Assessment of *Cryptosporidium* and norovirus risk associated with de facto wastewater reuse in Trinity River, Texas

Keah-Ying Lim, Yiwen Wu, Sunny C. Jiang*

Civil and Environmental Engineering, University of California, Irvine

*Correspondence: 844E Engineering Tower, Civil and Environmental Engineering, University of California, Irvine, CA 92697; email: sjiang@uci.edu; Tel: 949-824-5527

Running title: QMRA of De Facto Reuse

Abbreviations:

AGI: Acute gastrointestinal illnesses

DALYs: disability-adjusted life years

DWTP: drinking water treatment plant

IPR: indirect potable reuse

LT2 rule: Long Term 2 Enhanced Surface Water Treatment Rule

MNV: murine norovirus

NoV-GI: norovirus genome type I

NoV-GII: norovirus genome type II

pppy: per person per year

QMRA: quantitative microbial risk assessment

U.S. EPA: United States Environmental Protection Agency

WHO: World Health Organization

WWTP: wastewater treatment plant

Abstract

De facto reuse refers to the use of surface water that contains a considerable portion of wastewater effluent from upstream communities as a source water for drinking water supplies. In contrast to the highly regulated domestic wastewater reuse practices in the U.S., not much is known regarding the human health risk associated with de facto reuse. Trinity River, Texas, a wastewater dominated river that is used as the main source of drinking water for Houston, is used as an exemplar to quantify the health risk associated with exposure to *Cryptosporidium* and norovirus through drinking water contaminated with wastewater effluents. The results show that the annual infection risks exceed the U.S. EPA recommended annual safe drinking water health risk of 10^{-4} per person per year (pppy) for all scenarios considered. However, a comparison of the corresponding disease burdens with the World Health Organization's annual disease burden for safe drinking water of 10^{-6} DALYs pppy indicates the disease burdens are mostly within the acceptable range of the guideline. This difference in risk interpretations reflects the difference in the fundamental definition of the two risk benchmarks as well as the gaps in data and risk models (including the conversion from infection to illness risk and expected DALY per infection). The results also imply that the current safe drinking water regulatory framework should be re-examined to best achieve the goal of human health protection. The outcomes of this study provide an overall picture of the potential risk associated with de facto reuse, which contributes to a better understanding of the practice and decisions in wastewater reuse in the U.S. and the rest of the world.

Keywords: De facto reuse; QMRA; *Cryptosporidium*; norovirus; drinking water

Introduction

The rapid growth of the global economy and population is placing tremendous pressure on water resources in many parts of the world. In particular, regions in the southwestern United States, Australia, the Middle East, and the Mediterranean are experiencing higher than average water stress [1]. Some metropolises in water stressed regions have relied on importing water from distant water reservoir(s) through extensive water networks of conveyance and distribution systems [2]. For example, Southern California is served by three large water conveyance systems — the State Water Project, the Los Angeles Aqueduct, and the Colorado River Aqueduct — to transfer over 5 million acre-feet of water per year to the region. Yet, the increasing water demands in these regions due to urban sprawling and population growth have presented ever increasing water challenges as the construction and expansion of water distribution systems is no longer feasible due to their significant impact on the environment [3]. In response to these issues, domestic wastewater has been recognized as a new water resource, wherein the wastewater is treated using advanced treatment technologies to meet the increasing water demand [4-7].

Wastewater reuse is classified into two broad categories: non-potable and potable reuse. To protect public health in reuse practices, each category of wastewater reuse usually follows a different set of water treatment requirements and water quality criteria. For example, in the State of California non-potable wastewater reuse is regulated based on Title 22 California Code of Regulations [8, 9]. Based on this code, the intended end uses of the treated wastewater should be used to guide the selection of engineering technologies for treating wastewater.

Almost all current potable reuse practices in the U.S. and other developed countries are categorized as indirect potable reuse (IPR), wherein the highly treated wastewater is stored in an environmental buffer such as a groundwater recharge basin or a surface reservoir and blended with the primary drinking water source. The blended water source is then withdrawn for drinking water production at a later time [2]. Most IPR projects add additional engineering technologies beyond the conventional secondary wastewater treatment processes, which may include microfiltration, reverse osmosis (RO), ultraviolet (UV) radiation, and addition of hydrogen peroxide (H_2O_2) to produce highly purified effluent. The quality of the effluent designated for IPR often surpasses the U.S. EPA and WHO drinking water quality standards [10]. In addition, IPR practices are also often aided by intensive water quality monitoring of effluent and environment reservoir, where the effluent is blended with the primary drinking water source. For example, Orange County Water District in Southern California — the largest IPR producer in the world — operates a vast network of monitoring wells within the groundwater basin receiving IPR effluent and uses (mathematical) models to determine the water levels, the water quality, and the travel time of the IPR effluent from injection sites to drinking water production wells [11].

In contrast to the vast capital investment and intense monitoring requirements of IPR, an unofficial form of wastewater reuse — termed as “de facto reuse” — that is not regulated or monitored by governmental agencies is practiced in many parts in the U.S. and Europe. De facto reuse refers to the use of surface water that contains a considerable portion of wastewater effluent from upstream communities as a source for drinking water supplies. This water practice is common in the lower reaches of Mississippi River, the lower watershed of Trinity River in Texas, and the downstream of Schuylkill River in Pennsylvania [12], wherein the fraction of wastewater effluent in surface water varies regionally and seasonally. Although the wastewater effluent is treated by conventional wastewater treatment plants (WWTPs) to meet the U.S. EPA

surface water discharge requirements, it is not meant to be used as a source for drinking water production.

Past decades have witnessed a considerable increase of de facto reuse in the U.S. In 1980 when the practice was first identified in an U.S. EPA's report, the top 25 most impacted drinking water treatment plants (DWTPs) in the nation had used source water that contained 2% –16% wastewater effluents from upstream locations under average streamflow conditions [13]. Nearly three decades later in 2008, the portion of effluent in the source water used by these DWTPs have increased to a range of 7–100% under low streamflow conditions due to the increase in municipal flows upstream of the DWTPs intakes [1]. The recognition of de facto reuse in drinking water supplies has raised public concerns on its potential risks to human health, which makes the risk assessment of de facto reuse a research priority for human and environmental health protection in the U.S. [5].

The primary objective of this study is to assess the human health risk associated with de facto reuse. Trinity River in Texas, a river that supplies over 50% of the water to a Houston drinking water reservoir, is used as a case example for this study. Trinity River is heavily influenced by upstream wastewater discharge that, at times, constitutes 100% of the river flow [5]. Quantitative microbial risk assessment (QMRA) is carried out to quantify the human health risk from exposure to *Cryptosporidium* (Crypto) and human noroviruses (NoV) — two important waterborne pathogens commonly found in wastewater — through drinking water supply. Site-specific information on streamflow, volume of effluent discharge, pathogen concentration, decay rate, and water residence time are considered in the assessment. A discussion on model uncertainties is then presented to identify data and knowledge gaps that can help improve the health risk assessment. The outcomes of this study provide a comparison of potential health risks associated with de facto reuse with the current safe drinking water guidelines established by the regulatory agencies.

Materials and Methods

Site description

Trinity River is the major Texan river that travels from south central Texas near Dallas/Fort Worth over 200 miles to Lake Livingston due north of Houston. The lake water is used as the source water for drinking water supply in Houston. Under baseflow conditions, Trinity River is dominated by wastewater effluent [5]. A study using flow models indicated that the river water may stay in Lake Livingston for up to a year [12], wherein nearly half of the lake water is made up of wastewater effluent [5] before it is withdrawn for drinking water treatment and distributed to consumers in Houston. However, both the fraction of wastewater effluent in lake water and the water residence time vary significantly with season.

Reference pathogens

Two reference pathogens, Crypto and NoV, are chosen for the risk assessment because they are among the top 15 pathogens causing the highest level of acute gastrointestinal illness (AGI) in the U.S. [12]. In fact, Crypto, an enteric protozoan, is used as the main reference pathogen for the development of EPA's Long Term 2 Enhanced Surface Water Treatment Rule (known as LT2 rule) that is used for regulating the safety of drinking water [14]. Crypto is an intestinal parasite, which caused the largest waterborne outbreak ever documented in the U.S. — over

400,000 people were affected by the outbreak in Milwaukee, WI — when a malfunctioning drinking water treatment plant exposed the communities to the parasite through its water distribution network [15].

Although no specific enteric virus is used as a reference pathogen for the development of LT2 rule, EPA requires all public water utilities to remove or inactivate all enteric viruses from drinking water supplies and to demonstrate the absence of virus in finished water for distribution. Among enteric viruses, NoV is the number one causative agent of AGI in the U.S. that attributed to ~20,800,000 episodes and 569 deaths in the year 2006 [16]. Typical symptoms of NoV-induced AGI usually develop 12 to 24 hours after a person is exposed to NoV, which may include acute-onset of vomiting, watery, non-bloody diarrhea with abdominal cramps and/or nausea, low-grade fever, headaches and/or myalgias [17].

Both Crypto and NoV are typically transmitted to humans via the ingestion of contaminated water and food. However, it is also possible for NoV to be transmitted to humans through inhalation when the NoV source is aerosolized [18]. As the first exemplar of risk assessment for de facto reuse, only the risk from direct ingestion of the water is assessed in this study.

Pathogen concentrations in wastewater effluent

The major WWTPs discharging effluents into Trinity River are managed by the Central Regional Wastewater System (CRWS) located in west Dallas, which collect and treat sewage generated by approximately 3 million people in the Dallas/Fort Worth area. Effluent discharged by these WWTPs undergo a series of conventional treatment processes that consists of preliminary screening, primary sedimentation, activated sludge treatment, and disinfection [19]. Although the water quality of effluent discharging into the Trinity River is monitored to comply with the discharge requirements set by the state of Texas, pathogen concentration in the effluent is not monitored for practical reasons [20].

As an approximation of the pathogen concentration in the effluents discharging to Trinity River, we identified literature reporting the concentration of Crypto and NoV in effluent treated by WWTPs that employed similar conventional activated sludge treatment processes as those managed by the CRWS. Pathogen concentrations reported in these literature were then extracted from graphs and compiled to generate empirical cumulative probability distributions for each pathogen.

The Crypto concentrations in wastewater effluent, $C_{Cryp.-eff}$, as reported in the literature (n=99) are shown in Figure 1 (see APPENDIX Table A.1. for source data and references). In compiling the concentration data for NoV, two genotypes of NoV — NoV GI and NoV GII — are identified. Further studies indicate that although much attention has been focused on NoV GII-related disease outbreaks, NoV GI co-circulates in the human population and is frequently linked to the disease outbreak as well [21]. In fact, the infectivity of NoV GI and GII as estimated from outbreak data are similar to each other [21]. When NoV GI and NoV GII concentration data from wastewater effluent are paired together (n=76 for each NoV genotype), Pearson's correlation indicates a coefficient, r^2 , of 0.93 between the two genotypes — suggesting/supporting the co-occurrence of NoV GI and GII in wastewater effluent (see Figure 2, Panel A). Because NoV GI and NoV GII share similar infectivity and correlate with each other, the sum of the two NoV genotypes concentration is used to represent the total NoV genome concentration. In simulating the total NoV concentration in the effluent, the correlation coefficient between the NoV GI and

NoV GII is used to generate paired GI and GII concentration, where the sum of each pair is used as an overall NoV concentration (see Figure 2, Panel B). The total NoV genome concentrations in wastewater effluent, $C_{NoV-eff}$, as reported in the literature are shown in Figure 1 (see APPENDIX Table A.2. for source data and references). Although a recent scientific breakthrough has made culturing of human NoV possible [22], all the available data for NoV concentration in wastewater effluent are based on quantitative PCR (qPCR) methods — the infectivity of these NoV in the wastewater effluent is unknown. However, it must also be noted that the NoV dose-response data derived from clinical trials were also quantified using qPCR. The similarity between the quantification methods for NoV concentration in wastewater effluents and clinical trial samples reduces the uncertainties in adopting clinical results to environmental exposure, which were often caused by the unknown ratio of genome number of NoV to number of infectious NoV.

Pathogen concentrations in the lake

The pathogen concentration in Lake Livingston is influenced by the volume of wastewater effluent and other water sources flowing into the lake, both of which are subjected to seasonal variation. By assuming that all pathogens originate from the effluent, a simple estimation of pathogen concentration in the lake water, $C_{rp-lake}$, is computed as the product of the pathogen concentration in the wastewater effluent and a dilution rate to account for the fraction of effluent in the total lake water (Equation 1). Three different dilution rates — 15%, 30%, and 45% — are used to represent the variability of the parameter, and also for assessing the sensitivity of the final risk outputs to the parameter. This range, although hypothetical, reflects the knowledge of seasonal variability of effluent volume and the N.R.C. 2012 report indicating up to 50% of lake water consists of wastewater effluent [5].

Pathogen decay from the time of effluent discharge to Trinity River to drinking water uptake from Lake Livingston is calculated based on water residence time in the environment and pathogen-specific decay coefficient. The pathogen decay rates are affected by a number of factors such as water temperature, predation, and UV inactivation. For simplicity, a constant pathogen decay rate under typical water temperature is used to characterize the environmental inactivation of the Crypto and NoV.

The decay rate of Crypto, $k_{Cryp.}$, of 0.0155 day^{-1} in water at temperature of 25°C as reported in a lab-scale study is used in this study [23]. On the other hand, the decay rate of murine norovirus (MNV) is used as a surrogate for human NoV as study based on human NoV is currently not available [24, 25]. The median decay rate of $0.01 \log_{10} \text{ day}^{-1}$ is adopted for NoV, k_{NoV} , which reflects the decay rate of MNV in water at 18°C [26]. These decay rates are adopted as they are the most relevant to the water temperature range in Lake Livingston. Although the actual temperature in Lake Livingston varies seasonally, this variation is not considered in our risk assessment.

Wastewater effluent residence time before drinking water uptake includes the effluent traveling time from Dallas/Fort Worth to Lake Livingston and residence time in the Lake. Three different residence times — 270, 315, and 360 days — are considered, based on the N.R.C. 2012 report indicating that the effluent may reside in the Lake for up to one year before withdrawal for drinking water treatment, but the duration varies significantly with the season. This hypothetical range is adopted to capture the natural variability and to assess the sensitivity of the parameter to

the final risk outcome. Subsequently, the pathogen concentration in the source water withdrawn for drinking water treatment, C_{rp-IN} , is estimated using (Equation 2) to incorporate the pathogen inactivation factor.

Exposure to pathogens

The final pathogen concentration in drinking water delivered to households is determined by incorporating the pathogen removal rate in the DWTP. Since no direct measurement is routinely performed in the DWTP, the pathogen removal rate required by the EPA LT2 rule of open reservoirs intake is applied. A 3-log₁₀ removal credit of Crypto and a 4-log₁₀ removal credit of NoV are assigned to the DWTPs in Houston for treating Lake Livingston's intake water to its finished drinking water [14]. Thus, the pathogen concentration in the finished drinking water, C_{rp-F} , is estimated using (Equation 3). Subsequently, the daily human exposure to pathogens, $Dose_{rp}$, is determined by multiplying C_{rp-F} with the daily volume of water consumption, V (Equation 4).

Daily infection and illness Risk

The health risks as a result of exposure to the Crypto and NoV are expressed in terms of infection and illness risk, which are estimated using their respective dose-response models. In assessing the infection risk of Crypto, $P_{Cryp.-inf}$, the fractional Poisson infection dose-response model developed by Messner et al. [27] is adopted in this study (Equation 5). This model has shown a better fit than past models based on the deviance information criterion. On the other hand, the infection risk of NoV, $P_{NoV-inf}$, is estimated based on the dose-response model developed by Teunis et al. [28] for the non-aggregated form of Norwalk virus (Equation 6). The model is based on human challenge studies using NoV GI. However, it has been frequently applied using combined NoV-GI and GII data for risk assessment [29, 30], especially considering the similar infectivity of GI and GII [21].

Since not all infection cases develop into illness (clinical symptoms), illness risk is frequently characterized as a fraction of the corresponding infection risk. In estimating the illness risk of Crypto, 50% of all Crypto infection cases are assumed to result in illness as proposed in [30]. The daily illness risk of Crypto, $P_{Cryp.-ill}$ is then calculated as the product of the conditional probability of illness, $P_{Cryp.}(ill | inf)$, and the infection risk (Equation 8).

Similarly, the illness risk of NoV, $P_{NoV-ill}$, is conditional on both the infection risk and the NoV dose. Since the illness risk of NoV depends both on the presence of host receptor and host immunity, a conditional illness dose-response model based on the non-aggregated NoV dose (Equation 7) is identified by [28] based on the human clinical trial studies and is adopted in our assessment for NoV illness risk. The illness risk of NoV is calculated using (Equation 8) (see APPENDIX for details of justification of model selection).

Risk characterization

The U.S. EPA safe drinking water annual infection risk of $\leq 10^{-4}$ per person per year (pppy) [14], and the WHO annual disease burden of $\leq 10^{-6}$ DALYs pppy (disability-adjusted life years pppy) [31] are used as benchmarks to characterize the risk of the de facto reuse practice. The annual infection risk takes into account the overall infection cases due to a target pathogen, including

asymptomatic cases that do not exhibit any clinical symptoms. On the other hand, the disease burden metric only accounts for symptomatic infections (i.e. illness), wherein the impacts of the disease are quantified in the form of “life-years” lost.

To facilitate the benchmarking, the daily infection risk due to each reference pathogen is converted to the annual infection risk, $P_{rp-inf,annual}$, following the approach used in [32] — assuming independence of the daily risk from one day to another, and multiple exposure events to the pathogen over a year (Equation 9). This same theory is also applied for calculating the overall infection risk, $P_{overall\ inf,annual}$, attributed to both Crypto and NoV, wherein the infection risk of Crypto is assumed to be independent of the NoV infection risk (Equation 10).

In determining the annual disease burden due to each reference pathogen, $DALY_{rp}$, the annual illness risk (computed using Equation 11) is used in conjunction with the disease burden per illness case for each corresponding pathogen. The disease burden per illness case value of 0.0012 is used for Crypto [33] and 0.00095 for NoV [34]. The overall disease burden due to both Crypto and NoV is the sum of the disease burden due to the individual reference pathogens (Equation 13).

A Monte Carlo approach is used for sampling a set of 10,000 values of pathogen concentration from the corresponding distribution of each reference pathogen. Each set of pathogen concentration is then used in conjunction with various sets of input parameters as described in Table 1 for estimating annual infection risks and disease burdens. All calculation procedures are performed using MATLAB R2014b (The Mathworks, Inc., MA).

Results

Annual infection risk

The annual infection risks due to Crypto, NoV, and both Crypto and NoV as a function of different portion of wastewater effluent in Lake Livingston and lake residence time are shown in Figure 3. Intuitively, the infection risks are negatively correlated to the residence time in the Lake and positively correlated to the fraction of effluent in the Lake. From Figure 3, it can be inferred that the lake water residence time is a more sensitive parameter than the fraction of effluent in the Lake in estimating the infection risk of Crypto and NoV. For context, the median value of Crypto annual infection risk decreased by approximately $0.7 \log_{10}$ pppy with each step wise increase in lake water residence time (45 days) when the portion of waste effluent in the lake is held constant; while only $\leq 0.3 \log_{10}$ pppy decrease was observed with each step-wise decrease in portion of wastewater effluent from 45% to 30% and 15% when the lake water residence time is held constant. Similar trends were observed for NoV, where $> 0.3 \log_{10}$ pppy decrease in annual infection risk is observed with each step-wise increase in lake water residence time, as compared to $< 0.2 \log_{10}$ pppy decrease in annual infection risk with each step-wise decrease in portion of wastewater effluent in the lake.

When the annual infection risks for either pathogen (and both pathogens) are compared with the U.S. EPA annual infection risk benchmark of $\leq 10^{-4}$ pppy, none of the scenarios considered can adequately meet the safe drinking water benchmark. The median values of the annual infection risk of Crypto ranged from 1.2×10^{-4} to 8.8×10^{-3} pppy, where the upper value is almost two orders-of-magnitude higher than the EPA benchmark. As for NoV, the median values of its

annual infection risk ranged from 4.4×10^{-2} to 6.4×10^{-1} pppy, which is two to three orders-of-magnitude higher than the EPA benchmark. The annual infection risk due to both pathogens is nearly identical to that due to NoV alone, suggesting the dominance of NoV infection.

Annual disease burdens

The annual disease burdens due to Crypto, NoV, and both Crypto and NoV as a function of different fraction of wastewater effluent in Lake Livingston and lake residence time are shown in Figure 4. Similar to some of the trends observed in the annual infection risk results, lake water residence time is deemed as a more sensitive parameter than the portion of wastewater effluent in lake water in the calculation of disease burden. However, the comparison of the disease burden results with the WHO's annual disease burden benchmark of $\leq 10^{-6}$ DALYs pppy indicate that disease burdens for many of the scenarios considered met the WHO's benchmark adequately. These outcomes differ considerably from the risk picture presented when annual infection risk is used as a regulatory metric.

In addition, the disease burden of NoV is much lower than that of Crypto; a trend that is opposite of the annual infection risk (see discussion for more details). The median values of the disease burdens of Crypto range from 7.1×10^{-8} to 5.3×10^{-6} DALYs pppy, wherein the upper value is within the same order-of-magnitude as the WHO benchmark. In contrast, the median values of the disease burdens of NoV range from 6.2×10^{-11} to 3.0×10^{-8} DALYs pppy, which is at least two orders-of-magnitude lower than the WHO benchmark. The total disease burdens due to both Crypto and NoV are almost identical to that due to Crypto alone.

Overall, the disease burden results present an opposite risk outcome to that of the annual infection risk. The disease burden results imply a comparatively safe case for de facto reuse of the water whereas the infection risk results imply an unacceptable risk level for public health protection.

Discussion

Although de facto reuse has been practiced by many water utilities in the U.S. since the last century, its potential health risk posted to the often-unknowing consumers has not been carefully examined. The application of de facto reuse in DWTP practice is drastically different than that of planned reuse of wastewater, for which public health risk has been investigated thoroughly before its acceptance by the public in the 2000s. Unlike planned reuse, wherein highly treated wastewater effluents from the treatment plants are recycled for designated uses (i.e., industrial use, open irrigation of fields or groundwater recharge), water affected by de facto reuse is primarily used for producing drinking water often without the knowledge of the consumers. Continuous and direct human exposure to drinking water produced from the de facto reuse of wastewater therefore presents a public health risk that should be addressed in a regulatory manner similar to that of the risk management of planned wastewater reuse.

Risk interpretation

As shown by these results, the annual infection risks of Crypto and NoV associated with de facto reuse of Trinity River are higher than the benchmark for annual infection risk under all the scenarios considered. As implied by the results, prolonging the storage of the source water before its use for the production of drinking water is presumably more important in safeguarding public health than controlling the contribution of waste effluent to the drinking water source. This

prolonged storage time is similar to the protective approach used in the IPR regulation that relies on a groundwater basin or a surface reservoir for further environmental inactivation of pathogens before use for drinking water treatment [35]. Nevertheless, it is also important to note that this observation is derived from the simplistic assumptions of constant water temperature and pathogen decay rate. A more complicated model would take into account the complex interactions of multiple factors, including the precise water intake point of the DWTP from the lake, the seasonal variation of water temperature with respect to the different thermoclines, the lake water's circulation pattern, the fate and transport of pathogens in the lake, and the variation of pathogen inactivation due to the above factors. Thus, it should be further emphasized that our QMRA work is aimed at estimating the magnitude of the risks where the aforementioned complex interactions are idealized by varying the water storage time and contribution of wastewater effluent to the drinking water source.

With that in mind, it is also important to remember that LT2 rule requires source water monitoring over a 2-year period, thus measures that exceed the source water quality requirements would likely trigger remedial actions to address the problem. Local DWTPs may already have mitigation actions in place to deal with the issue. On the other hand, the variability of the wastewater effluent volume and residence time may challenge the water quality testing outcomes. Under the scenario of higher effluent contribution to the lake water and shorter water storage time (i.e. 45% wastewater effluent contribution and 270 days water storage time), the annual infection risk can increase sharply and is more than 100 times higher than the EPA's benchmark. With the ongoing climate change and population growth, the water level of the reservoir will likely drop in response to the increasing water demands and droughts, which effectively increases the contribution of wastewater effluent (which is constantly generated by humans) to the reservoir. In short, it is speculated that the scenarios with higher wastewater effluents in lake water and shorter water storage time are likely to become the norm in the future.

The public health risk of de facto reuse is of even greater concern when the infection risks of NoV is added into the risk picture, where the estimated annual infection risk of NoV is at least two orders-of-magnitude higher than the EPA's benchmark. As discussed later in model comparisons of NoV risk estimation, the risk outcomes presented here are considered conservative estimates. The worst scenario (i.e. lowest dilution of wastewater effluent and shortest duration of environmental inactivation of pathogens) examined in this study implies a 64% chance of NoV infection over a year among the people who consume drinking water affected by de facto reuse. In reviewing this outcome, one may argue that the infection rate indicated by the study outcome has not been observed among population in Houston area as indicated by the lack of reports of NoV disease. However, it is important to note that infection risk does not represent illness risk. As demonstrated in NoV annual illness risk results and presented later in the discussion of disease burden, the NoV disease burden is much lower and falls within the safety margin of WHO guideline. The illness cases observed at the community level are likely a reflection of the disease burden of the de facto reuse. So far, there has not been a biomarker-based study (i.e. antibody to NoV) to examine the NoV infection rate in the area.

All factors taken into consideration, the development of dose-response models is still an active research area with many important questions yet to be addressed [36]. Several different dose-response models for the same pathogen have been proposed by different researchers, which can lead to very different outcomes. To better understand the risk outcomes and their implications, it

is necessary to include a more detailed discussion of uncertainties associated with the risk outcomes.

Uncertainties of the risk outcomes

Intrinsic to all QMRA, there are many uncertainties, including the pathogen concentration in the wastewater effluent, the decay of pathogen in the environment, dose of pathogen exposure to humans and the quantitative dose-response relationship, associated with the risk assessment outcomes. Among all these factors, the dose-response models play a critical role in determining the risk outcomes, yet most of these models are developed based on limited clinical trial data that made it difficult for reaching consensus of the “best-fit” model. This is especially problematic for NoV in comparison to Crypto, for which clinical data from multiple human trials have been gathered to refine model parameters. So far, only two clinical trial studies of human NoV were used for the development of dose-response models [28, 37]. Adding to these uncertainties, there are some subtleties of human’s responses to infection and of disease manifestation upon infection. For example, the “secretor” status of the human subjects is a main factor for determining the subjects’ susceptibility to NoV infection — non-secretors who lack the receptors on intestinal epithelial cells to which NoV bind to as a mean of infection are virtually immune to NoV infection [21, 28, 38]. The dose-response models for NoV are entirely based on dose-response data for the susceptible “secretors” only. The exact portion of non-secretors among the general population is unknown, but is estimated to be around 20% according to Lindesmith et al. [39] and Marionneau et al. [40]. This portion of non-secretors in the general population is not factored in our risk estimate for NoV, which leads to a higher risk estimate.

Furthermore, the development of the dose-response models for NoV is also hampered by aggregation of the viruses in the inoculum used for human challenge studies [28]. The confluent hypergeometric dose-response model for disaggregated NoV was selected for our risk assessment because the aggregation state of NoV in wastewater effluent is not known. This model is also a more conservative model yielding higher risk estimates, which may lead to a more cautious risk management approach. The same model selection decision was also made by many QMRA models involving NoV [29, 30, 41, 42]. However, future research is urgently needed to fill the data gap and to improve the understanding of NoV dose-response for an improved risk assessment result.

As discussed by McBride [42] and Schmidt [36], neglecting the aggregation state of NoV has a big impact on the risk estimate of NoV. In the context of the risk outcomes presented here, the use of disaggregated NoV dose-response model resulted in infection risk that is two to three orders-of-magnitude higher than that for the same dosage of NoV that is aggregated (i.e. using the aggregated NoV dose-response model). Without considering an aggregation factor, Schmidt [36] demonstrated that the same set of NoV dose-response data can be fitted and explained equally well by considering partial immunity against NoV infection among the susceptible “secretors”. On a similar note, Messner et al. have developed dose-response models that are characterized by a single immunity parameter (i.e. fractional Poisson model) for both NoV and Crypto and found that such models usually demonstrate comparable fits to dose-response data than complicated models with more parameters [27, 43]. More details on model fittings to NoV dose-response relationship can be found in [27, 28, 36, 43], and will be not repeated here. Nevertheless, with additional clinical trials and epidemiological data, a new model may emerge to replace the current models for a better estimation of the risk outcome. Ideally, low-dose

clinical trials for pathogens of concern (i.e., both NoV and Crypto), should be designed and conducted to validate the different dose-response models [27, 36, 43].

Disease burdens vs Infection Risks

The WHO annual disease burden for safe drinking water provides another angle of risk interpretation, and in the case of this study, a very different risk interpretation. As shown by comparing the disease burdens and annual infection risks of Crypto, the disease burdens imply much more acceptable risk outcomes under all scenarios as compared to its annual infection risk counterparts. The comparison is even more dramatic for the NoV results — the annual infection risks of NoV are orders of magnitude higher than the U.S. EPA benchmark, but the corresponding NoV disease burdens are within the acceptable range of the WHO disease burden guideline. This observation is mostly due to the conversion of infection to disease risk, where a fixed portion of the infection is used to imply disease risk for Crypto while a conditional illness dose-response model is used to calculate illness risk directly for NoV. The DALY per illness for Crypto and NoV is not that dramatically different.

The two different risk interpretations based on different regulatory frameworks deserve further research, but are beyond the scope of the current study. A simple explanation of the differences may be that the annual infection risk is an overly cautious measure of public health risk, which does not account for the real impacts of the infection (i.e. disease). The illness risk could be much lower than the infection risk. An individual who did not get sick upon repeated exposures to low dose of pathogen may also obtain partial immunity against the pathogen. Furthermore, not much is known of the pathogen dose-dependent relationship of illness except for the NoV study carried out by [28]. Illness risk calculated as a fixed portion of infection risk is usually higher than that based on the dose of pathogen. If the disease burdens of NoV are computed based on a fixed fraction of infection risk as illness risk (i.e. 50% as used by most QMRA study), all the disease burden outcomes would exceed the WHO's DALYs benchmark. Moreover, the disease burden is not a commonly used regulatory metric in the U.S. and there is a lack of region-specific illness data [44]. The conversion of disease burden from infectivity using data collected elsewhere may not be applicable because disease burden of each target pathogen could vary even among developed countries.

Despite its shortcomings, the disease burden approach is an important complement to the infection risk approach. Infection risk due to de facto reuse takes into account all infected people who may or may not exhibit symptoms, which sometimes leads to an overly cautious risk management approach. This is of particular significance when the portion of ill subjects among the infected is trivial or the overall burdens do not disrupt the daily life of the human subject. Disease burden is thus used as a measure for the likely impacts of the illness upon infection, which also acts as a more tangible measure for infection risks.

Conclusion

The public health risk in drinking water affected by de facto reuse has been a neglected issue that needs to be addressed. Using Trinity River in Houston area as an exemplar, our risk assessment results highlighted the considerably high infection risks associated with de facto reuse with respect to the U.S. national benchmark for safe drinking water, but acceptable disease burden with respect to WHO benchmark. So far, there has not been a biomarker-based approach to

verify the infection rates predicated by the risk assessment. The sporadic illness cases that are below the level of current regulatory guideline are also easily missed by the disease surveillance system. The uncertainties of the models and parameters should be improved through additional data collection and analysis. More specifically, the uncertainties of the NoV dose-response models are singled out as the key component to be addressed.

In conclusion, an accurate risk assessment of de facto reuse is needed for devising additional risk management of practices in order to safeguard public health, such as requiring additional water treatment for de facto reuse. In doing so, de facto reuse should be redefined from an “unofficial” planning state to an “official” planning state to improve water quality management. Despite being rejected by the general public sometimes in the U.S., wastewater reuse is deemed as the best solution to supplementing the dwindling water resources in many water stressed cities. Due to its long history and unofficial acceptance by the public, the successful management of de facto reuse would present the ideal buffer in advancing the state of wastewater reuse in the U.S. and other parts of the world.

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Figure Legends

Figure 1. Empirical cumulative probability distribution of *Cryptosporidium* oocyst and norovirus genome concentration in wastewater effluents from the conventional wastewater treatment plants using activated sludge processes.

Figure 2. Panel A: Correlation between norovirus GI and norovirus GII genome concentration in wastewater effluent. The data used for plotting were extracted from the literature. Panel B, Simulated norovirus GI and GII data based on the correlation coefficient (10,000 samples).

Figure 3. Annual infection risks due to the *Cryptosporidium* and norovirus through drinking water impacted by de facto reuse.

Figure 4. Annual disease burdens due to *Cryptosporidium* and norovirus through drinking water impacted by de facto reuse.

Table 1. Parameters used in the model equations

Parameter Description	unit	Symbol	Point estimate(s) or Probability Distribution	Reference(s)
Effluent concentration of Crypto	$\log_{10}(\text{oocyst/L})$	$C_{Cryp.-eff}$	Empirical distribution	[45-49]
Effluent concentration of NoV	$\log_{10}(\text{gc/L})$	$C_{NoV-eff}$	Empirical distribution	[50-53]
Dilution factor for effluent in lake water	dimensionless	$Dilution$	15%, 30%, and 45%	
Environmental decimal decay rate of Crypto	day^{-1}	$k_{Cryp.}$	0.0155	[23]
Environmental decimal decay rate of NoV	day^{-1}	k_{NoV}	0.01	[26]
Duration of environmental decay	days	t	270, 315, and 360	
Daily consumption rate of drinking water	L per person	V	2	[54]
$\log_{10}$ reduction of Crypto by DWTP treatment processes	dimensionless	$LR_{Cryp.}$	3	[14]
$\log_{10}$ reduction of NoV by DWTP treatment processes	dimensionless	LR_{NoV}	4	[14]
Infection dose-response model parameter for Crypto	dimensionless	$P_{Cryp.}$	0.739	[27]
Infection dose-response model parameter for NoV	dimensionless	α	0.04	[28]
	dimensionless	β	0.055	
Conditional illness dose-response model parameter for NoV	dimensionless	r_{NoV}	0.00255	[28]
	dimensionless	η_{NoV}	0.086	
Risk of illness conditional on infection risk due to Crypto	illness case per infection case	$P_{Cryp.}(ill inf)$	0.5	[30]

Disability-adjusted life years due to a single illness case caused by Crypto	DALYs per illness case	$\left(\frac{DALY}{\text{illness case}} \right)_{Cryp.}$	0.0012	[33]
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Disability-adjusted life years due to a single illness case caused by NoV	DALYs per illness case	$\left(\frac{DALY}{\text{illness case}} \right)_{NoV}$	0.00095	[34]
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Table 2. Equations used for modeling the risks of de facto reuse

Description	Unit (abbreviation)	Equation	Equation no.	Reference
Pathogen concentration in the lake	oocyst/L for Crypto, gc/L for NoV	$C_{rp-lake} = C_{rp-eff} \times Dilution$	1 ^a	
Pathogen concentration in intake water for DWTP	oocyst/L for Crypto, gc/L for NoV	$C_{rp-IN} = C_{rp-lake} \times 10^{-k_{rp} \times t}$	2 ^a	
Pathogen concentration in the drinking water	oocyst/L for Crypto, gc/L for NoV	$C_{rp-F} = C_{rp-IN} \times 10^{-LR_{rp}}$	3 ^a	
Daily dose of pathogen ingested through drinking water	oocyst/day for Crypto, gc/day for NoV	$Dose_{rp} = C_{rp-F} \times V$	4 ^a	
Daily risk of infection due to Crypto	infection case per person per day (inf pppd)	$P_{Cryp.-inf} = P_{Cryp.} \times (1 - e^{-Dose_{Cryp.}})$	5	[55]
Daily risk of infection due to NoV	infection case per person per day (inf pppd)	$P_{NoV-inf} = 1 - {}_1F_1(\alpha, \alpha + \beta, -Dose_{NoV})$	6	[28]
Conditional illness risk due to NoV	illness case per infection case (ill pppd)	$P_{NoV}(ill inf) = 1 - (1 + \eta_{NoV} \times Dose_{NoV})^{-r_{NoV}}$	7	[28]
Daily illness risk due to reference pathogen	illness case per day (ill pppd)	$P_{rp-ill} = P_{rp}(ill inf) \times P_{rp-inf}$	8 ^a	
Annual infection risk due to reference pathogen	infection case per year (ill pppy)	$P_{rp-inf,annual} = 1 - \prod_{i=1}^{n=365} [1 - D(P_{rp-inf})_i]$	9 ^{a,b}	[32]
Annual infection risk due to both Crypto and NoV	infection case per year (ill pppy)	$P_{overall\ inf,annual} = 1 - \left(\prod_{i=1}^{n=365} [1 - D(P_{Cryp.-inf})_i] \times \prod_{i=1}^{n=365} [1 - D(P_{NoV-inf})_i] \right)$	10 ^b	[55]
Annual illness risk due to reference pathogen	illness case per person per year (ill pppy)	$P_{rp-ill,annual} = 1 - \prod_{i=1}^{n=365} [1 - D(P_{rp-ill})_i]$	11 ^{a,b}	[32]

Annual disease burden due to reference pathogen	DALY per person per year (DALY pppy)	$DALY_{rp} = \left(\frac{DALY}{\text{illness case}} \right)_{rp} \times P_{rp-ill, annual}$	12 ^a	[56]
Annual disease burden due to both Crypto and NoV	DALY per person per year (DALY pppy)	$DALY_{overall} = DALY_{Cryp.} + DALY_{NoV}$	13	

^aThese equations are generalized for both reference pathogens (Crypto and NoV), wherein the specific forms are obtained through substituting the parameters with subscript ‘*rp*’ with either that subscripted with ‘*Cryp.*’ or ‘*NoV*’ (See Table 1).

^b*D* is the symbol for distribution, from which random samples are generated.

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Panel A

Panel B