bacteria). Methodology has been established for the detection and quantitation of four antimicrobials in wastewater (flucloxacillin, erythromycin, roxythromycin and doxycycline). Analysis of spatial and temporal trends in the presence of these antimicrobials is in progress. Additional antimicrobials are also being added to the analysis, some of which require a different extraction method (eg, amoxicillin). In a second approach, HiSeq-based short read metagenomic sequencing is being used to identify AMR genes in wastewater samples. In a pilot study 120-150 putative AMR genes were identified, including genes conferring resistance to clinically relevant antimicrobials such as colistin, beta-lactams, including carbapenems and cephalosporins, and aminoglycosides such as gentamicin and streptomycin. In the third approach, clinically relevant groups of AMR bacteria have been isolated from wastewater. These include Extended Spectrum Beta-Lactamase (ESBL)-producing and carbapenemase-producing Gram-negative bacteria. Whole genome sequencing has been undertaken on a subset of isolates, and isolates have been tested for antimicrobial susceptibility. These three approaches for assessing AMR through wastewater will provide valuable insights into trends in community antimicrobial usage and community prevalence of AMR.

2.2.6 Environmental contaminants

In the context of this report, ‘environmental contaminants’ refers to chemical substances humans may be exposed to in the environment, particularly those which may cause adverse health effects. These include chemicals found in personal care products, pesticides, industrial chemicals and surfactants (Luo et al 2014). A wide range of these chemicals can be detected in wastewater, and therefore could be target biomarkers for wastewater monitoring. A range of chemicals which have been shown to be detectable in wastewater are summarised below:

- **Personal care products**

These include insect repellents such as DEET (N,N-Diethyl-meta-toluamide) (Terzić et al 2008), the UV filter BP-3 (benzophenone-3) found in sunscreens and cosmetics (Kim and Choi 2014), galaxolide and tonalide found in some musk fragrances (Terzić et al 2008), and disinfectants such as triclosan (Behera et al 2011).

[28] https://www.globalsurveillance.eu/

- **Pesticides**

These include herbicides such as diuron and atrazine (Campo et al 2013), insecticides such as diazinon (Campo et al 2013), and fungicides such as clotrimazole and tebuconazole (Kahle et al 2008).

- **Industrial chemicals**

These include plasticizers such as BPA (bisphenol-A), DBP (dibutyl phthalate), DEHP (di(2-ethylhexyl) phthalate) and DMP (dimethyl phthalate) (Clara et al 2005, 2010), and fire retardants such as TCEP (tris(2-carboxyethyl) phosphine) and TCPP (tris (1-chloro-2-propyl) phosphate) (Loos et al 2013).

- **Surfactants**

These are chemicals that reduce the surface tension of liquids and include nonylphenol and octylphenol (Terzić et al 2008).

In New Zealand, programmes are currently in place to screen the general population for exposure to persistent organic pollutants (POPs) and other selected chemicals of concern (SCoC). Persistent organic pollutants are chemicals that are resistant to degradation and as such, remain in the environment for long periods of time. They are known to accumulate within the fatty tissues of the body and have been linked to cancer, birth defects and many other significant health conditions[29]. Selected chemicals of concern are a range of additional environmental contaminants not considered POPs, but of similar public health concern. These include heavy metals, phenols, phthalate metabolites, fluoride and the nicotine metabolite cotinine (Mannetje et al 2018). Several of the contaminants listed above are SCoC including triclosan, BPA, BP-3, DMP, DEP, DBP and DEHP (Mannetje et al 2018). The screening programmes for POPs and SCoC will be discussed in more detail in Section 4 of this report, with reference to the potential for monitoring for the presence of these chemicals in wastewater.

2.2.7 Disease biomarkers

Most wastewater monitoring is focused on screening for chemical or biological determinants of disease, such as illicit substances and infectious agents. However, much could be gained from monitoring for endogenous human biomarkers indicative of disease. For example, if there are high biomarkers for a given disease in a certain region, this may suggest there is a causal link within the environment which would need to be investigated. This concept of screening wastewater for indicators of disease has been referred to as BioSCIM (sewage chemical-information mining) (Daughton 2018). Candidate disease biomarkers excreted in urine or faeces include diacetyl-polyamines, which are linked to cancer, kidney disease and diabetes (Daughton 2018); C-reactive protein, which is linked to kidney disease, peripheral artery

[29] http://www.pops.int/TheConvention/Overview/tabid/3351/Default.aspx

disease, stroke and heart attacks (Stuveling et al 2003); interleukin-8, which is a biomarker of urinary tract infections and general inflammation (Rao et al 2001; Taha et al 2003); and a range of potential biomarkers for brain disorders (An and Gao 2015). This field of wastewater monitoring is in its infancy and it remains to be seen how successful this approach will prove.

3. FRAMEWORK FOR EVALUATING HEALTH DETERMINANTS FOR INCLUSION IN WASTEWATER MONITORING

3.1 PHASE 1: EVALUATION OF CANDIDATE HEALTH DETERMINANTS

The list of potential public health-associated characteristics, or determinants, which could be examined using wastewater monitoring is almost limitless. These could include hazards such as illicit substance abuse and infectious diseases, but also non-hazardous characteristics such as prescription medication use or vitamin C consumption. However, when evaluating which determinants to include in any public health wastewater monitoring, several aspects need to be taken into consideration. Firstly, candidate selection should be guided by the goals of the New Zealand Environmental Health Surveillance Program. This is a joint program between ESR and the Ministry of Health, with support from diagnostic laboratories. It involves the continual collection and analysis of data related to certain health outcomes to direct planning, implementation and evaluation of public health programmes. Selection of candidate health determinants should also be informed by guidelines used in selection of the New Zealand Environmental Health Indicators (EHI) (Table A3; Mason et al 2018). Environmental Health Indicators describe the link between the environment and health and are based on known or plausible cause-and-effect relationships between environmental factors and a person's health (Mason et al 2018). These indicators are developed by the Environmental Health Intelligence New Zealand (EHINZ) team based at Massey University, on behalf of the Ministry of Health[30]. The criteria used in selecting EHIs are discussed below with regards to their applicability in assessing health determinants for wastewater monitoring as the first phase in an evaluation framework.

3.1.1 Availability of data

The EHI must have data that can be easily and reliably extracted.

With regards to wastewater analysis, this means there must be a suitable chemical or biological substance (biomarker) for the chosen health determinant which can be relatively easily and reliably detected. Criteria for evaluation of suitable biomarkers will form phase 2 of the framework as discussed below.

[30] https://www.ehinz.ac.nz/indicators/

3.1.2 Scientific validity

The EHI must have an established, scientifically-sound link to the environmental health issue.

This criterion implies that changes in levels of a biomarker, or detection of a previously absent biomarker, infers changes in the prevalence of the associated health determinant. For example, increased presence of illicit drugs or their metabolites in wastewater would imply increased illicit substance abuse within the catchment zone (Chappell and Ashmore 2018). Similarly, detection of SARS-CoV-2 RNA fragments in wastewater indicates the presence of COVID-19 cases within the catchment zone (WHO 2020b).

3.1.3 Sensitivity to changes

The EHI should respond relatively quickly and noticeably to changes but not show false movements.

This criterion means that levels of biomarker excreted into wastewater should change in response to a change in the associated health determinant. For example, if more people within a given catchment starting consuming methamphetamine, the level of methamphetamine detected in wastewater from that catchment should also increase. Conversely, this criterion also implies that biomarker levels should not change unless there is a change in the associated health determinant. Abnormally high or low wastewater flow could lead to perceived changes in biomarker concentration not representative of true changes in consumption. Thus, it is very important that all wastewater monitoring data is adjusted based on the flow rate. Influx of stormwater into the wastewater system will result in increased flow through the wastewater network, particularly where wastewater and stormwater systems are combined. This will result in biomarker dilution, which may mean low abundance biomarkers are too dilute to be detected. The effect of flow rate is taken into consideration during back calculations of biomarker abundance. For example, when calculating drug consumption:

$$\text{Drug use} = \frac{\text{concentration} \times \text{flow rate} \times \text{excretion rate}}{\text{Population adjustment}}$$ (Chappell and Ashmore, 2018)

3.1.4 Consistency

The EHI should be consistent with those used in other indicator programmes (including internationally) so comparisons can be made.

This criterion has two implications with regards to wastewater monitoring. Firstly, it implies that where wastewater monitoring is to be used as a complimentary analysis to another method already in use, the information it provides must be able to be compared easily with results from the other methods. For example, wastewater monitoring for COVID-19 looks for the presence of SARS-CoV-2 RNA fragments, which are detected after PCR (polymerase chain reaction) amplification (Ahmed et al 2020a; Randazzo et al 2020; Rimoldi et al 2020). This is complimentary to nasopharyngeal swab testing done at COVID-19 testing stations and swab/saliva testing of border workers, which also rely on PCR of amplification of viral

particles[31]. This allows results from these two monitoring programmes to be compared, such that wastewater detection would be expected in regions where cases have been identified by clinical testing and unexpected in regions where no cases have been identified. However, as discussed above COVID-19 detection in wastewater can pre-empt clinical detection, in which case it would provide support for a rapid public health response to identify the source.

Secondly, this criterion implies that when selecting biomarkers for monitoring a given health determinant, consideration should be given to comparable wastewater monitoring occurring internationally. If this health determinant is already the target of wastewater monitoring overseas, ideally the same biomarker should be employed in New Zealand to allow direct study comparisons.

3.1.5 Comparability

The EHI should be consistent to allow comparisons over time.

This criterion implies that the relationship between a health determinant and its chosen biomarker should not change, allowing for informative comparisons over time. For example, in the national wastewater drug testing programme the ability to monitor changes in drug/metabolite excretion over time and establish patterns of drug usage is particularly important for Police and Customs officials[32]. New psychoactive substances pose a particular challenge as their composition is regularly being altered to avoid detection, achieve new outcomes or due to manufacturing changes. As such, new NPS are constantly entering the market, resulting in a generally short lifespan for most NPS and reducing the usefulness of long-term analysis. Moreover, some illicit substances and NPS share common breakdown products, further confounding analysis. Special consideration must also be given with regards to infectious diseases as these often mutate, giving rise to new variants which have the potential to impact on monitoring if the mutation changes features of the biomarker that affect its detection. However, a recent study has revealed that wastewater monitoring can also be useful for examining the rise of novel mutations in infectious diseases. Izquierdo-Lara et al (2021) performed whole genome sequencing of wastewater samples to examine the diversity of SARS-CoV-2 in the community, allowing them to detect several novel mutations. Lastly, it is also feasible for novel infectious diseases to arise which share a common biomarker with one already monitored, thereby confounding interpretation as the biomarker would then relate to more than one health determinant.

3.1.6 Methodologically sound measurement

The EHI measurement needs to be methodologically sound.

There are many factors to consider during wastewater monitoring to ensure resulting measurements are accurate. Methodologies for sample collection, processing and analyte determination must be optimised and validated for each biomarker. The biomarker must be

[31] https://www.health.govt.nz/our-work/diseases-and-conditions/covid-19-novel-coronavirus/covid-19-health-advice-public/assessment-and-testing-covid-19/how-covid-19-testing-works
[32] https://www.police.govt.nz/about-us/publication/national-wastewater-testing-programme-quarter-1-2020

sufficiently stable for measurements to be meaningful. This is discussed in more detail in phase 2 below. In cases where a human-specific metabolite of the biomarker is monitored, back-calculations must include consideration of metabolism rates of the parent compound (Gracia-Lor et al 2016). Lastly, one of the most challenging steps in wastewater monitoring is estimation of the size of the contributing population to allow for calculation of biomarker abundance per capita (Rico et al 2017). Methods include using the design capacity of the WWTP, or extrapolation based on information from census data, from hydrochemical parameters, or based on concentrations of nitrogen, oxygen or phosphorus (van Nuijs et al 2011a; Castiglioni et al 2014). However, each of these approaches have considerable limitations. Recently, a range of human urine biomarkers have been tested to identify biomarkers which may act as a good proxy for population size (Rico et al 2017). The importance of accurately measuring population size also depends on whether qualitative or quantitative measurements are required for the chosen health determinant.

3.1.7 Intelligible and easily interpreted

The EHI should be sufficiently simple to be interpreted in practice and be intuitive in the sense that it is obvious what the indicator is measuring.

As discussed above, there are a multitude of putative health determinants that could be examined using wastewater monitoring. However, it is important to consider what results from wastewater monitoring of that health determinant would mean. This will depend on the overall purpose of the monitoring – is it to gain information around spatial and temporal changes in levels of the biomarker (a quantitative approach) or is it for surveillance purposes to detect the presence of a previously absent biomarker (a qualitative approach)? For some health determinants, the meaning of wastewater monitoring results is clear. For example, detection of illicit drugs or their metabolites within wastewater indicates there has been consumption of that drug by people within the catchment region, and changes in the levels detected can be monitored over time and compared across different geographic areas[33]. This can then allow conclusions around trends in drug consumption on different days of the week or times of the year, and differences across regions to be drawn. This information can then be used by Police and border officials for law enforcement, allow health authorities to deliver harm reduction programmes more efficiently, and inform policy changes. Similarly, detection of SARS-CoV-2 RNA fragments in wastewater indicates the presence of COVID-19 cases within the catchment area (WHO 2020b). This information can then be used to direct public health measures. However, for some health determinants the meaning of results from wastewater monitoring might not be so clear. For example, monitoring testosterone levels in wastewater could give information about average testosterone excretion within a catchment zone. However, as results are normalised for population size it would be impossible to tell whether there were certain individuals within the catchment with very high levels of excretion. Additionally, there would be no information around gender distribution in the contributing population which would obviously confound any conclusions, given males generally produce twenty-times more testosterone than females (Southren et al 1967). There are also considerations around this

[33] https://www.police.govt.nz/about-us/publication/national-wastewater-testing-programme-quarter-1-2020

criterion relating to the specificity, sensitivity of detection and stability of biomarkers which will be discussed in phase 2 of this framework.

3.1.8 Is the indicator able to be disaggregated?

The EHI needs to be able to be broken down into population subgroups or areas of particular interest, such as ethnic groups or regional areas.

This criterion has considerable limitations with regards to wastewater monitoring as samples are reflective of the entire population within the catchment and it is impossible to break this down into subgroups. Specific regions can be targeted by altering sampling locations to focus on certain zones within the catchment region, however the social and ethical risks of sampling specific sub-catchments must be considered. For health determinants where it is essential to have information broken down by population demographics, wastewater-based monitoring is unsuitable.

3.1.9 Timely

Data needs to be collected and reported regularly and frequently to ensure it is reflecting current and not historical trends.

When considering this criterion with regards to wastewater monitoring it is important to note that by its very nature, wastewater monitoring reflects the current situation due to the continual flow of wastewater past the sampling site. However, delays could exist post-sampling within the analysis stage of the workflow, and the turnaround time from sampling to reporting would need to take into consideration the urgency of the output. For example, reporting on detection of COVID-19 needs to be rapid to allow an appropriate public health response. Similarly, in cases where wastewater monitoring performs a surveillance role for new infectious diseases or NPS, for example, rapid reporting would also be essential. In contrast, more long-term monitoring programmes such as the drugs in wastewater programme would have less urgency associated with reporting and information on historic trends would still be useful.

3.1.10 Public health impact

The EHI needs to relate to an environmental health issue of significant public health impact to New Zealand. This health impact may include affecting a large number of people, a vulnerable population, or Māori health; or having substantial policy relevance.

This criterion implies that any health determinant chosen for wastewater monitoring must relate to a significant issue for public health in New Zealand. Specifically, it should relate to: an issue affecting a large number of people, more vulnerable groups within society (such as the elderly), or having large health inequalities (such as conditions over-represented in certain groups such as Māori or Pasifika); a serious illness or condition such as severe illnesses, those with long-term repercussions, and/or those with risk of death; issues where there is potential for changes in public health measures or policy change to enhance overall public health (Mason et al 2018).

3.1.11 Presence of alternative methodologies for monitoring the health determinant

In addition to the above EHI criteria, it is also important to examine what other methodologies, if any, are currently being employed to monitor the chosen health determinant. Important questions to consider include: Can wastewater monitoring provide faster, cheaper or more reliable information about this health determinant than current methodologies? Can the biomarker for the chosen health determinant be added into an existing analysis workflow? For example, in the drugs in wastewater programme it is possible to add new compounds into the analysis workflow and gain substantial information for very little additional cost. This approach could mean a health determinant not worthy of the considerable cost associated with establishing/ maintaining a new monitoring workflow can still be examined due to the significantly reduced cost associated with adding it to an existing workflow. Can wastewater monitoring provide complementary or corroborative information? For example, COVID-19 is currently monitored through nasopharyngeal swab testing at community testing centres and routine testing of border workers. However, wastewater monitoring provides a complementary 'surveillance' safety net to detect the presence of cases which may have been missed by these other approaches possibly due to the sometimes-symptomless nature of COVID-19, or community complacency. As discussed above, COVID-19 wastewater monitoring is also capable of detecting cases several days before they are evident from clinical testing (WHO 2020b). If data from wastewater monitoring will not add significantly to data already being collected for the chosen health determinant by other methodologies it is not a good candidate as resources would be better spent on health determinants not already being monitored.

3.2 PHASE 2: IDENTIFICATION AND EVALUATION OF BIOMARKERS

Once a health determinant has been selected as a potential candidate for wastewater-based monitoring, there are several considerations that form the second phase of the evaluation framework:

3.2.1 Identification of potential biomarkers

The first step is to determine if a suitable biomarker exists for the chosen health determinant. The term biomarker with regards to WBE simply refers to a specific human excretion product. The nature of the biomarker will differ depending on whether the health determinant being monitored is chemical or biological in nature. For example, in wastewater monitoring of COVID-19, non-infective RNA fragments of SARS-CoV-2 act as the biomarker (WHO 2020b). In contrast, in the New Zealand wastewater drug testing programme, illicit drugs and/or their metabolites act as the biomarkers (Chappell and Ashmore 2018). It is important to choose a biomarker for which there is considerable pre-existing scientific knowledge, particularly around its metabolism. For example, a drug which is almost completely broken down by the body or is broken down into a wide variety of metabolites may not be suitable as a biomarker. Similarly, biomarkers related to multiple health determinants may also not be suitable as this could confound analyses.

3.2.2 Is the biomarker excreted?

Perhaps the most important consideration in the selection of a biomarker for wastewater monitoring is whether it is excreted in urine or faeces. For ease of analysis, biomarkers that are excreted in urine are preferential (Chen et al 2014). However, methodology can be adapted for those biomarkers excreted in faeces or sorbed onto particulate matter. Ideally, excretion levels should also correlate with dosage or level of exposure to the health determinant. If the chosen biomarker is not excreted or is excreted in insufficient quantities to be detected, then it is unsuitable for wastewater monitoring. It has been suggested that to be detectable a given biomarker should be excreted in μg/L concentrations (Chen et al 2014). However, this is obviously dependent on how many people within the catchment are excreting the biomarker. Pre-existing knowledge of the rate or timing of excretion of the chosen biomarker is crucial. This can often vary between individuals or across population demographics (age, ethnicity, health status) so it is beneficial to have extensive quantitative data across different groups. Additionally, in the case of infectious diseases not every infected individual may shed the biomarker. For example, only around 40% of people infected with COVID-19 shed viral RNA in their faeces (Parasa et al 2020). The relative importance of this depends on whether qualitative or quantitative data is desired. For example, in the case of COVID-19, the potential public health benefits provided by the extra level of surveillance conveyed by wastewater monitoring outweighs the limitation of not all cases being detectable via this method. In contrast, for health determinants where quantitative data is required, it may be inappropriate to use a biomarker not excreted by every individual exposed to that health determinant.

3.2.3 Is the biomarker stable?

The stability of a chosen biomarker is a key consideration for WBE studies. This includes stability in-sewer; during collection, transport and storage; and during analysis (McCall et al 2016). Several studies have been published assessing the stability of various biomarkers in wastewater. However, it is important to acknowledge that many of these studies have focused on biomarker degradation in laboratory sewer reactors rather than directly in-sewer, which can lead to an overestimation of the degradation rate (Choi et al 2020b). Additionally, degradation can vary dramatically between laboratory reactor types (rising main and gravity sewer), as seen for the antihistamine cetirizine (Choi et al 2018). In-sewer stability will also vary with changing environmental conditions, including temperature and pH (McCall et al 2016). It has been proposed that for ‘best practice’ the mean hydraulic retention time of wastewater in sewers should be less than the time taken for 10% degradation of the chosen biomarker ($t_{10\%}$) (O’Brien et al 2017). However, many biomarkers can still provide useful information even if they degrade at a faster rate than this, particularly for qualitative studies (Choi et al 2020a). For example, Choi et al (2018) found that cetirizine degraded by 10% within 45 minutes in a rising main laboratory sewer reactor, but useful information could still be gained from the study. In contrast, some biomarkers will prove too unstable for useful wastewater monitoring. Examples include anserine and carnosine (biomarkers of meat consumption), and HPMA (biomarker of exposure to the toxin acrolein) which are almost completely degraded within one hour in a rising main reactor (Choi et al 2020a). It is important to note that lack of information on biomarker stability does not necessarily preclude the possibility for wastewater monitoring but rather may necessitate pilot studies to determine stability in wastewater.

3.2.4 Is the biomarker specific for human exposure to the health determinant?

To be useful for wastewater monitoring studies, a biomarker must be specifically released into wastewater via human excretion in response to exposure to the chosen health determinant (Gracia-Lor et al 2017b). This means there should not be any other exogenous source contributing to its presence in wastewater, be it as an input or through microbial activity in-sewer. This can be particularly difficult as wastewater often contains not only excreted matter (black water) but also water from showers/baths, sinks, dishwashers and washing machines (grey water). Many biomarkers are present in a wide range of plant or animal tissues or can be produced (or consumed) by microbes (Choi et al 2020a). Additionally, chemical substances can be intentionally added to wastewater, for example caffeine from leftover coffee poured down the drain, or illicit substances flushed down the toilet. One approach to overcome these problems is to monitor for a human-specific metabolite of the biomarker of interest. For example, Gracia-Lor et al (2017a) in their study of caffeine in wastewater monitored for the caffeine specific metabolite 1,7-dimethyluric acid. They then used data from pharmacokinetic studies to back-calculate caffeine consumption. Similarly, in the drugs in wastewater programme, the presence of cocaine is estimated by monitoring its metabolite benzoylecgonine and performing appropriate back-calculations (Chappell and Ashmore 2018). To determine if biomarker presence in wastewater is solely due to human excretion it is possible to compare levels in wastewater with data on average human excretion levels (Choi et al 2020a). In cases where levels in wastewater exceed that which would be expected based on excretion levels in urine/faeces and given the population size, it can be assumed that there are exogenous sources contributing to biomarker presence. For example, Choi et al (2020a) found that riboflavin (vitamin B2) levels in Australian wastewater were more than twice that expected based on average human excretion rates, indicating significant non-human sources. Where there are known significant exogenous sources for a biomarker it may be possible to account for their contribution and subtract it from the total level measured to still obtain useful information on consumption/exposure levels. However, biomarkers for which insufficient information is available to quantify contribution from exogenous sources would be unsuitable for WBE studies.

3.2.5 Can you detect/extract the biomarker from wastewater

Obviously, in order to examine the presence of a biomarker in wastewater, and potentially measure its levels, it must be detectable in wastewater. Wastewater is a complex matrix, comprised of solids, dissolved particles, heavy metals, nutrients, microbes, and other micropollutants (Warwick et al 2013). As such, multiple refining steps are typically required before detection of a biomarker is possible in order to remove any inhibitory substances and concentrate the sample. These steps vary depending on the biomarker being monitored and the detection method. For example, when monitoring for illicit drugs, samples are generally vacuum filtered, the pH adjusted, internal standards for each metabolite being analysed are added, solid phase extraction (SPE) is performed, and the extract is analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS) (Bade et al 2020). In contrast, methods used in SARS-CoV-2 monitoring may involve ultrafiltration, in which samples are passed through centrifugal filters, concentrated, then the RNA extracted directly from the concentrate and used for PCR analysis (Ikner et al 2011); or direct extraction from electronegative membranes, where samples are pH adjusted, passed through the membrane,

then the RNA extracted from the membrane and used for PCR analysis (Ahmed et al 2015). New methodologies are constantly being developed to optimise wastewater monitoring. Interesting progress has also been made into monitoring proteins in wastewater using polymer probes combined with untargeted proteomics, allowing insight into the proteomic profiles of wastewater (Carrascal et al 2020). It is important to note that limits of detection (LOD) for a biomarker will depend on the methodology employed. For example, in the New Zealand wastewater drug testing programme samples are extracted by solid phase extraction (SPE) and analysed by liquid chromatography mass spectrometry (LC-MS/MS) as detailed above. Using this approach, the LOD for benzoylecgonine, a metabolite of cocaine, and methamphetamine is 0.00125 μg/L, whereas for heroin it is 0.0025 μg/L (Chappell and Ashmore 2018). In cases where only very low amounts of biomarker are present in wastewater it may be insufficient to be detected. It is recommended that to be efficiently detected biomarkers should be excreted in the µg/L range (Chen et al 2014).

The method of sample collection will also potentially affect the detectability of a given biomarker. Common wastewater sampling methods include grab, composite and passive sampling. The decision around which methodology to use will depend on the resources available, and consideration of the abundance of the biomarker of interest and proportion of the population excreting it. Grab samples consist of a single sample taken at a given point in time. As such, they provide a 'snapshot' of what is present in wastewater from the sampling location at sampling time (Clesceri et al 1998). Biomarkers excreted by only a small number of people in the population are easily missed if the 'contribution' from these people is added to the wastewater system too far in advance or after the sampling time. In contrast, composite samples consist of multiple sub-samples which are collected either manually as a series of grab samples or by an autosampler (Clesceri et al 1998; Duncan et al 2007). Composite samples can be either flow- or time-weighted. Time-weighted samples are obtained by taking sub-samples of set volume at set intervals over a chosen sampling period, eg, 200 mL every 30 min over a 24-hour period (Hewitt et al 2021). In contrast, flow-weighted samples consist of multiple sub-samples of either varying volume or taken at varying time intervals proportional to the influent flow rate (Duncan et al 2007). Composite samples are therefore representative of the sampling period over which the sub-samples were taken, with biomarker concentration in these samples being an average of the different concentrations present over that time (NZWERF 2002). Sampling frequency can be altered depending on the predicted abundance of the biomarker of interest. For biomarkers of predicted low abundance or where very few people within the population are excreting them, samples will need to be taken more frequently. Similarly, the closer to the expected source that samples are collected, the more frequently you need to sample. For example, when sampling for SARS-CoV-2 RNA directly outside a managed isolation and quarantine (MIQ) facility where there were known COVID-19 cases, Hewitt et al (2021) collected 60 mL samples every 10 min over a 24-hour period. During the same study, samples collected downstream at the inlet to the wastewater treatment plant consisted of 200 mL samples taken every 30 min over the same 24-hour period.

In passive sampling, a sampling device is installed at a site within the wastewater system (eg, manhole, pumping station or wastewater treatment plant) and left to interact with the wastewater for a certain period, which depends on the abundance of the biomarker of interest. The device is then retrieved and analysed. The most classic passive sampling device is the Moore swab, which is essentially a piece of gauze with a string that is left in the wastewater

system for between 1-7 days (Moore 1951). More recently, a promising new passive sampling device has been developed in Australia and validated for detection of SARS-CoV-2 (Schang et al 2021).

The size of the population contributing to the wastewater catchment being monitored may also impact on detectability and should be taken into consideration. For example, when monitoring for SARS-CoV-2 RNA, detection of a single case in a catchment containing 5,000 people (prevalence of 0.02%) is more likely than detection of a single case in a catchment of 100,000 people (prevalence of 0.001%).

3.2.6 Accuracy and validation

For wastewater monitoring to be considered a reliable public health measure, it is essential that results are as accurate as possible. This includes ensuring any new extraction and detection methods are validated to confirm that the biomarker is being efficiently extracted and able to be detected, and that the risk of false negatives and false positives is minimised. To this end it is important to include internal controls in each extraction, as wastewater is dynamic and as such there may be instances where high levels of inhibitors are present which prevent extraction/detection, or inevitable human error, and without controls it could be wrongly assumed that the biomarker is absent (false negative results). In the New Zealand wastewater drug testing programme internal standards for each metabolite to be measured are added to all samples as described above. Similarly, in the COVID-19 wastewater monitoring programme, a cat coronavirus is added to all samples as an internal control[34]. It is also of equal importance to mitigate the risk of false positives, which are particularly relevant for qualitative surveillance monitoring. For example, when reporting on detection of SARS-CoV-2 it is essential that there are no false positive results, which could cause undue concern and potentially an unnecessary public health response. To this end, any positive results must be verified through repeated PCR analysis and repeated extraction and analysis of a stored replicate sample[7].

The accuracy of results from wastewater monitoring are also dependent on excretion profiles of the contributing population. For biomarkers where rates of excretion vary considerably between individuals, the lower the number of people within the population who are excreting the biomarker, the greater the impact of this variability in excretion rates. As the number of people excreting the biomarker increases, the variation in excretion rates will average out to give more consistent and reliable measurements.

Other factors that could lead to sampling bias should also be considered. For example, some biomarkers adhere to the wastewater pipes, leading to perceived lower abundance. Additionally, biomarkers associated with the wastewater solid phase often require extra extraction steps (Hewitt et al 2021) which are essential to ensure detection is not weighted towards those biomarkers preferentially present in the liquid phase.

[34] https://www.esr.cri.nz/our-expertise/covid-19-response/other-covid-19-work/wastewater-faqs/

3.3 CONCLUSIONS

This framework forms a basis for evaluation of putative health determinant candidates being assessed for suitability for wastewater monitoring. Not every criterion will apply to every health determinant, and failure to satisfy a criterion will not necessarily be means for exclusion from wastewater monitoring. However, there are some ‘stop/go’ criterion which must be met for the health determinant to be considered further. This includes the necessity for there to be a suitable, excreted and detectable biomarker for the chosen health determinant. The link between the health determinant and its biomarker must be scientifically valid. Crucially, the health determinant must relate to a significant New Zealand public health issue. Overall, this framework is meant as a guide to inform decisions on health determinant selection, by highlighting those candidates which are best suited to wastewater monitoring.

4. EVALUATION OF HEALTH DETERMINANT CANDIDATES VIA THE WASTEWATER MONITORING FRAMEWORK

In this section, several candidate health determinants will be assessed by the evaluation framework to determine their suitability for wastewater monitoring. This may not involve a complete analysis of all criterion where the candidate health determinant is deemed unsuitable based on partial evaluation.

4.1 PERSISTANT ORGANIC POLLUTANTS AND SELECTED CHEMICALS OF CONCERN BIOMONITORING SURVEYS

Interest has been expressed by the Ministry of Health as to whether wastewater monitoring could replace the New Zealand persistent organic pollutants (POPs) and selected chemicals of concern (SCoC) biomonitoring surveys. In 2004, New Zealand signed the Stockholm Convention on Persistent Organic Pollutants[35]. Signatories are required to set in place systems to reduce or eliminate the release of chemicals listed in the convention into the environment (Table A4). The Stockholm Convention takes into consideration the fact that these chemicals can easily become geographically widely distributed, so protecting the environment and human health requires a global response. Signatories are required to collect monitoring data for the presence of POPs within the population. This data should be comparable, and as such the World Health Organisation (WHO) has established protocol guidelines that they encourage signatories to adhere to for both reliability and comparability (WHO 2007). Prior to signing the Stockholm convention, New Zealand had already begun monitoring for POPs, with the Ministry for the Environment establishing in 1995 the national organochlorines programme to determine the level of contamination of the New Zealand environment. To date, three studies investigating the contamination of breast milk in New Zealand mothers have been published. Sampling for these was carried out in 1987-1988 (Bates et al 1994), 1998 (Bates et al 2002) and 2007-2010 (Mannetje et al 2013). Whilst these studies were informative, they included very small sample sizes (n = 38, 53 and 39 respectively), and only reflect biological accumulation of POPs within a small subset of the general population (breast-feeding women). In 2004, the results of the first study examining levels of POPs in blood serum of the general (non-occupationally exposed) New Zealand adult population were published (Bates et al 2004). Samples were taken during 1996-1997, and results represented data from 1034 females and 800 males from a range of ages, geographical locations and ethnicity (Māori/non-Māori). Persistent organic pollutants detected in these studies are summarised in Table A5. These studies showed that the background body burden for chlorinated POPs, PCDDs and PCBs is low, especially in comparison to international data

[35] www.pops.int

(Mannetje et al 2013). The breast milk studies show that control mechanisms have been effective, with a two-thirds decrease in chlorinated POPs detected in breast milk between 1988-1998, and a further 50% decrease between 1998-2008 (Mannetje et al 2013). Concentrations of the insecticides DDT (or its metabolite DDE) and dieldrin are of more concern as they were detected at mid-range concentrations, higher than reported in some countries (Mannetje et al 2013). In the blood serum study, DDE was found at levels 50-100 times that seen for other pesticides analysed (Bates et al 2004).

In addition to the POPs studies, the Ministry of Health commissioned the Massey University Centre for Public Health Research (CPHR) to assess the levels of selected chemicals of concern (SCoC) in the blood and urine of New Zealanders (Mannetje et al 2018). Samples were taken during 2014-2016 from 319 adults and 303 children, including a range of geographic regions, both genders, Māori and non-Māori. Blood samples were analysed for lead and mercury. Urine samples were more extensively analysed for metals and metalloids (chromium, arsenic, cadmium, thallium, antimony), cotinine (the predominant metabolite of nicotine), fluoride, environmental phenols (BPA, parabens), and phthalate metabolites. Results of this study are summarised in Table A6. In general, concentrations of these chemicals were comparable to those found overseas. However, a main aim of this study was to determine reference values for these chemicals and establish a benchmark for levels in New Zealand to which future studies can be compared. The ability to monitor differences in concentration in different age groups, genders, geographic locations and ethnicities (Māori/non-Māori) was of key importance for this study.

4.1.1 Framework evaluation – POP surveys

The first important point to make when considering the suitability of wastewater monitoring for analysing POP contamination is that given the directive that signatories of the Stockholm Convention should aim to adhere to the protocol guidelines for monitoring set out by the WHO, wastewater monitoring should not replace the breast milk and serum analyses. However, wastewater monitoring could provide a complimentary approach. For this reason, its suitability should still be evaluated by the framework. It is also important to note that POPs are not one health determinant, with over 30 separate chemicals currently listed in the Stockholm Convention[36] (see Appendix); each chemical would therefore need to be considered separately to determine its suitability for wastewater monitoring. An important point to consider is whether population-level data would be informative. With individual testing, the exact chemical concentration burden of each person can be determined. However, with wastewater monitoring the concentrations would be normalised across the entire population, diluting the effects of some individuals having very high levels. Given that the toxicity of many of these chemicals is dose-dependent a wastewater monitoring approach may not be suitable. Nevertheless, two selected POPs have been examined by the framework to highlight those additional criteria which will be most important for assessment, with particular focus on the ‘stop/go’ criteria. The two selected POPs are the pesticides dieldrin and DDT, as they were shown to be present at concerning levels in the biomonitoring surveys.

[36] http://www.pops.int/TheConvention/ThePOPs/AllPOPs/tabid/2509/Default.aspx

The first step in assessing these two chemicals is to determine if a suitable biomarker exists for each. This requires information about their metabolism to determine whether the chemical itself or a metabolite would be suitable for monitoring. Dieldrin is an Annex A chemical in the Stockholm convention meaning that signatories should take steps to eliminate its production and use. Dieldrin is a pesticide in its own right but can also be formed via the rapid metabolism of another POP, aldrin (de Jong 1991; ATSDR 1993). Dieldrin is slowly metabolised to form 9-hydroxydieldrin, which is excreted primarily in faeces and to a lesser extent in urine and could therefore be a suitable biomarker[37].

DDT is an Annex B chemical, meaning that signatories should take steps to restrict its production and use. DDT is excreted in urine in its unmetabolized form but is also metabolised to DDA, which is then excreted (Baselt 1982). DDT can also be metabolised to DDE, but this metabolite is generally stored in adipose tissue rather than excreted. DDT is very slowly eliminated from the body at the rate of around 1% of the stored levels per day. DDA was found to be undetectable in unexposed individuals, and average DDT concentration in unexposed individuals was only 0.007 mg/L (Baselt 1982). Whilst this indicates that DDT and/or DDA could be used as a biomarker as they are both excreted in urine, further analysis would be needed to determine if their low-level excretion would be detectable in wastewater.

Low-level excretion of DDT due to its storage within the body highlights an important caveat for use of wastewater monitoring for POPs. These chemicals are often stored in fatty tissues and so studies of serum are informative as cumulative exposure can be assessed. Wastewater monitoring would only be able to assess the level of POPs which are being actively excreted by the body and not stored, providing no information on cumulative exposure. This may also impact on the ability of these biomarkers to be sensitive to changes in exposure levels, in regard to their abundance in wastewater. Both 9-hydroxydieldrin and DDT would need to be further assessed by the remaining framework criteria to determine if they are specific for the health determinant, if there are other sources in wastewater, if methodology exists for their extraction and detection, and if they are stable in wastewater. Given there is already a requirement to monitor exposure to these chemicals as part of the Stockholm Convention, they clearly meet the criterion regarding public health significance. However, an important feature of the biomonitoring surveys is the ability to disaggregate the data to provide information on levels within different age, gender, ethnic groups and geographical location. Given the nature of wastewater monitoring, it is impossible to gain this information, with the exception of broad geographical information based on sampling locations. Future analysis of the feasibility of monitoring POPs in wastewater should consider whether there is potential for a combined sampling, extraction and analysis workflow for these chemicals, as monitoring for each chemical independently would likely be prohibitively costly.

4.1.2 Framework evaluation – selected chemicals of concern surveys

Similar to POPs, the selected chemicals of concern surveys monitor a number of different chemicals, which must all be considered potential health determinants and assessed independently through the framework. It is important to note from the outset that the Mannetje et al (2018) biomonitoring survey placed particular emphasis on the importance of being able

[37] http://www.inchem.org/documents/pims/chemical/pim573.htm

to examine differences in contamination levels across different age groups, genders, locations and ethnicities. As this is not possible with wastewater monitoring, apart from geographic location based on sampling sites, it may not be a suitable replacement for the biomonitoring surveys. However, wastewater monitoring may provide a valuable complementary approach. For this reason, the suitability of the selected chemicals of concern as candidates for wastewater monitoring will still be analysed using the framework. Additionally, as for the POPs, the value of population-level data versus concentration data for individuals would need to be assessed to determine whether wastewater monitoring would be informative, or whether any effects would be diluted out once values were normalised for population size. With the exclusion of mercury and lead, the chemicals of concern were all measured in urine in the first biomonitoring survey (Mannetje et al, 2018). Mercury is known to be excreted in urine and faeces (Nuttall 2004), and metallic or elemental mercury and inorganic mercury salts are often assessed in urine[38]. However, organic mercury compounds and methyl mercury are generally assessed in blood. As these are the most common dietary sources of mercury contamination, this was the method employed in the biomonitoring survey (Mannetje et al 2018). Wastewater monitoring may still be useful for measuring mercury contamination from metallic/elemental mercury and inorganic mercury salts, in addition to the blood biomonitoring surveys. Lead is also excreted in urine; however, its excretion is influenced by urinary flow rate (Sommar et al 2014) and may not be fully indicative of recent exposure due to storage within the body (Mannetje et al 2018). For these reasons, lead would likely be unsuitable for wastewater monitoring.

Similarly to the POPs evaluation, two candidates from the selected chemicals of concern will be assessed by the framework. These are the heavy metal cadmium and the nicotine metabolite, cotinine. Both chemicals are known to be detectable in wastewater (Castiglioni et al 2015b; Agoro et al 2020). However, it is important to determine if there are other sources in addition to human excretion contributing to their presence in wastewater. For example, cadmium is detectable at relatively high levels in wastewater, but this has been attributed to it entering via a variety of non-human sources including food waste, detergents and body care products, impurities in galvanised pipes, and run-off from roads and farms entering stormwater drains that feed into the wastewater system (Agoro et al 2020). As it would be impossible to distinguish human and non-human sources, cadmium would not be a suitable candidate for wastewater monitoring.

In contrast to cadmium, cotinine has already been the subject of numerous international WBE studies into tobacco consumption, as it is a key metabolite of nicotine (Lopes et al 2014; Rodríguez-Álvarez et al 2014; Castiglioni et al 2015b; Baz-Lomba et al 2016b; Lai et al 2018; Mackie et al 2019). As there is a precedent for monitoring cotinine in wastewater, it is a member of the selected chemicals of concern, and it is indicative of nicotine consumption it is a very good candidate to consider further for wastewater monitoring in New Zealand.

In assessment of the remaining selected chemicals of concern, it will be crucial to initially determine if they are stable and detectable in wastewater, and if there are any other non-human sources which may be contributing to their presence in wastewater before further assessment is made. As for the POPs, the possibility of assessing many of these chemicals using the same sampling, extraction and analysis workflow should be considered.

[38] https://labtestsonline.org/tests/mercury

4.2 ILLICIT SUBSTANCES

As described above, wastewater monitoring for illicit substances is well-established world-wide. The New Zealand programme provides invaluable information for the New Zealand Police, Customs and Ministry of Health on patterns and changes in usage of these substances across the country. For example, heroin is generally undetectable in samples due to low opiate use within New Zealand[39]. Increased detection could signal important changes to the New Zealand illicit drugs market. Fentanyl has also only recently been added to the illicit drugs analysis workflow and a baseline for its consumption is still being determined so that any changes in usage can be monitored. This is confounded by fentanyl also being legally prescribed for analgesia and sedation[40]. An important feature of the illicit drug monitoring programme is that multiple metabolites can be analysed using the same sampling, extraction and analysis workflow, making it both time and cost effective. Methamphetamine (4-hydroxy-N-methylamphetamine), cocaine (benzoylecgonine and ecgonine methyl ester), fentanyl (norfentanyl), heroin (6-acetylmorphine and morphine) and MDMA (4-hydroxy-3-methoxymethamphetamine), and their metabolites (indicated in brackets), are all collectively extracted from wastewater by solid phase extraction (SPE) then analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS) (Chappell and Ashmore 2018). The different metabolites are then distinguished based on expected signal patterns and inclusion of reference standards. Not all substances covered by the Misuse of Drugs Act 1975 are currently monitored in wastewater in New Zealand. Two examples, morphine and lysergic acid diethylamide (LSD) have been analysed by the evaluation framework for their suitability for inclusion.

4.2.1 Framework evaluation – morphine

Morphine is a complicated biomarker with regards to wastewater monitoring. Whilst not strictly monitored for its usage as a class B drug it its own right, it is monitored as a metabolite of heroin. Metabolism of heroin produces 6-acetylmorphine (6-MAM) and morphine. 6-MAM is a heroin-specific metabolite but represents only around 1% of total heroin metabolised. This highlights a key limitation of wastewater monitoring for morphine with regards to the framework, as it is not specific to consumption of morphine. Additionally, morphine is also frequently legally prescribed for therapeutic uses[41], meaning there are other sources in wastewater than just from illicit drug consumption. These limitations as they apply to heroin are addressed by the New Zealand programme, where in the absence of 6-MAM detection, back calculations of consumption are not performed using morphine (Chappell and Ashmore 2018). As 6-MAM represents only 1% of heroin metabolised, and heroin usage in New Zealand is low, 6-MAM is generally not detected. Given the morphine present in wastewater is not solely due its consumption as an illicit substance, it is unsuitable for wastewater monitoring.

[39] https://www.police.govt.nz/about-us/publication/national-wastewater-testing-programme-quarter-1-2019
[40] https://www.medsafe.govt.nz/profs/Datasheet/f/Fentanylinj.pdf
[41] https://www.medsafe.govt.nz/profs/Datasheet/l/lamorphtab.pdf

4.2.2 Framework evaluation - LSD

Despite LSD being an important class A drug, there are very few wastewater monitoring studies which include LSD. This is likely due to LSD having a very low active dose, with effects seen from around 15 µg, and a heavy dose being only 300 µg (Baquiran and Khalili 2021). For example, a 2014 study analysing wastewaters in Sweden reported that LSD could not be detected above the limit of quantification of 10 ng/L (Östman et al 2014). With regards to the evaluation framework, this means that LSD would likely not be reliably detectable in wastewater, particularly in the absence of high consumption. For this reason, LSD is not a suitable candidate for wastewater monitoring.

4.3 NEW PSYCHOACTIVE SUBSTANCES

New psychoactive substances have already been the subject of two WBE studies in New Zealand. In a pilot study looking at illicit substances in wastewater in Auckland, the synthetic cannabinoid JWH-018 and methylone, a common ecstasy substitute were detected, albeit at relatively low levels (Lai et al 2017). Interestingly, mephedrone (bath salts), arguably one of the most popular NPS in New Zealand based on data from the Illicit Drug Monitoring System (IDMS), was not detected (Wilkins et al 2015). Although this has been suggested to be due to sampling during winter, when there were no events (eg, festivals) that may be associated with increased NPS consumption. The second more recent study focused on NPS consumption at four holiday destinations around New Zealand as part of a larger study into NPS usage over New Years in New Zealand, Australia, China, Spain, Italy, The Netherlands, Norway and the USA (Bade et al 2021). This study screened for the presence of over 200 NPS, with 26 being quantifiably examined. N-ethylpentylone, mephedrone, methylone, and eutylone were all detected in New Zealand wastewaters. Ketamine, and its metabolite norketamine, while not strictly NPS, were also detected. Another round of sampling was undertaken during the 2020-2021 New Year's period, and results are expected towards the end of 2021.

4.3.1 Framework evaluation

Similar to POPs, NPS include a potentially limitless number of biomarkers that could be examined by wastewater monitoring. However, in contrast to POPs, monitoring for NPS has the advantage that these can often be extracted and analysed together, and then individual NPS detected based on their expected peak in the mass spectrum. Moreover, to a certain extent you do not necessarily need to know exactly what you are looking for when running your analysis. For example, it is possible to screen mass spectra for peaks indicative of slight changes to the chemistry of known NPS, which may indicate the presence of a novel NPS which could then be interrogated further. It would not be possible to quantify these substances as this would require inclusion of a standard, but they may be useful as a qualitative measure. Additionally, it may also be possible to retrospectively screen spectra for a given NPS in light of new information such as a hospital admission or police seizure. The caveat is that absence of a peak may not mean the NPS was absent but rather that it was not extracted. It is important to note that this non-directed approach would only be possible for NPS that are amenable to the same extraction and detection method. There are also several limitations to the use of wastewater monitoring for NPS with respect to the evaluation framework. Firstly, very little is known about the metabolism of most NPS, so monitoring relies on detection of the parent

compound as the biomarker. If the NPS is well metabolised there will be very little parent compound left and it will likely not be detected. Detection is a major limitation of wastewater monitoring for NPS as these substances often have very high potency and so people do not need to use much to experience their effects. Combined with the relatively small number of people using a given NPS, they can be very difficult to detect and may fall below the limits of detection of the methodology used. Lastly, the fact that NPS are constantly changing means that comparability is often difficult. New NPS constantly enter the market and the prevalence of established NPS also varies making it difficult to examine long-term trends.

4.4 INFECTIOUS DISEASES

ESR is already monitoring wastewater for SARS-CoV-2 as part of New Zealand's COVID-19 surveillance strategy[42]. Considerable scope exists for expansion of this monitoring into a border health role. Monitoring of wastewater from international aircraft could provide an indirect means of assessing all new arrivals, complementing the current managed isolation nasopharyngeal testing regime. SARS-CoV-2 has already been shown to be detectable in wastewater from a commercial aircraft and cruise ship (Ahmed et al, 2020b). Furthermore, wastewater specifically from international airports could be monitored to indicate the potential arrival of infected individuals or spread of cases within border/airport workers.

The wastewater monitoring infrastructure that has been established in New Zealand as part of the COVID-19 response has the potential to be applied to a diverse range of infectious diseases. These could include diseases already present in the country, with the aim to track regional outbreaks. For example, wastewater monitoring has been used to track measles outbreaks in the Netherlands (Benschop et al 2017). Wastewater monitoring could also be used for early detection of new emerging diseases identified overseas. In this case, a border health surveillance approach as detailed above could be particularly relevant.

4.4.1 Framework evaluation – COVID-19

As COVID-19 is already the subject of wastewater monitoring programmes around the globe, this evaluation will be used to highlight the major advantages and limitations it presents with regards to the framework. The first important point is that given the very small number of COVID-19 cases in New Zealand, the monitoring fulfils more of a qualitative surveillance role rather than providing quantitative information on absolute number of cases. For this reason, considerations around the most accurate method for normalising results for population size are less crucial than they may be in other parts of the world, or indeed for other infectious diseases, where absolute number of cases is important (Polo et al 2020).

With regards to detection, wastewater monitoring is unlikely to reproducibly detect a single case within a catchment zone as the viral particles would be too diluted[43]. However, this is dependent on the catchment size and the rate at which the virus is shed, which is often highly variable between individuals (reviewed in Jones et al 2020). In New Zealand, recent analysis has estimated the probability of detection of 10 cases per 120,000 people (0.01% prevalence)

[42] https://www.esr.cri.nz/our-expertise/covid-19-response/other-covid-19-work/wastewater-faqs/
[43] https://www.esr.cri.nz/our-expertise/covid-19-response/other-covid-19-work/wastewater-faqs/

as 78% (Hewitt et al 2021). It is possible that detection would still be observed with fewer cases than this, but it is dependent on how much virus the infected individuals are shedding. Given this variability it is impossible to determine exactly how many infected individuals are present in a catchment zone based on the level of SARS-CoV-2 RNA detected. Nor is it possible to identify infected individuals based on monitoring data. Additionally, not all individuals infected with COVID-19 shed detectable levels of viral RNA in their faeces, so some cases will not be detected in wastewater (Parasa et al 2020).

Monitoring for COVID-19 is highly specific and any detection is confirmed using both internal standards and repeat analyses. This ensures that any reported presence of COVID-19 within a catchment is accurate. However, the caveat is that some people continue to shed viral particles after they have recovered and are no longer infectious. This can lead to detection in community wastewater samples once that person has been released from quarantine.

The major advantages of wastewater monitoring for COVID-19 as a complementary surveillance approach is that, as discussed above, it can detect cases within the population in advance of detection by clinical screening (WHO 2020b). This can alert regional health authorities to the potential need for elevated screening. Additionally, it allows potentially millions of people to be non-invasively screened en masse. This obviously allows many more individuals to be screened than could be feasible by clinical screening approaches. Thus, despite the limitations of wastewater monitoring for COVID-19, it still forms an essential part of the New Zealand surveillance program. This highlights the fact that wastewater monitoring for infectious diseases can still be very useful despite potential limitations, and decisions around whether a candidate disease is suitable for monitoring must weigh the putative benefits against the limitations.

5. CONCLUSIONS

Wastewater-Based Epidemiology has emerged globally as a powerful tool to monitor exposure to a wide range of substances and organisms. Wastewater monitoring can provide population-level, unbiased information on the overall health of a community, and can be used to inform public health responses and policy changes. In New Zealand, it is currently used to monitor illicit drug consumption and plays an important surveillance role in New Zealand's COVID-19 response; however, significant potential exists to expand wastewater monitoring in New Zealand to a wide range of other applications.

Several possible health determinants for wastewater monitoring have been presented in this report, and some promising candidates for further investigation have been identified. These include potential expansion of the drugs in wastewater programme to include new psychoactive substances. Successful pilot studies have already been performed in conjunction with Australian researchers. This programme could be further expanded to include legal substances such as tobacco (nicotine) and alcohol. Monitoring of tobacco consumption across the country could provide important information on usage patterns and inform the Smokefree Aotearoa 2025 programme. Similarly, information from monitoring of spatial and temporal alcohol consumption patterns could identify potentially 'at-risk' regions with high rates of consumption, directing the need for additional support in these areas.

Global health is facing perhaps its biggest challenge with the rise of antimicrobial resistance. Wastewater monitoring is proving invaluable for gaining a comprehensive picture of the diversity of AMR genes present in the environment. Although not yet a major problem in New Zealand, the threat posed by AMR is imminent, particularly given antibiotic usage in New Zealand healthcare supersedes that of many other developed nations. Wastewater monitoring of antimicrobial consumption in concert with surveillance for AMR could provide a powerful approach to direct education and public health responses, and potentially inform policy changes.

The value of wastewater monitoring as a component of infectious disease surveillance has been demonstrated by its essential role in global COVID-19 responses. Potential exists to expand this monitoring into the border health area by targeting wastewater from inbound international aircraft and international airports. Substantial infrastructure has been established both in New Zealand and globally to support wastewater monitoring for SARS-CoV-2. This provides an ideal opportunity for expansion of the COVID-19 wastewater monitoring programme to a wide range of infectious diseases, including diseases known to be present in New Zealand (eg, measles, common enteric viruses) and new emerging diseases. As such, WBE has huge potential to be developed as a complementary public health tool in New Zealand for monitoring spread of infectious diseases. This area of WBE is rapidly developing and has a very promising future as an important global health tool.

Wastewater-Based Epidemiology approaches for monitoring environmental contaminants and endogenous biomarkers of disease are still relatively in their infancy. Whilst these approaches are promising, caution must be taken to ensure they are scientifically sound before they are adopted in New Zealand.

There are many potential candidates for wastewater monitoring in New Zealand. However, limited resources necessitate important decisions around which are the most beneficial from a public health perspective. This report provides a framework for evaluating the suitability of candidate health determinants for wastewater monitoring. All decisions will need to weigh perceived benefits against the limitations outlined in this report.

Finally, but very importantly, any decisions around expansion of wastewater monitoring in New Zealand will need to include careful consideration of any social and ethical implications of the chosen programme, including responsibilities to Māori under Te Tiriti o Waitangi.

APPENDIX

Table 2. Summary of global New Psychoactive Substances (NPS) in wastewater monitoring studies. Adapted from Gent and Paul (2021).

Year	Country	NPS	Reference
2013	United Kingdom	Methcathinone, mephedrone, butylone, 4-FPP, 2-MEOPP, MBZP	Mwenesongole et al (2013)
	The Netherlands	Ketamine	Bijlsma et al (2013)
	The Netherlands	MDA, fentanyl, ketamine, methcathinone	Van der Aa et al (2013)
	Norway	Cathine, JWH-018 N-5-hydroxypentyl	Reid et al (2014)
	Australia	BZP, mephedrone, methylone	Lai et al (2013)
	Australia	Mephedrone, methylone, MDPV, BZP, methcathinone, TFMPP	Chen et al (2013)
	Belgium	Ketamine	Van Nuijs et al (2013)
2014	United Kingdom	TFMPP, MDA, ephedrine, ketamine, norketamine	Baker et al (2014)
	Finland	MDPV	Kankaanpää et al (2014)
	Finland	MDPV	Vuori et al (2014)

2015	Belgium and Switzerland	methoxetamine, butylone, ethylone, PMMA	Kinyua et al (2015)
	Greece	JWH-210, JWH-122, CP47-497, α-PVP	Borova et al (2015)
	Italy	Ketamine, mephedrone	Castiglioni et al (2015a)
	Croatia	Flephedrone, methylone, methedrone, mephedrone, ketamine, norketamine	Senta et al (2015)
2016	United Kingdom, Spain and Italy	MEPH, METC, 4-FMC, 4-MEC, ETHL, MDPV, DCAT	González-Mariño et al (2016a)
	United Kingdom	MDA, MDEA, ephedrine, mephedrone, ketamine	Castrignanò et al (2016)
	Italy	Buphedrone, 4-MEC, pentedrone	González-Mariño et al (2016b)
	Norway	Methylone, ketamine, methoxetamine, JWH-073, XLR-11, UR-144, AM-2201, JWH-018, MAM-2201, JWH-122, JWH-018 N-pentanoic acid, JWH 018 N-5-hydroxypentyl, JWH-073 N-butanoic acid, JWH-073 N-4-hydroxybutyl, JWH-122 N-5-hydroxypentyl, AM-2201 N-4-hydroxypentyl and RCS-4 N-5-hydroxypentyl	Baz-Lomba et al (2016a)
	Australia	Methylone, ketamine	Van Dyken et al (2016)
	Australia	Methylone, methcathinone, MDPV, mephedrone, TFMPP, BZP, α-PVP	Tscharke et al (2016b)
	Australia	Methylone	Thai et al (2016)
	Poland	Mephedrone, 4-MEC	Styszko et al (2016)
2017	United Kingdom, Belgium, Spain, Norway, Denmark, Italy, The Netherlands and Switzerland	Mephedrone, methylone, MDPV	Bade et al (2017)
	China	BZP, MDPV	Gao et al (2017)

	The Netherlands	MMA, 4-FA, MDEA, mCPP, 2C-B, fentanyl, 'L-759,633	Causanilles et al (2017)
	Spain	PMA, BUF, 4-MeO-PCP	Andrés-Costa et al (2017)
	Spain	Methylone, flephedrone, buphedrone, B = butylone, 4-MEP, mephedrone, pentedrone,3,4-DMMC, α-PVP, MDPV	Fontanals et al (2017)
	Australia	MDPV, MDA, 25H-NBome, α-PVP, ketamine	Bade et al (2018)
	USA	Mephedrone, PB-22, JWH-073, JWH-018	Asimakopoulos et al (2017)
	New Zealand	JWH-018, methylone	Lai et al (2017)
	South Africa	Mephedrone	Archer et al (2018)
2018	Norway, United Kingdom, Denmark, The Netherlands, Belgium, Switzerland, Italy and Spain	Mephedrone, MDA	Castrignanò et al (2018)
	China	Ephedrine, PMMA, ketamine, methcathinone, TFMPP, 2C-I	Chen et al (2018)
	Australia	Butylone, ethylone, a-PVP, methcathinone, MDPV, pentylone, mephedrone	Bade et al (2019b)
2019	Germany, Italy, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, the Netherlands, Ukraine	3,4-DMeO-α-PVP, 25H-NBoMe, 2-MA, DOIP, AMT, PMA, 2-PEA, N-methyl-2AI, DOIP, AMT, 25E-NBoMe, HDMP-28, isopropyl phenidate, AMB-FUBINACA, diphenidine	Salgueiro-González et al (2019)
	Greece	MDAI, methoxetamine, 2-Phenethylamine, N-Ethyl-Amphetamine, PMA, PMMA, O-Desmethyl-Tramadol, diphenhydramine, gabapentin, GHB, orphenadrine, pregabalin, quetiapine, venlafaxine, MePPP, MBZP, ethylphenidate,	Diamanti et al (2019)

		memantine, bufotenin (5-OH-DMT), AB--CHMINACA, methedrone, methoxyphenamine, DMAA, DMT	
	Spain	Ketamine, dipentylone, butylone, mephedrone, methedrone, methylone	Celma et al (2019)
	Australia	Mephedrone, methylone	Bannwarth et al (2019)
	Australia	4-FA, MDA, 5F-APINACA, 5F-APINACA monohydroxylated, AM-2201, JWH-018, JWH-073, UR-144, UR-144 N-pentanoic acid, 4-FMC, 4-MEC, α-PVP, butylone, ethylone, mephedrone, methcathinone, methylone, pentedrone, pentylone, U-47700, methiopropamine, methoxetamine.	Bade et al (2019c)
	Australia	TFMPP, MDA, a-PVP, ethylone, mephedrone, methcathinone, MDPV, PMA	Bade et al (2019a)
2020	Australia	Butylone, butyryl fentanyl, furanyl fentanyl, methoxetamine, N-ethylpentylone, valeryl fentanyl	Bade et al (2020)
	Maldives	Methylone, butylone, ethylone, mephedrone	Fallati et al (2020)
2021	New Zealand, Australia, China, Spain, Italy, The Netherlands, Norway, USA	25B–NBOMe, 25C–NBOMe, 25I–NBOMe, 3-EMC, 3-methylbuphedrone, 3-MMC, 4-FA, 4-FMC, 4-methylbuphedrone, 4-MEC, AH-7921, buphedrone, butyryl fentanyl, butylone, ethylone, furanyl fentanyl, mephedrone, methcathinone, methiopropamine, methoxetamine, MDPV, methylone, N-ethylpentylone, pentylone, U-47700, valeryl fentanyl	Bade et al (2021)

Table 3. Environmental Health Indicator selection criteria. Adapted from Mason et al (2018).

Indicator selection criteria	Explanation
Available data	Indicator must have data that can be easily and reliably extracted.
Scientifically valid	Indicator must have an established, scientifically-sound link to the environmental health issue.
Sensitive	Indicator should respond relatively quickly and noticeably to changes, but not show false movements.
Consistent	Indicator should be consistent with those used in other indicator monitoring programmes (including internationally), so comparisons can be made.
Comparable	Indicator should be consistent to allow comparisons over time.
Methodologically sound measurement	Indicator measurements need to be methodologically sound.
Intelligible and easily interpreted	Indicator should be sufficiently simple to be interpreted in practice and be intuitive in the sense that it is obvious what the indicator is measuring.
Able to be disaggregated	Indicator needs to be able to be broken down into population subgroups or areas of particular interest, such as ethnic groups or regional areas.
Timely	Data needs to be collected and reported regularly and frequently, to ensure it is reflecting current and not historical trends.
Public Health impact	Indicator needs to relate to an environmental health issue of significant public health impact to New Zealand. This health impact may include affecting a large number of people, a vulnerable population, or Māori health; or having substantial policy relevance.

Table 4. Persistent Organic Pollutants (POPs) listed in the Stockholm Convention.

	Pesticide	Industrial Chemical
Annex A (Elimination)	Aldrin	Hexabromodiphenyl and heptabromodiphenyl ethers (commercial octabromodiphenyl ether)
	Chlordane	Hexabromocyclododecane (HBCDD)
	Chlordecone	Decabromodiphenyl ether (commercial mixture, c-decaBDE)
	Dicofol	Hexabromophenyl
	Dieldrin	Hexachlorobenzene (HCB)
	Endrin	Hexachlorobutadiene (HCBD)
	Heptachlor	Pentachlorobenzene
	Alpha hexachlorocyclohexane	Polychlorinated biphenyls (PCB)
	Beta hexachlorocyclohexane	Polychlorinated naphthalenes (PCNs)
	Lindane	Perfluorooctanoic acid (PFOA), its salts and related compounds
	Mirex	Short-chained chlorinated paraffins
	Pentachlorobenzene	Tetrabromodiphenyl ether and pentabromodiphenyl ether (commercial pentabromodiphenyl ether)
	Pentachlorophenol, its salts and esters (PCP)	
	Technical endosulfan and its related isomers	
	Toxaphene	
Annex B (Restriction)	DDT	Perfluorooctane sulfonic acid (PFOS), its salts and PFOS-fluoride (PFOS-F) (also an insecticide)
Annex C (unintentional production)	Hexachlorobenzene	Hexachlorobutadiene (HCBD)
	Pentachlorobenzene (PeCB)	Polychlorinated biphenyls (PCB)
	Polychlorinated dibenzo-p-dioxins (PCDD)	Polychlorinated dibenzofurans (PCDF)
	Polychlorinated naphthalenes (PCNs)	

Annex C chemicals are all unintentional by-products.

Table 5. Persistent organic pollutants (POPs) detected in New Zealand breast milk and serum biomonitoring surveys.

Class of Substance	POPs Tested	POPs Detected*	Chemical names	Reference
OCPs	13	3	Hexachlorobenzene (HCB), dieldrin, DDT	Bates et al (1994)
PCBs	16	3	138, 153, 180	Bates et al (1994)
PCDDs/PCDFs	15	11	2,3,7,8-TCDF, 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDF/ 1,2,3,6,7,8-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,7,8-HxCDD/ 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,6,7,8-HpCDD, OCDD	Bates et al (1994)
OCPs	4	4	β- HCH, HCB, dieldrin, DDT	Bates et al (2002)
PCBs	6	6	74, 118, 138, 153, 170, 180	Bates et al (2002)
PCDDs/PCDFs	12	12	2,3,7,8-TCDF, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDD, 1,2,3,7,8,9-HxCDF, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, 1,2,3,4,6,7,8-HpCDD, OCDF, OCDD	Bates et al (2002)
OCPs	13	8	α-HCH, β-HCH, HCB, dieldrin, heptachlor, chlordane, DDT, mirex	Mannetje et al (2013)
PCBs	43	35	15, 28, 37, 44, 49, 52, 70, 74, 99, 101, 105, 110, 114, 118, 123, 126, 138, 153, 155, 156, 157, 167, 169, 170, 180, 183, 187, 189, 194, 196/203, 202, 205, 206, 208, 209	Mannetje et al (2013)
PCDDs/PCDFs	17	9	2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD	Mannetje et al (2013)
OCPs	9	3	β-HCH, dieldrin, DDT (detected as DDE)	Bates et al (2004)
PCBs	10	10	74, 118, 126, 138/158, 153, 169, 170, 180, 187, 194	Bates et al (2004)

PCDDs/PCDFs	17	14	2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF	Bates et al (2004)

*Above LOD in majority of samples tested. Detection of metabolites of a POP are treated as detection of that POP. Eg, DDE detection is indicative of DDT. PCB, polychlorinated biphenyls; PCDD, polychlorinated dibenzo-*p*-dioxins; PCDF, polychlorinated dibenzofurans; OCP, organochlorine pesticides. HCH, hexachlorocyclohexane.

Table 6. Frequency of detection of selected chemicals of concern in adult and child blood and urine.

Class of Substance	Chemical name	FOD* adults (%)	GM conc. adult (µg/L)	FOD child	GM conc. child (µg/L)
Metal/metalloids	Lead	100	13	100	8
	Mercury	99	1.6	93	0.9
	Arsenic – inorganic	79	4.2	74	3.2
	Arsenic - organic	68	3.5	53	1.5
	Cadmium	89	0.19	53	0.07
	Chromium	59	0.05	44	0.03
	Thallium	97	0.2	79	0.05
	Antimony	90	0.06	99	0.09
Cotinine	Cotinine	11	563	2	12
Fluoride	Fluoride	100	760	100	630
Phenols	BPA	93	1.8	89	2.2
	Triclosan	85	4.8	92	3.9
	BP-3	100	18.4	100	20.8
	tOP	3	-	3	-
Parabens	Methylparaben	100	17.5	100	11.9
	n-propylparaben	100	3.4	99	2.1
	Ethylparaben	100	1.4	98	0.7
	Butylparaben	32	-	33	-

Phthalate metabolites	DMP	4	-	10	-
	DEP	96	19.1	64	12.9
	DBP	100	3.7	100	60.6
	BBzP	82	4.2	93	7.7
	mEHP	82	2	90	3
	mEOHP	99	7	100	14
	mEHHP	96	9	98	18
	DCHP	1	-	0	-
	DOP	63	2.4	76	3.3
	DiNP	1	-	0	-

*FOD, frequency of detection. GM, geometric mean. ND, not determined. mEHP, mEOHP and mEHHP are all metabolites of DEHP. BPA, bisphenol A; BP-3, benzophenone-3; tOP, 4-tert-octylphenol; DMP, dimethyl phthalate; DEP, diethyl phthalate; DBP, dibutyl phthalate; BBzP, butylbenzyl phthalate; DEHP, di(2-ethylhexyl) phthalate; mEHP, mono-2-ethylhexyl phthalate; mEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; mEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; DCHP, dicyclohexyl phthalate; DOP, di-n-octyl phthalate; DiNP, di-iso-nonyl phthalate. Lead and mercury were analysed in blood. The remaining chemicals were analysed in urine.

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