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Quantitative Microbial Risk Assessment of De Facto Water Reuse Practice: A Case
Study of Trinity River, Texas

THESIS

Submitted in partial satisfaction of the requirements
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ABSTRACT OF THE THESIS

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Master of Science in Environmental Engineering

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[Water reuse have been adopted in regions with limited natural water resources to alleviate water supply stress caused by rapid population and economic growth. The form of water reuse varies, can include non-potable, indirect potable and possibly direct potable reuse in the near future. Each category of water reuse has been highly regulated and controlled in the United States and other developed countries. However, there is an unofficial form of water reuse, sometimes called de facto reuse, where the drinking water source of downstream communities contains a significant portion of wastewater effluent from upstream communities. These effluents were treated to meet the EPA surface water discharge standards, which are not intended for use as the source for drinking water supplies. This study examined the health risk of de facto reuse by Quantitative Microbial Risk Assessment (QMRA) using Trinity River, Texas as a case example. The concentrations of Cryptosporidium and norovirus in wastewater effluents upstream of Trinity River were estimated using literature data that fitted by normal and bimodal distribution function, respectively. The exposure assessment considered the portion of wastewater effluent in

drinking water source, pathogen decay during storage, and removal rates in water treatment plants. Health risks were computed using Monte Carlo simulation with 10,000 iterations. Results indicated that the annual infection risk of norovirus are exceeding the suggested safety level set by the U.S. EPA, while cryptosporidium risks can only meet the safety benchmark under some circumstances. The disease burden for both pathogens also exceed the WHO DALY-based tolerance level under some circumstances. This study risks concerns of de facto reuse in high portion of effluent and insufficient storage time.]

1. INTRODUCTION AND BACKGROUND

1.1 Current State of Water Supply

The rapid growth of global economy and population has placed tremendous pressure on water supplies in regions with limited natural water resource. Southwestern United States, Australia, the Middle East, and the Mediterranean are experiencing the higher than average water stress in comparison with many other regions (Rice, Wutich et al. 2013). In addition, the change of precipitation and climate patterns due to both anthropogenic and natural influences further worsen the stress of water supplies. The chances of tapping into new water supplies for metropolitan areas (i.e. through long distance water transport) are getting more and more difficult (Leverenz, Tchobanoglous et al. 2011). To sustain the rapid economic and social development, wastewater reuse has been evaluated, permitted and implemented as a "new source" of water supply to meet the ever-increasing demand in many states in U.S and Australia (NWQMS 2008, EPA 2012, CDPH 2014).

1.2 Wastewater Treatment and Water Reuse

Conventional wastewater treatment process includes a preliminary treatment to remove debris, a primary treatment to separate solids and greases, a secondary treatment to remove organic matter, and chlorination treatment to remove disease-causing organisms followed by de-chlorination before discharge. Many treatment plants also include advanced treatment to further remove nitrogen and phosphorus from wastewater. In the United States, wastewater discharge to surface water is regulated by National Pollutant Discharge Elimination System (NPDES) permits and water quality in the discharge effluent must meet

the requirement established in the Clean Water Act. The effluent was not intended for use as a source of drinking water in the U.S. or other developed countries.

Reuse of wastewater effluent, however, for non-potable purpose has a long history and is still practiced in many developing countries (N.R.C 2012). Sewage farming was once considered a beneficial practice to take advantage of nutrients harbored in sewage. The practice was phased out in the developed nations due to the potential transmission of infectious disease through consumption of sewage contaminated crops and exposure to farm workers (N.R.C 2012). Non-portable reuse of treated sewage is now regulated in many states in the U.S., which requires the reclaimed water to be treated to a level that meet the water quality criteria for human health protection. In the State of the California, the non-portable water reuse is regulated by Title 22 California Code of Regulations (CHL 2001, CDPH 2013), which links engineering treatment technology applied with the end-use purpose of the water. Both disinfected secondary effluent and tertiary effluent were permitted for various non-potable applications in California.

Non-potable water reuse is loosely defined as reclaimed water that is treated to fit for purposes that do not require water of drinking quality standard. Reclaimed water for industrial uses or municipal irrigation was developed and applied mostly in the areas where recycled water can be used to substitute the demand for drinking water. The distribution of the recycled non-portable water, however, will require a separate pipeline from the drinking water distribution system, which adds tremendous capital cost and engineering challenges (Leverenz, Tchobanoglous et al. 2011). The construction of the secondary distribution system is often unfeasible in the well-established urban centers. Furthermore, even for non-drinking purpose, non-potable water reuse has met with public resistance regarding the

water quality and safety of the applications (Marks 2006, Dolnicar and Schäfer 2009). Non-potable reuse is only gaining acceptance in the recent years (Dolnicar, Hurlimann et al. 2011, Fielding and Roiko 2014).

In spite of the important contributions of non-potable reuse effort to reduce water scarcity, the limitation on its fit-for-purpose applications, the lack of storage capacity and the new distribution pipelines have hindered the further development of the practice. In fact, a large portion of recycled water produced by Southern California water reclamation plants is discharged to surface waters during the rainy season due to the lack of demand for non-portable irrigation water and the short of storage capacity to bank the recycled water. Indirect Potable Reuse (IPR) has been developed to directly supplement the diminishing drinking water supply.
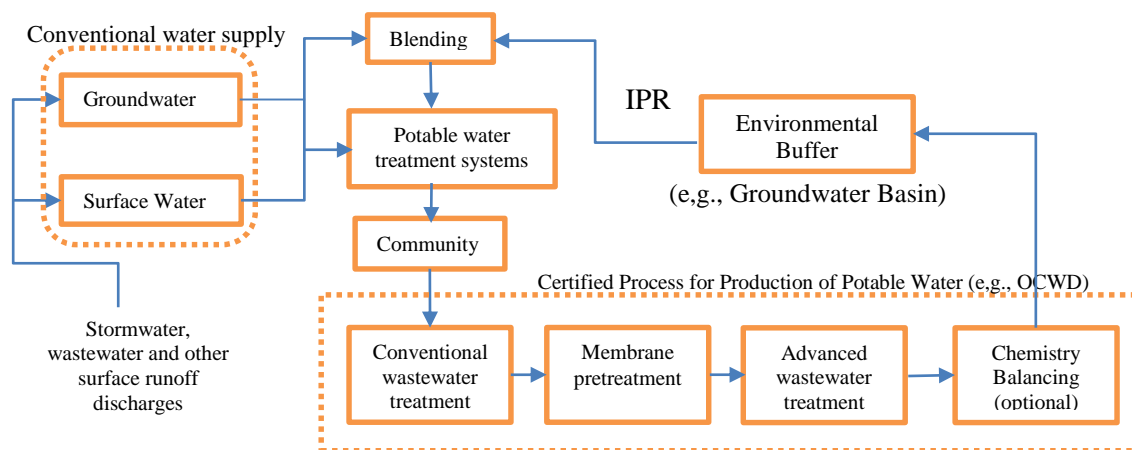
Figure 1.1 Indirect Portable Reuse Process Example (OCWD)

As illustrated in figure 1.1, IPR is defined as a water reuse practice that treats wastewater using advanced engineering approaches, which may include microfiltration, reverse

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osmosis, UV and H_2O_2. In addition, an environmental buffer such as a groundwater recharge basin or a surface reservoir is required to store and blend the highly treated water with natural water before intake by potable water treatment facilities (Leverenz, Tchobanoglous et al. 2011). In the case of OCWD's Groundwater Replenishment System (GWRS), a groundwater recharge basin is used as environmental buffer, where the treated water is store in the ground for at least 6 months before it is pumped for drinking water treatment as currently required by the California Drinking Water Division (former California Department of Public Health) (CDPH 2011). The OCWD's GWRS project is the largest in the world (www.gwrsystem.com), costs over $481 million (U.S. Dollars) in initial design and construct. In addition to the large cost for construction, intense monitoring of water quality in finished water and groundwater near the spreading basins are also required to ensure water quality protection. OCWD operates a vast network of monitoring wells within the groundwater basin and conducts modeling to determine water levels, water quality and recharged water travel time from spreading basins to drinking water production wells (www.gwrsystem.com).

1.3 De facto reuse

In contrast to the extraordinary capital investment and intense monitoring requirements, an unofficial form of water reuse that is not regulated or monitored by governmental agencies, termed de facto reuse, is practiced in many parts in the U.S. De facto reuse describes the use of surface water that contains a large portion of wastewater effluent from upstream communities as source water for drinking water supplies. De facto reuse is common in the lower reaches of Mississippi River, the lower watershed of Trinity River in TX, and the

downstream of Schuylkill River in PA (Trussell, Salveson et al. 2013), although the portion of wastewater effluent varies with regions and seasons. In these scenarios, effluents from upstream community were treated by conventional wastewater treatment plants to meet the EPA surface water discharge standards, but were not designed for use as the source for drinking water supplies. The downstream communities have little control over the water quality of their intakes.

Past decades have witted the increasing of de facto wastewater reuse in parts of U.S. As it was first identified in EPA's report in 1980, among all investigated drinking water treatment plants (DWTPs) impacted by upstream wastewater treatment plant in U.S., the top 25 most impacted DWTPs contained between 2% and 16% wastewater discharges from upstream locations under average streamflow conditions (Swayne, Boone et al. 1980). A study in 2013 provides an update to the 1980 EPA analysis, pointing out that de facto reuse had increased for 17 of the 25 DWTPs from 1980 to 2008, as municipal flows upstream of the sites increased by 68%, and that under low streamflow conditions, de facto reuse in DWTP supplies range from 7% to 100% (Rice, Wutich et al. 2013). The non-ignorance of de facto reuse in sustainable water supplies has raised public concerns on its potential risks to human health, and made the quantification of extent of de facto reuse the number one research need for human health, social, and environmental studies in U.S. (N.R.C 2012).

1.4 Quantitative Microbial Risk Assessment (QMRA)

Quantitative Microbial Risk Assessment (QMRA) is often used to study human health risk associated with the use of reclaimed water. Unlike the direct toxicological studies (N.R.C 1998) that require vast knowledge and further development to be broadly applied, or

epidemiological studies that often fail to identify the level of risks considered significant from a population-based perspective (Trussell, Salveson et al. 2013), QMRA enables measurements of risks due to exposure to various pathogens during drinking water exposure. The QMRA risk outcomes can then be used to develop tolerable guidelines for water and food that may be the source of microbial pathogen exposure to humans. Four main steps are involved in QMRA: 1) Hazard Identification, 2) Exposure assessment, 3) Dose Response Assessment and 4) Risk Characterization (HAAS;, ROSE; et al. 2014). Hazard identification includes selection of reference pathogens, and the description on physical systems of infection. Exposure assessment focuses on the pathway and the quantity of these pathogens to reach human body. Dose response assessment refers to study on the probability of getting infected or showing illness symptoms with different dose of pathogen exposed. Risk characterization is the process of comparing the calculated risk to existing laws and policies, such as U.S. EPA annual infection risk for drinking water (10^{-4} per person per year (pppy)). Through QMRA, risk can be identified; quantified; and used by decision makers to assess whether the estimated likelihood of harm is socially acceptable.

During the last decade, QMRA has played an important role in assessing pathogen reduction requirements for wastewater recycling for various purposes, including non-potable reuse, IPR and DPR scenarios (Barker, Packer et al. 2013). The risk assessment based on dose-response models was first used by U.S. EPA for the development of the Surface Water Treatment Rule for Giardia and viruses (Haas, Rose et al. 1993). (Teunis, Havelaar et al. 1994) also used formal QMRA procedures for pathogenic microorganisms focusing on water and food, and risk comparisons of different sources of hazards. In 2013, the U.S. EPA together with the U.S. Department of Agriculture (USDA) published a guideline for QMRA for food and

water (http://www.epa.gov/raf/files/mra-guideline-final.pdf). In addition, WHO has also developed guidelines for QMRA to address not only drinking water but also recreational and reclaimed waters. The use of recycled wastewater for irrigation of both food and nonfood crops was addressed using QMRA that includes six case studies (http://qmrawiki.msu.edu/index.php?title=Case_Studies). However, so far there has not been a QMRA on de facto reuse, and public concerns on the safety of de facto reuse has not been well responded to, leaving a significant gap in sustainable water reuse management systems.

1.5 Water Reuse in Trinity River Basin

The Trinity River in Texas is used here as an exemplar to investigate the health risk of de facto water reuse (Figure 1.2). Trinity River is the major river flow from south central Texas near Dallas/Forth Worth to Houston. Houston gets roughly a third of its drinking water supply from the Trinity River through Lake Livingston (http://www.publicworks.houstontx.gov/). The surface waters are treated through a conventional water purification plant in east Houston before delivered to consumers. The other two-thirds of Houston's water comes from Lake Houston, which is part of the San Jacinto River watershed and from ground aquifers.

Trinity River is an effluent-dominated surface water system (N.R.C 2012), the section of the river south of Dallas/Forth Worth consists almost entirely of wastewater effluent under baseflow conditions. The Trinity flows past Dallas and travels south over 200 miles to Lake Livingston, at which point over half of flow cascades down the lake's spillway are wastewater effluent (https://stateimpact.npr.org/texas/). The resident time at Lake Livingston was

estimated for up to a year (Trussell, Salveson et al. 2013) before withdrew by conventional

drinking water treatment plant and is delivered to consumers in Houston.

Figure 1.2 Trinity River Basin (N.R.C 2012)

1.6 Study Goals

This study aimed to provide a case study on human health risk of drinking water through de

facto reuse and to compare the risk outcome with other water and food related health risk.

The quantified risk outcomes contribute to a better understanding of the current reuse

regulations, and the comparison of risks in different water and food practices provides policy

makers with tools to evaluate a sustainable water management practice. The discussion on

model uncertainties can also help to interpret and compare results with epidemiological data

(or guide the epidemiological study) in Houston area. The results also shed the light and

research gaps in health risk assessment through QMRA.

2. MATERIALS AND METHODS

2.1 Scenario Settings

To set the scenarios for de facto reuse, the wastewater travel route was postulated and illustrated as shown in Figure 3.1. Based on the literature (N.R.C 2012), WWTP effluent discharged to the Trinity River is traveled to Lake Livingston in two weeks and stayed in the lake for up to one year before pumped for treatment as source of drinking water. At times, Trinity River is nearly 100% sewage effluent. For risk estimates of de facto reuse, we set three scenarios for wastewater dilution by Trinity River baseflow at 45% wastewater; 30% wastewater and 15% wastewater. Assuming a constant decay factor for pathogen inactivation in the environment during the course of the water flow from Trinity River to Lake Livingston, we also set three scenarios for the pathogen resident time in the environment to be 295 day, 315 days and 365 days.

```
┌──────────┐      ┌──────────┐  Travel for  ┌──────────┐  Stay for   ┌──────────┐      ┌──────────┐
│   WWTP   │ ───> │  Trinity │   2 weeks    │   Lake   │ up to 1 year│   WTP    │ ───> │ Consumers│
│ Effluent │      │   River  │ ───────────> │Livingston│ ──────────> │  intake  │      │          │
└──────────┘      └──────────┘              └──────────┘             └──────────┘      └──────────┘
```

Figure 2.1 Wastewater Travel Route

2.2 Target pathogens

We chose norovirus and *Cryptosporidium* as our target pathogens for risk assessment because they are among the top 15 pathogens causing the highest level of acute gastrointestinal illness (AGI) in the U.S. (Trussell, Salveson et al. 2013). Their importance in public health also rendered them the most investigated and quantified enteric pathogens in

water. Norovirus is the number one cause of AGI in the U.S. that attributed to 20,796,079 episodes and 569 deaths in the year 2006 (Scallan, Hoekstra et al. 2011). Typical symptoms of AGI in a person may develop 12 to 24 hours after being exposed to norovirus, which may include acute-onset of vomiting, watery, non-bloody diarrhea with abdominal cramps and/or nausea, low-grade fever, headaches and/or myalgias (http://www.cdc.gov/norovirus). *Cryptosporidium* is an intestinal parasite found worldwide and it has caused the largest waterborne outbreak ever documented in the U.S., where over 400,000 people became ill in Milwaukee, WI when a drinking water treatment plant malfunctioned (Costa and Mackenzie 1994). The most common symptom of cryptosporidiosis is watery diarrhea, which generally begins 2 to 10 (average 7) days after becoming infected with the parasite. Other symptoms include stomach cramps or pain, dehydration, nausea, vomiting, fever and/or weight loss (http://www.cdc.gov/parasites/crypto). Both norovirus and *cryptosporidium* infect human via ingestion of water and food. They are quantifiable using Quantitative Polymerase Chain Reaction (Q-PCR) (Trussell, Salveson et al. 2013). Cryptosporidium can also be quantified by fluorescence microscopy methods or cultivation methods. However, there has not been an infectivity assay for norovirus due to the lack of a sensitive tissue culture cell line. Dose-response models for both organisms have been developed using clinical data and applied in other water and food risk assessment practices.

2.3 Pathogen concentration in WWTP effluent

Detection and quantification of pathogens in WWTP effluent have been carried out by many researchers in many parts of the world but they are not part of required water quality

monitoring practice for wastewater treatment utilities. As a result there is no specific pathogen concentration information from the WWTPs that directly discharge into the Trinity River. As an approximation to the pathogen concentration in WWTP effluents in the region, we collected information on the WWTP from the Trinity River Authority of Texas (http://www.trinityra.org/), which gives details of WWTP treatment technologies used in the area, source of wastewater and treatment capacity in order to identify the similar WWTPs where pathogen data were monitored and reported. The main wastewater treatment facility discharging effluents into Trinity River is managed by Central Regional Wastewater System (CRWS), a system, located in west Dallas, serving approximately 3 million people in the Dallas/Fort Worth area treating domestic wastewater collected through more than 200 miles of collection pipelines.

The treatment plants of CRWS includes preliminary treatment, primary treatment, secondary treatment, and final disinfection before discharging effluent into Trinity River (http://www.gptx.org/) (Mancl 2012). Water quality parameters including chemical oxygen demand (COD), biochemical oxygen demand (BOD), nitrogen, phosphorus, metals and pathogen indicator bacteria (*E. coli* in this case) are monitored in the effluent to evaluate the compliance of effluent with the requirements of the Texas Commission on Environmental Quality for discharging to the Trinity River (http://www.tceq.texas.gov/).

The concept of using indicator bacteria as monitoring surrogates for microbial pathogens is based on its relative simplicity and short turn-around time for assessing the microbial characteristics of a sample (Rose, Farrah et al. 2004). However, many recent studies have pointed out that indicator bacteria do not represent all disease-causing pathogens well, and that *Cryptosporidium* and norovirus are both more resistant to water treatment processes

than indicators (Assavasiliavansukul, Harrington et al. 2008, Shirasaki, Matsushita et al. 2010, Barker, Packer et al. 2013). Therefore pathogen concentrations should be detected separately for the purpose of risk assessment.

In accordance with the wastewater treatment technologies and scales of treatment in Dallas/Fort Worth regional WWTPs, we identified literature reports of the concentration of *Cryptosporidium* and norovirus in WWTP effluents using the similar treatment processes. Data were compiled and described in the following sections.

2.3.1 *Cryptosporidium* Concentration in WWTP effluent

To estimate the concentration of *Cryptosporidium* in effluent discharged into Trinity River, 99 datasets from literature reporting effluent concentration in various WWTPs using activated sludge and disinfection processes were collected (Appendix I). Based on these datasets，*Cryptosporidium* oocysts concentration was $\log_{10}$ transformed and ranked. The histogram of $\log_{10}$ oocysts concentration was generated using bin size of 0.1 $\log_{10}$ unit. The maximum likelihood estimation was used to fit the $\log_{10}$ transformed *Cryptosporidium* concentrations using normal distribution function as shown in equation 1.

$$\log_{10} C_{C-eff} \text{ oocysts} \sim N(\mu, \sigma^2) \qquad (1)$$

Where

C_{C-eff}: Concentration of *Cryptosporidium* in WWTP effluent

N: Normal distribution function

μ: Expectation;

σ: Standard Deviation;

The best fit parameters μ and σ for normal distribution function of *Cryptosporidium* concentration were used in simulated distribution to estimate the goodness of fit using 2000 simulated data sets generated by MATLAB R2014b (The Mathworks, Inc., MA).

2.3.2   Norovirus Concentration in WWTP effluent

In comparison with literature reports on *Cryptosporidium* concentrations, norovirus concentrations in WWTP effluents reported in the literature are sparse due to technical difficulties of viral concentration and quantification using existing technologies. The literature reports also suffer from the inconsistent in viral concentration method, recovery rates and methods used for quantification, making the comparison and compiling of norovirus concentration data challenging. (Da Silva, Le Saux et al. 2007) reported the most comprehensive norovirus concentration dataset that includes concentrations in influents and effluents of four WWTPs monitored over 12 months. Among these four plants, two plants used activated sludge as the secondary treatment process, which are consistent with the wastewater treatment technology used in the CRWS in the Dallas/Fort Worth region. The norovirus concentration data from these two plants (Appendix II), which include both norovirus genotype I (GI) and genotype II (GII), were $\log_{10}$ transformed and ranked. The minimum detection limitation of genome copy (gc) is 5000 gc/L for norovirus GI and 200 gc/L for norovirus GII for the reported study. Only 1 out of 42 samples for norovirus GI and 2 out of 43 samples for Norovirus GII was reported as non-detect. We used ½ of the detection limit to set non-detect value, which is 2500 gc/L for GI and 100 gc/L for GII. The histogram of $\log_{10}$ gc/L for each norovirus genotype was generated using bin size of 0.1 $\log_{10}$ gc/L and fitted using bimodal distribution function shown in equation 2.

$$\log_{10} C_{N-eff} \text{ gc/L} \sim \alpha * N(\mu_1, \sigma_1^2) + (1 - \alpha) * N(\mu_2, \sigma_2^2) \qquad (2)$$

where

C_{N-eff}: Concentration of norovirus in effluent

N: Normal distribution function

α: Ratio factor, $\alpha \in (0,1)$;

μ_1: Expectation of model 1;

σ_1: Standard Deviation of model 1;

μ_2: Expectation of model 2;

σ_2: Standard Deviation of model 2.

The best-fit parameters for bimodal distribution of each norovirus genotype were used in simulated distribution to estimate the goodness of fit using 2000 simulated datasets in MATLAB. The simulated datasets for norovirus GI and norovirus GII, was then combined into 2000 new datasets for total norovirus using a Monte-Carlo simulation process in MATLAB to serve as total norovirus concentration for use in dose-response assessment.

2.4 Dilution ratio

Due to the seasonal variability of precipitation, trans-evaporation and the volume of the wastewater effluent discharge, the portion of wastewater effluent in Lake Livingston is highly variable. The current available information indicates the section of the Trinity River south of Dallas/Forth worth consists almost entirely of wastewater effluent under base flow conditions (N.R.C 2012), and little dilution of the effluent-dominated waters occurs as the water travels from Dallas/Fort Worth to Lake Livingston (Fono, Kolodziej et al. 2006). However, other water sources for Lake Livingston brought various levels of dilution to the wastewater from Trinity River, among which the most important is precipitation in the local area. Literatures suggest that up to half of the water flowing into Lake Livingston is derived from precipitation (N.R.C 2012), but no datasets or calculated results were provided to support this value. In the absence of the exact precipitation or evaporation data, we chose 45%, 30% and 15% as our assumption of the portion of wastewater effluent contribution to the Livingston Lake after dilution by other sources of water from small tributaries of the Lake. Based on the above assumptions, the concentration of *Cryptosporidium* and norovirus in Lake Livingston is estimated using equation 3 without the inclusion of pathogen decay.

$$C_{DL} = C_{eff} * DR\% \qquad (3)$$

C_{DL}: Concentration of pathogens in Lake Livingston after dilution with other waters

(oocysts/L for *Cryptosporidium* and gc/L for Norovirus)

C_{eff}: Concentration of pathogens in WWTP effluent

$DR\%$: Dilution Rate, 45%, 30% and 15%

2.5 Decay of pathogens during storage

Both *Cryptosporidium* and norovirus are highly resistant to environmental decay as compared with other enteric pathogens transmitted by water and food. The decay rate of *Cryptosporidium k* of 0.0155 day^{-1} was adopted based on Olson's 1999 experiments (Olson, Goh et al. 1999). This value was derived from the *Cryptosporidium* survival data in water at temperature of 25 ℃. Although the actual water temperature in Lake Livingston varies dramatically from hot summer to the cold winter, which results in either higher or lower decay rate, for the simplicity of this study, only a single decay rate is applied. Other environment factors including predation and sedimentation contribution to Cryptosporidium decay are not included in this study due to the large degree of uncertainties. The norovirus inactivation and survival in various environments are challenged by the lack of a cultivation method for assessment the infectivity of the viruses. Instead, its surrogate murine norovirus (MNV) is often used to represent the decay of human norovirus (Karst, Wobus et al. 2003) (Katayama, Hansman et al. 2006). (Lee, Zoh et al. 2008) reported the decay rate of MNV in 40 days in 18℃ water ranged from 0~0.02-log$_{10}$ day^{-1}. The median value of 0.01 log$_{10}$ day^{-1} was chosen to represent the decay rate k for norovirus in this study. In computation of the final pathogen concentration in the intake water for drinking water treatment from Lake Livingston, we incorporated the two weeks' travel of WWTP effluent in Trinity River before reaching Lake Livingston and the water resident time in the Lake with

the estimated decay rate. Pathogen inactivation and the concentration of pathogens can be expressed using an exponential function of time shown in equation 4.

$$C_{in} = C_{DL} * 10^{-kt} \qquad (4)$$

Where

C_{in}: Concentration of pathogen in drinking water intake;

 (oocysts/L for *Cryptosporidium* and gc/L for Norovirus)

C_{DL}: Concentration of pathogens in Lake Livingston after dilution with other waters from equation 3;

 (oocysts/L for *Cryptosporidium* and gc/L for Norovirus)

k: Decay rate (day^{-1})

t: Time for storage (days)

As presented in scenarios setting, three different t value, 270, 315 and 360 days were chosen to serve as various time series for storage. Through comparison of three time-varied cases we were able to analyze on the affection of duration time on risk outputs.

2.6 Pathogen concentration in WTP finished water

After withdrawal from Lake Livingston by WTP, water is treated using standard drinking water treatment process to meet the drinking water requirements. During this process, both *Cryptosporidium* and norovirus are removed through various processes. The final concentration of pathogens in WTP finished water is expressed as:

$$C_f = C_{in} * 10^{-PR} \qquad (5)$$

Where

C_f: Concentration of pathogen in finished water (from WTP)

(oocysts/L for *Cryptosporidium* and gc/L for Norovirus)

C_{in}: Concentration of pathogen in intake water from equation (4);

(oocysts/L for *Cryptosporidium* and gc/L for Norovirus)

PR: Log$_{10}$ Removals of Pathogen; $10^{-PR} \in (0，1)$

For *Cryptosporidium*, 1 to 5 log10 pathogen reduction was achieved across various water treatment plants, depending on raw water quality, treatment operations and the influent concentration of pathogens; in this study, we adopted the median 3.0 log10 as the *PR* value, as suggested by (N.R.C 2012) . The Norovirus *PR* value we adopted was 4.0 log10 according to (N.R.C 2012) as no other data is available to provide any better value.

2.7 Dose of pathogen ingested

Pathogen ingestion is estimated using pathogen concentration and daily volume of water consumed, each of the parameters is assumed to be independent of each other. It is expressed as:

$$Dose = C_f * V \qquad (6)$$

Where

Dose: Dose of pathogen ingested (oocysts/day for *Cryptosporidium*; gc/day for norovirus)

C_f: Concentration of pathogen in drinking supply water from equation (5)

(oocysts/L for *Cryptosporidium* and gc/L for Norovirus)

V: Volume of water people drinks per day (2 L*day^{-1}*person^{-1})

The volume of water people dinks per day is assumed to be 2 L/day as U.S.EPA suggested for an adult weighed 70kg.

2.8 Daily Infection Risk

The daily infection risk, P_{inf}, is quantified as number of infection case per person per day. The calculation of daily infection risk varies by different pathogens, with their virulence and infection dose involved.

The dose-response relationship for *Cryptosporidium* is widely recognized as an exponential function (Teunis, Medema et al. 1997) in equation 8.

$$P_{C-inf} = 1 - e^{-r_c * Dose} \qquad (8)$$

Where

P_{C-inf}: Probability of infection by *Cryptosporidium* per day

r_c : Dose response parameter for *Cryptosporidium*

Dose: Dose of *Cryptosporidium* ingested

The best-fit value for r_c in this function is 0.05 based on the combining clinical trials data (McBride, Stott et al. 2013).

The calculation of infection risk of norovirus is based on the dose-response model generated by Teunis (Teunis, Moe et al. 2008) on Norwalk, one of norovirus GI. The relation was applied for combined norovirus GI and norovirus GII, since there is no separate dose-response model for each norovirus genotype. The best-fit distribution for

non-aggregated (1F1) form of Norwalk virus from clinical trials of infection is shown in equation 9, in which $\alpha = 0.040, \beta = 0.055$ is the best-fit parameters for the does-response curve.

$$P_{N-inf} = 1 - {_1F_1}(\alpha, \alpha + \beta, -Dose) \quad (9)$$

Where

P_{N-inf}: Probability of infection by norovirus per day

${_1F_1}$: Kummer confluent hypergeometric function

α, β: Shape and location parameters for Kummer function

Dose: Dose of norovirus ingested (gc/L)

2.9 Daily Illness Risk

Infection does not usually always lead to illness, therefore illness risk for each pathogen needs to be calculated to better understand the potential burden of disease through water consumption. The calculation of the illness risk can either be transferred from an infection risk using an assumed fixed proportion of infection resulting in illness, or using a dose-response for illness for specific pathogens when the data is available.

For *Cryptosporidium*, (McBride, Stott et al. 2013) suggests the probability of illness after infected by *Cryptosporidium* is 50%, and the probability of illness (P_{C-ill}) can be expressed in equation 10.

$$P_{C-ill} = P_c(ill|\text{inf}) * P_{C-inf} = 50\% P_{C-inf} \quad (10)$$

Where

P_{C-ill}: Probability of illness by *Cryptosporidium* per day

P_{C-inf}: Probability of infection by *Cryptosporidium* per day

$P_C(ill|\text{inf})$: Probability of illness after infection by *Cryptosporidium*

The illness risk of norovirus, on the other hand, is accessed via a dose-illness relationship developed by (Teunis, Moe et al. 2008) using clinical trail data and shown in equation 11.

$$P_{N-ill} = 1 - (1 + \eta * Dose)^{-r} \qquad (11)$$

Where

P_{N-ill}: Probability of illness by norovirus per day

η, r are shape and location parameters, $\eta = 0.00255; \ r = 0.086;$

Dose: Dose of norovirus ingested (gc/L)

2.10 Risk Characterization

In order to compare the risk of the de facto water reuse practice with the current water health risk benchmark in the U.S. and the world, the outputs for both daily infection/illness risk were further adjusted to annual infection or illness risk, $P_{inf,annul}$ or $P_{ill,annul}$, through equation 12 or 13, based on the theorem of independence of probability (Haas et al., 1999).

$$P_{inf,annual} = 1 - \prod_{i=1}^{n}[1 - D(P_{inf})_i] \qquad (12)$$

$$P_{ill,annual} = 1 - \prod_{i=1}^{n}[1 - D(P_{ill})_i] \qquad (13)$$

The subscript i represents the i-th iteration of Equition 12 or 13 and n represents the total number of iterations (the total number of exposure events in a year, which is 365 in this study). $D(P_{inf}) / D(P_{ill})$ represents distribution of infection/illness.

The annual risk is characterized by comparison with US EPA's acceptable annual infection risk associated with drinking water of 10^{-4} per person per year (pppy) and the WHO Disability-Adjusted Life Years (DALYs) of 10^{-6} DALYs (santé 2011). To apply DALYs as another benchmark, the annual illness risk was further transferred into DALYs metrics through equation 14.

$$DALYs = \frac{DALY}{illness\ case_{pathogen}} * P_{ill_{pathogen}}\ (14)$$

The DALY/ill ratio, $\frac{DALY}{illness\ case_{pathogen}}$, varies for different pathogens.

$\frac{DALY}{illness\ case_{cryptosporidium}}$, the DALY/ill ratio for Cryptosporidium, equals to 0.0012, as suggested by (Murray, Vos et al. 2013); for norovirus, the DALY/ill ratio,

$\frac{DALY}{illness\ case_{cryptosporidium}}$, is computed as 0.00095 by dividing total DALYs per year by total number of incidence cases as recommended by (Kemmeren, Mangen et al. 2006).

3. Results

3.1 Pathogen concentrations in WWTP effluent

3.1.1 *Cryptosporidium* concentrations

The best fit curve for the concentration distributions of *Cryptosporidium* is shown in Figure 3.1, where the simulated curve (blue line) is normal distribution $N(\mu, \sigma^2)$ with the μ=2.689 and σ=0.625. The concentration of *Cryptosporidium* in WTTP effluents in Trinity River south of Dallas/Fort Worth is then expressed as $Log_{10}C_{C-eff} \sim N(2.689, 0.625^2)$ oocysts/L.
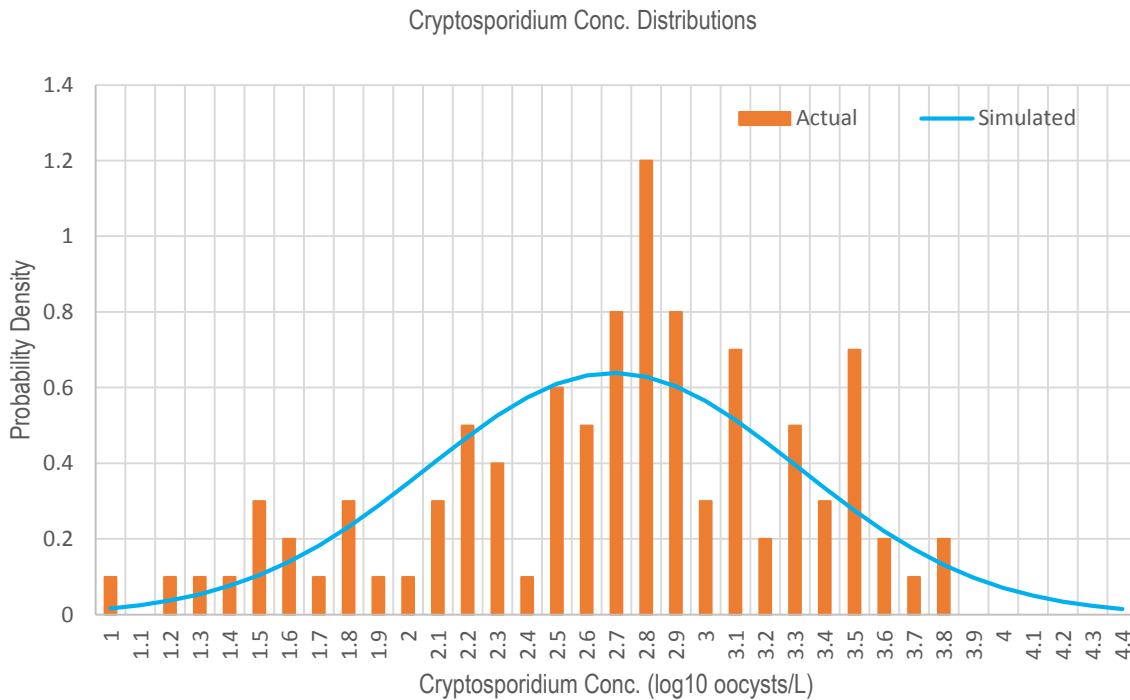

Figure 3.1 *Cryptosporidium* Concentration Distributions

3.1.2 Norovirus Concentrations

Figure 3.2(a), 3.2(b) shows the distributions fitting curve of the concentration of norovirus GI and GII in WWTP effluents, where the lines show the best fit bimodal curves.
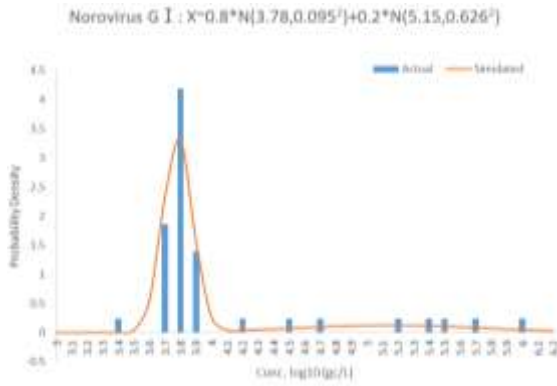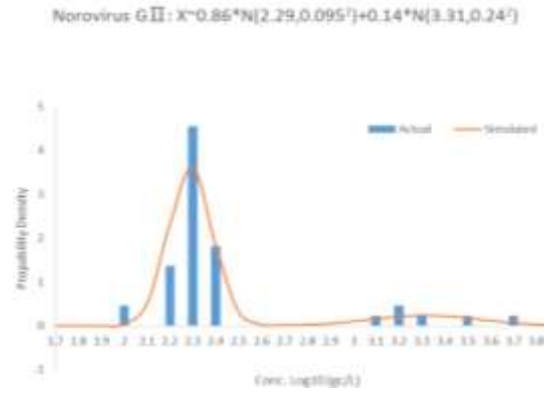

Figure 3.2(a) Norovirus GI concentrations

Figure 3.2(b) Norovirus GII concentrations

Based on the best-fit curves, the concentration of norovirus GI and GII follows the bimodal distribution:

$$Log_{10}C_{N-GI-eff} \sim 0.8 * N(3.78,0.095^2) + 0.2 * N(5.15,0.626^2) \qquad gc/L$$

$$Log_{10}C_{N-GII-eff} \sim 0.86 * N(2.29,0.095^2) + 0.2 * N(3.31,0.240^2) \qquad gc/L$$

Where

$N(\mu, \sigma^2)$: Normal distribution function,

$C_{N-GI-eff}$: Concentrations of Norovirus GI in WWTP effluents,

$C_{N-GII-eff}$: Concentration of norovirus GII in WWTP effluents.

3.2   Infection Risk

3.2.1   Infection Risk of *Cryptosporidium*

Figure 3.3(a) shows the infection risks of *Cryptosporidium* per day with various portion of effluent in Lake Livingston (45%, 30%, 15%) and storage times (after 270/315/360 days) in box plots. Various portion of wastewater are indicted by different color boxes, and the different storage times are marked on the x-axis. From the observation on storage time, we can conclude that the infection risks of *Cryptosporidium* per day follows the ascending order: 15%<30%<45%, suggesting the risks arises as the percentage of wastewater in Trinity River increases. Within the same storage time, there is minor difference (less than 0.2-log10 per person per day (pppd)) between the portions of effluent in the lake. For instance, the mean infection risk at 15%, 30% or 45% of effluent varies from $10^{-6.3}$, $10^{-6.1}$ to $10^{-5.9}$ pppd when storage time is set at 270 days.

Obviously, the infection risks decreases when the storage time increases for the same portion of effluent, following a descending order: 270 days>315 days>360 days. Difference in risks caused by the storage time is significant, a decrease of greater than 0.5 log10 pppd with each addition 1.5 month (45 days). For instance, with the same level of wastewater, 30%, the median risks for storage time at 270, 315, 350 days is $10^{-6.1}$/$10^{-6.8}$/$10^{-7.5}$ pppd.

It is also observed that median risks of all portions of wastewater and storage times are below the US EPA's Annual Infection Risk Benchmark (0.0001pppy), which is outlined by a dashed line in figure 3.3(a). However, this will not be the fair comparison between the daily risk with the annual risk since drinking water is used in every day of the year.
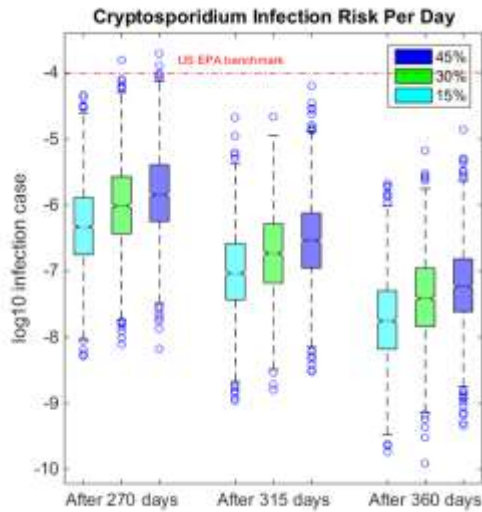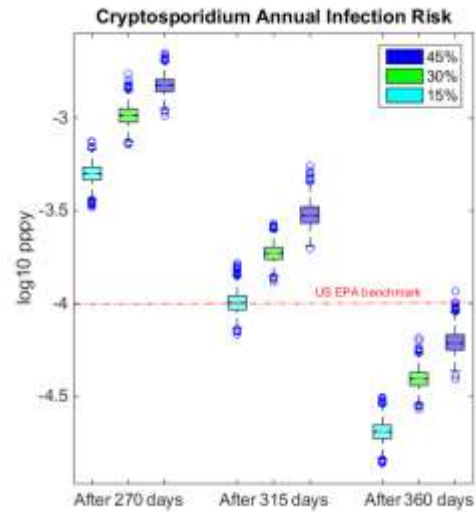
| Figure 3.3(a) | Figure 3.3(b) |

Conversion of daily risk to the annual infection risks of *Cryptosporidium* in various portions of wastewater and storage times is shown in Figure 3.3(b). The similar trends as in daily risk were observed for annual risks. This time more than half of the risks are above US EPA's Annual Infection Risk Benchmark line. Only after 360 days storage time of wastewater in the lake will reduce the annual risk to the acceptable range. The *cryptosporidium* infection risk outputs suggest the unsafety for drinking use in less storage time days (<360).

3.2.2 Infection Risk of Norovirus

Figure 3.4(a) presents the daily infection risks of Norovirus. Comparing risks of various wastewater contribution to the Lake and storage times, similar to that of *Cryptosporidium*, we can also conclude that with other conditions fixed, risks arises as the portion level of wastewater arises, but decreases as storage time increases. For storage time less 270 days, median risk of even the lowest portion of wastewater in the Lake will exceed the EPA annual

infectious risk of -4 $\log_{10}$. Increase storage time from 270 days to 315 and 360 will bring the median level of the risk below the 10^{-4} annual illness benchmark.
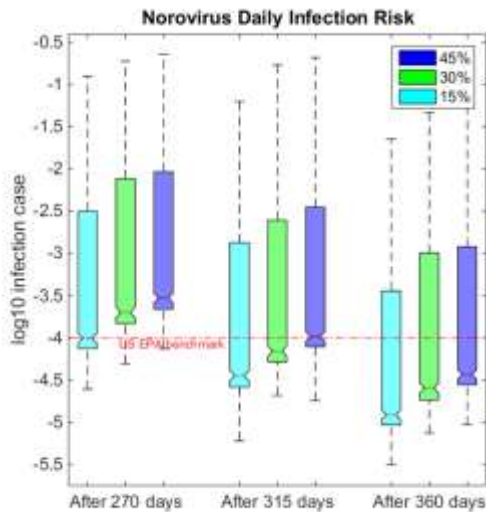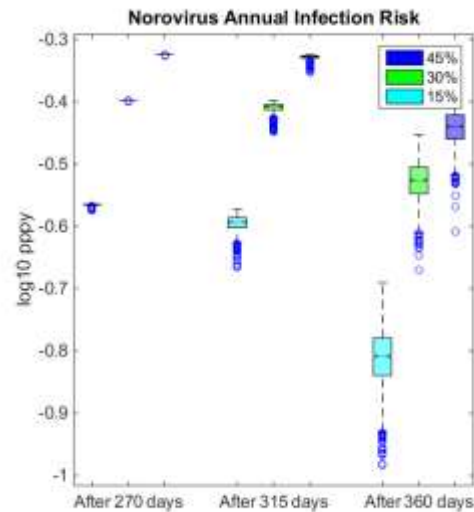

Figure 3.4(a)   Figure 3.4(b)

Converting the daily risks to annual infection risks of norovirus infection (Figure 3.4 (b)), indicated the infection risk is higher than US EPA Infection Benchmark by several orders of magnitude. The lowest annual risk of norovirus infection, when Lake contains only 15% of wastewater effluent and effluent resides in the Lake for about one year, is greater than 10^{-1} pppy, which is four-orders of magnitude higher than the benchmark of 10^{-4} pppy. Based on the risk outputs of norovirus, de facto reuse in Trinity River shows significant elevated infectious risk for drinking purpose. Based on its high infection risk of norovirus, the application of de facto reuse cannot response well to public health concerns.

3.3 Disease Burdens

Since only a subportion of the infection will translate to diseases, the direct examination of infectious risk may mislead the interpretation of public health. We adopted DALYs as the second benchmark to indicate the health risk of de facto reuse through the angle of disease burden risk analysis.

3.3.1 Disease Burdens of *Cryptosporidium*

Figure 3.5 (a) shows the disease burden risks of *Cryptosporidium* DALYs pppd with various portion of effluent in Lake Livingston and storage time. In accordance to the infection risks in Section 3.2.1, the daily disease-burden risk also arises as portion level of wastewater increases or storage time decreases. Compared to the WHO DALYs-based tolerance (< 10^{-6} DALYs pppy), risk outputs for all storage times and portion levels of wastewater is within the acceptable range.

Annual disease burden risks is shown on Figure 3.5(b). Using dashed line to mark the DALYs-based tolerance illness benchmark of *Cryptosporidium*, it is obvious that the majority of risks are below that line. Even for 45% portion of effluent and after 270 days storage time, where the WHO DALYs-based tolerance line is yet within 99.3% risk distribution range, the median risk ($10^{-6.1}$ pppy) is yet minor than 10^{-6} DALY pppy. In all other conditions, the whole risk distributions were far below the WHO tolerance line, suggesting a comparatively safety for drinking purpose. Disease burden risk outputs for *Cryptosporidium* indicates the practical of de facto reuse in specific conditions for Trinity River and a lower portion of wastewater and longer storage time is also recommended.
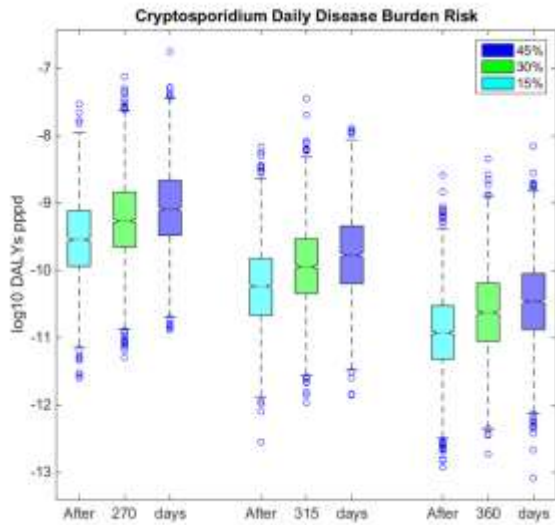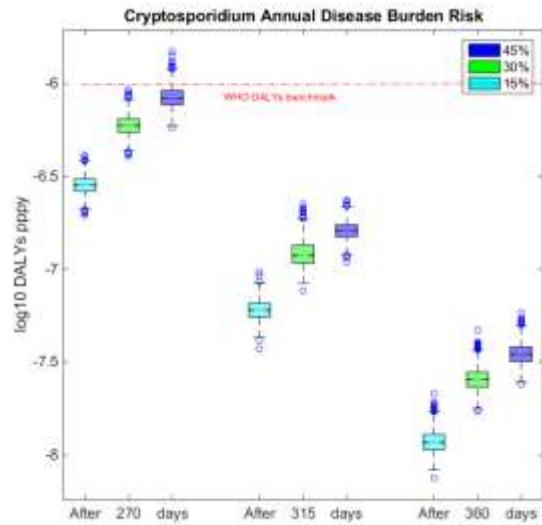
Figure 3.5(a)   Figure 3.5(b)

3.3.2 Disease Burdens of Norovirus

Figure 3.6(a) presents daily disease burdens of norovirus. Compared to the dashed line for WHO DALY-based tolerance dis-burden benchmark, risk mean of each dilution and storage time are lower than this line, though for 45% portion level of wastewater after 270 days, the bench mark are yet within 99.7% risk distribution range. The daily disease burden risk output suggested that for storage time more than 315 days and portion level of wastewater less than 30%, the disease burden risks are within the suggested safe level set by WHO.

Figure 3.6(b) shows the annual disease burden risk of norovirus. Although risk values remain a higher level compared to *cryptosporidium*, the annual disease burden risk seems more reasonable than annual infection risk. Compared to the dashed line representing DALYs-based tolerance benchmark, portion of risk distributions in 270/315 storage times are yet higher than the benchmark, while for 15% of effluent in 315 storage time the risks are completely below the line. After the storage time of 360 days, all risks are below the

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WHO's tolerance line. The disease burden risks of norovirus, varies from the high infection risk of norovirus, outlines the unsafety of de facto reuse in Trinity River in some cases and also indicates the potential of applying de facto reuse for drinking supply only after enough storage time (>=360 days).
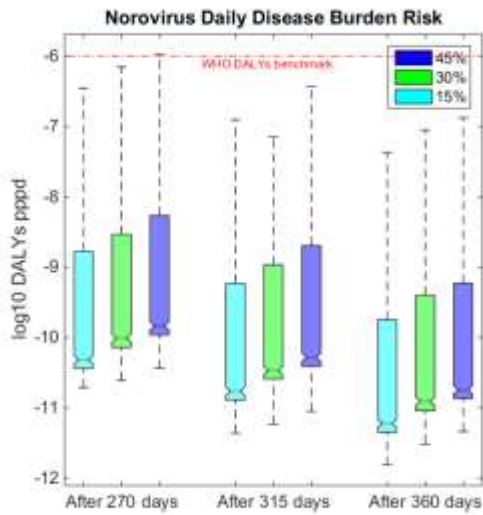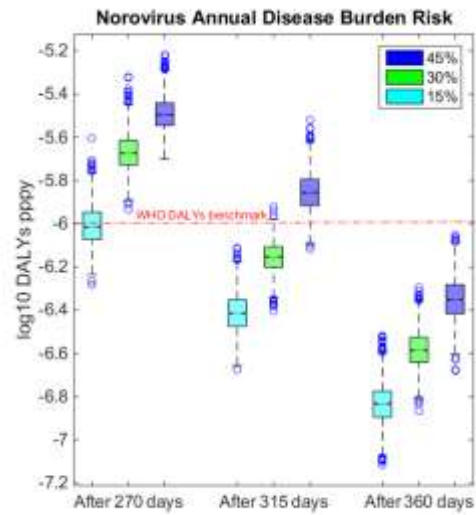

Figure 3.6(a)

Figure 3.6(b)

4. DISCUSSION AND CONCLUSION

This study responses to public concerns on health risk of de facto reuse for drinking purpose through a case study on Trinity River. Results suggest the non-ignorance effects of portions of effluent and storage time on the safety of de facto reuse for drinking purpose. Current conditions of de facto reuse in Trinity River, is comparatively unsafe for public health when the portion of its flow from WWTP effluent is comparatively high (>=30%) or the storage time is sufficient (<=315 days). The comparison of outputs point out the necessity of longer than 1 year storage time in reservoir for de facto reuse to reduce the health risk for drinking purpose to acceptable level. The results also indicate both the infection and illness risk associated with de facto reuse for drinking supply is more dominated by its storage time and a longer periods should be more considered to maintain the risks to lower levels. Also this study suggested a significantly higher infection risk of Norovirus than *Cryptosporidium*, and its value are far beyond U.S. EPA's suggested risk level for drinking purpose.

This study estimated the formula of concentration of two pathogens (*Cryptosporidium* and Norovirus) in WWTP effluents using literature datasets, with the assumption that the treatment level and efficiency of these WWTPs are similar to that in Trinity River. The actual effluent quality is affected by a variety of environmental factors, including climate change, seasonal alteration and geodynamic movement, all of which have non-ignorable and unexpected effects on pathogen concentrations. Besides, the methods we used to measure concentration of both pathogens is Q-PCR, which focus on RNA of pathogens and might not succeed in providing an accurate measurement on virus concentration. In the paper we used for estimate decay rate of norovirus (Lee, Zoh et al. 2008), the author pointed out that Q-PCR have disadvantage in confirming the presence of infectious viruses, and it overestimates the

number of infectious norovirus under most environment conditions. That unavoidable error could lead to extremely high results of Norovirus annual infection risks.

This study built a QMRA model to help calculate the risks using MATLAB R2014b (The Mathworks, Inc., MA) and that provided convincing results with math-based analysis, based on the simulated concentrations above and does relationships of both pathogens. As mentioned in materials and methods, the infections dose relationship for norovirus we adopted was from (Teunis, Moe et al. 2008), and the formula simulated by his experiments was only on Norwalk Virus. With no better literatures or experiments to compare with, we used the formulas for Norwalk Virus for all norovirus with the assumption that the dose-relationship also applied to Norovirus GII and other Norovirus GI.

The benchmarks we used to compare infection risks with is US EPA's annual infection benchmark, which was well applied as guideline in many risk assessment studies. However, this benchmark has also been questioned for long, for the reason of using one single benchmark for all pathogens without considering their biological varieties. Other benchmarks should be considered as well. As discussed in (Haas 1996), an annual infection risk of $<=10^{-3}$ for foodborne was more recommendable.

Besides, using a different risk benchmark, one for illness or disease burden risk, such as the WHO DALY-based tolerance benchmark, is recommended as another risk characterization method. We calculated the disease burden risks of cryptosporidium and norovirus by converting illness case into DALY and compared it to WHO tolerance line. Reflected by the disease burden results, using DALYs to explore disease burden benchmarks for different pathogens is also worth explored. However, the choosing of the DALY ratio for each pathogens, one important parameter for converting illness risks into DALYs, also matters to

the accuracy of the results because it is difficult to quantify as estimates vary widely and varies in different regions in different time. The DALY/illness-case ratio for cryptosporidium world widely is 0.00356 in 1990 but decreased to 0.00122 in 2010 (Murray, Vos et al. 2013), while regions like Netherlands remains higher ratios ranging from 0.0029 to 0.0031 in recent years (Havelaar, Haagsma et al. 2012, Mangen, Bouwknegt et al. 2015). In addition, the application of DALY only focus on the impact of illness, while an infection without clinical signs of illness has not been taken into consideration. DALY couldn't be used individually as benchmark for evaluating risks without further study on infection as well (Lim and Jiang 2013).

In the end, this study recommends a longer storage time (>=360 days) to reduce the health risk of de facto reuse and we expected more studies on norovirus concentration, as well as more accurate analysis methods and a separate dose-response relationship of each norovirus genotype. The exploration of a comprehensive risk benchmark on pathogens are also desired for a sustainable water resource management.

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Appendix I

Cryptosporidium Concentrations in WWTPs Effluents*

*All WWTPs in this appendix use activated Sludge as their 2nd treatment process

Cryptosporidium Concentrations in WWTPs Effluents (1/3)			
References	oocysts/100L	oocysts/L	Log_{10} oocysts/L
(Flapper;, Campbell; et al. 2012)	0.1	10	1.00
(Flapper;, Campbell; et al. 2012)	0.17	17	1.23
(Flapper;, Campbell; et al. 2012)	0.22	22	1.34
(Flapper;, Campbell; et al. 2012)	0.27	27	1.43
(Flapper;, Campbell; et al. 2012)	0.33	33	1.52
(Flapper;, Campbell; et al. 2012)	0.35	35	1.54
(Flapper;, Campbell; et al. 2012)	0.35	35	1.54
(Rose, Farrah et al. 2004)	0.372	37.2	1.57
(Flapper;, Campbell; et al. 2012)	0.41	41	1.61
(Flapper;, Campbell; et al. 2012)	0.5	50	1.70
(Rose, Farrah et al. 2004)	0.588	58.8	1.77
(Flapper;, Campbell; et al. 2012)	0.63	63	1.80
(Rose, Farrah et al. 2004)	0.676	67.6	1.83
(Rose, Farrah et al. 2004)	0.726	72.6	1.86
(Gennaccaro, McLaughlin et al. 2003)	1.12	112	2.05
(Flapper;, Campbell; et al. 2012)	1.19	119	2.08
(Flapper;, Campbell; et al. 2012)	1.28	128	2.11
(Flapper;, Campbell; et al. 2012)	1.33	133	2.12
(Flapper;, Campbell; et al. 2012)	1.49	149	2.17
(Rose, Farrah et al. 2004)	1.5	150	2.18
(Flapper;, Campbell; et al. 2012)	1.63	163	2.21
(Flapper;, Campbell; et al. 2012)	1.71	171	2.23
(Flapper;, Campbell; et al. 2012)	1.76	176	2.25
(Montemayor, Valero et al. 2005)	1.8	180	2.26
(Flapper;, Campbell; et al. 2012)	1.85	185	2.27
(Flapper;, Campbell; et al. 2012)	1.9	190	2.28
(Montemayor, Valero et al. 2005)	2.02	202	2.31
(Rose, Farrah et al. 2004)	2.25	225	2.35
(Flapper;, Campbell; et al. 2012)	2.86	286	2.46
(Flapper;, Campbell; et al. 2012)	2.98	298	2.47
(Flapper;, Campbell; et al. 2012)	3.2	320	2.51
(Flapper;, Campbell; et al. 2012)	3.33	333	2.52
(Flapper;, Campbell; et al. 2012)	3.35	335	2.53
(Flapper;, Campbell; et al. 2012)	3.47	347	2.54

Cryptosporidium Concentrations in WWTPs Effluents (2/3)			
References	oocysts/100L	oocysts/L	Log$_{10}$ oocysts/L
(Flapper;, Campbell; et al. 2012)	3.87	387	2.59
(Flapper;, Campbell; et al. 2012)	3.89	389	2.59
(Flapper;, Campbell; et al. 2012)	3.89	389	2.59
(Flapper;, Campbell; et al. 2012)	4.27	427	2.63
(Flapper;, Campbell; et al. 2012)	4.29	429	2.63
(Flapper;, Campbell; et al. 2012)	4.78	478	2.68
(Flapper;, Campbell; et al. 2012)	4.83	483	2.68
(Flapper;, Campbell; et al. 2012)	5.09	509	2.71
(Flapper;, Campbell; et al. 2012)	5.1	510	2.71
(Flapper;, Campbell; et al. 2012)	5.11	511	2.71
(Flapper;, Campbell; et al. 2012)	5.2	520	2.72
(Flapper;, Campbell; et al. 2012)	5.58	558	2.75
(Montemayor, Valero et al. 2005)	5.6	560	2.75
(Flapper;, Campbell; et al. 2012)	5.78	578	2.76
(Flapper;, Campbell; et al. 2012)	6.11	611	2.79
(Flapper;, Campbell; et al. 2012)	6.19	619	2.79
(Flapper;, Campbell; et al. 2012)	6.25	625	2.80
(Flapper;, Campbell; et al. 2012)	6.37	637	2.80
(Flapper;, Campbell; et al. 2012)	6.39	639	2.81
(Flapper;, Campbell; et al. 2012)	6.47	647	2.81
(Flapper;, Campbell; et al. 2012)	6.57	657	2.82
(Flapper;, Campbell; et al. 2012)	6.63	663	2.82
(Flapper;, Campbell; et al. 2012)	6.82	682	2.83
(Flapper;, Campbell; et al. 2012)	6.98	698	2.84
(Flapper;, Campbell; et al. 2012)	7	700	2.85
(Flapper;, Campbell; et al. 2012)	7.15	715	2.85
(Flapper;, Campbell; et al. 2012)	7.29	729	2.86
(Flapper;, Campbell; et al. 2012)	7.81	781	2.89
(Flapper;, Campbell; et al. 2012)	8.33	833	2.92
(Flapper;, Campbell; et al. 2012)	8.4	840	2.92
(Flapper;, Campbell; et al. 2012)	8.43	843	2.93
(Flapper;, Campbell; et al. 2012)	8.44	844	2.93
(Flapper;, Campbell; et al. 2012)	8.75	875	2.94
(Flapper;, Campbell; et al. 2012)	9.89	989	3.00
(Flapper;, Campbell; et al. 2012)	10.96	1096	3.04
(Flapper;, Campbell; et al. 2012)	11.13	1113	3.05
(Flapper;, Campbell; et al. 2012)	11.5	1150	3.06
(Flapper;, Campbell; et al. 2012)	11.7	1170	3.07
(Flapper;, Campbell; et al. 2012)	12.32	1232	3.09

Cryptosporidium Concentrations in WWTPs Effluents (3/3)			
References	oocysts/100L	oocysts/L	Log$_{10}$ oocysts/L
(Flapper;, Campbell; et al. 2012)	12.5	1250	3.10
(Robertson, Hermansen et al. 2006)	13.16	1316	3.12
(Flapper;, Campbell; et al. 2012)	13.16	1316	3.12
(Flapper;, Campbell; et al. 2012)	13.54	1354	3.13
(Flapper;, Campbell; et al. 2012)	15.64	1564	3.19
(Flapper;, Campbell; et al. 2012)	15.71	1571	3.20
(Flapper;, Campbell; et al. 2012)	18.13	1813	3.26
(Flapper;, Campbell; et al. 2012)	18.42	1842	3.27
(Flapper;, Campbell; et al. 2012)	20.4	2040	3.31
(Flapper;, Campbell; et al. 2012)	21.28	2128	3.33
(Flapper;, Campbell; et al. 2012)	21.91	2191	3.34
(Flapper;, Campbell; et al. 2012)	23.96	2396	3.38
(Flapper;, Campbell; et al. 2012)	26.71	2671	3.43
(Flapper;, Campbell; et al. 2012)	26.87	2687	3.43
(Flapper;, Campbell; et al. 2012)	28.53	2853	3.46
(Flapper;, Campbell; et al. 2012)	30.91	3091	3.49
(Flapper;, Campbell; et al. 2012)	31.43	3143	3.50
(Flapper;, Campbell; et al. 2012)	32.34	3234	3.51
(Flapper;, Campbell; et al. 2012)	33.71	3371	3.53
(Flapper;, Campbell; et al. 2012)	33.91	3391	3.53
(Flapper;, Campbell; et al. 2012)	34.65	3465	3.54
(Flapper;, Campbell; et al. 2012)	36.91	3691	3.57
(Flapper;, Campbell; et al. 2012)	39.37	3937	3.60
(Flapper;, Campbell; et al. 2012)	50.27	5027	3.70
(Flapper;, Campbell; et al. 2012)	60.94	6094	3.78
(Flapper;, Campbell; et al. 2012)	62.32	6232	3.79

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Appendix II

Norovirus GI Concentrations in WWTPs Effluents*

*All datasets are from (Da Silva, Le Saux et al. 2007):
> Da Silva, A. K., J.-C. Le Saux, S. Parnaudeau, M. Pommepuy, M. Elimelech and F. S. Le Guyader (2007). "*Removal of norovirus in wastewater treatment using real-time RT-PCR: different behavior of genogroup I and genogroup II.*" Applied and Environmental Microbiology

**Plant C is a large AS plant mentioned in the literature above, and plant B is a small AS plant.

***This data is half of detect limitation (2500) we used to represent undetected data.

Plant**	Log10 gc/l	Plant**	Log10 gc/l
C	3.4***	C	3.8
C	3.7	C	3.8
C	3.7	C	3.8
C	3.7	C	3.8
C	3.7	C	3.8
C	3.7	C	3.8
C	3.7	B	3.9
C	3.7	B	3.9
C	3.7	B	3.9
B	3.8	B	3.9
B	3.8	B	3.9
B	3.8	B	3.9
C	3.8	B	4.2
C	3.8	C	4.5
C	3.8	C	4.7
C	3.8	B	5.2
C	3.8	C	5.4
C	3.8	B	5.5
C	3.8	C	5.7
C	3.8	C	6
C	3.8		

Appendix III

Norovirus GII Concentrations in WWTPs Effluents

*All datasets are from (Da Silva, Le Saux et al. 2007):
    Da Silva, A. K., J.-C. Le Saux, S. Parnaudeau, M. Pommepuy, M. Elimelech and F. S. Le Guyader (2007).
    "Removal of norovirus in wastewater treatment using real-time RT-PCR: different behavior of genogroup I and genogroup II." Applied and Environmental Microbiology
**Plant C is a large AS plant mentioned in the literature above, and plant B is a small AS plant
***This data is half of detect limitation (100) we used to represent undetected data.

Plant**	Log10 gc/l	Plant**	Log10 gc/l
C	2***	C	2.3
C	2***	C	2.3
B	2.2	C	2.3
B	2.2	C	2.3
B	2.2	C	2.3
C	2.2	C	2.3
C	2.2	C	2.3
C	2.2	C	2.4
C	2.3	C	2.4
C	2.3	C	2.4
B	2.3	C	2.4
B	2.3	C	2.4
B	2.3	C	2.4
B	2.3	C	2.4
B	2.3	C	2.4
B	2.3	C	3.1
B	2.3	C	3.1
B	2.3	C	3.2
B	2.3	C	3.3
C	2.3	C	3.5