Microbial and chemical risk from reclaimed water use for residential irrigation

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ABSTRACT

Arid and semi-arid locations are increasingly utilizing nontraditional irrigation water including reclaimed wastewater. Human health risk associated with reclaimed wastewater use was determined by testing reservoir, distribution line and home spigot water \( n = 190 \) and 14 types of vegetables and fruits \( n = 90 \) harvested from 5 home gardens for 7 waterborne pathogens, 47 antibiotic resistance genes and 12 pharmaceuticals and personal care products (PPCPs). Based on surveys of the residents' use of the reclaimed wastewater, two exposure routes were modeled: irrigation of fruits and vegetables and drinking from irrigation hoses. Probabilistic quantitative microbial risk assessment indicated that consumption of raw vegetables and fruits exceeded a 0.015 benchmark illness rate due to adenovirus and enterococci. Chemical risk assessments indicated that consumption of tons of vegetables per day and hundreds to millions of gallons of water per day would be needed to reach an unacceptable risk among the 10 PPCPs detected in home spigot water, indicating de minimis risk from PPCPs. Eight different drug resistance gene families were detected in the water samples and crops indicating that antibiotic-resistant organisms are present on foods irrigated with reclaimed water containing pharmaceuticals. These results elucidate the combined risk from pathogens and PPCPs from reclaimed wastewater irrigation.

Key words: home gardens, irrigation, pathogens, pharmaceutical and personal care products, reclaimed wastewater, risk assessment

HIGHLIGHTS

- Reclaimed wastewater irrigation presents unacceptable microbial human health risks.
- Reclaimed wastewater used for irrigation fosters antibiotic resistance associated with PPCPs.
- Irrigation with reclaimed wastewater presents de minimis chemical health risk.
- Pathogens and PPCPs accumulate differentially in vegetable skin and flesh.

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INTRODUCTION

The availability of water and its sustainable management has become a critical issue for many drought-stricken or water resource-limited communities in the United States. Diminished water supplies have resulted in an increased use of treated municipal wastewater, commonly known as reclaimed wastewater, for reuse applications. Reclaimed wastewater is a valuable resource for crop irrigation, in particular in semi-arid and arid climates not only as a water source, but also as a source of nutrients. State regulatory authorities typically specify a certain level of treatment and monitoring of reclaimed wastewater to protect public health and the environment, in a fit-for-purpose approach (Chhipi-Shrestha et al. 2017). Water quality regulations for utilizing reclaimed wastewater for irrigation typically specify acceptable log reductions of microorganisms, physicochemical parameters (e.g., turbidity, oxygen demanding material, chlorine residual), agronomic parameters (e.g., salinity, sodium adsorption ratio, pH), and the number and types of treatment technologies required for the particular reuse scenario (Shoushtarian & Negahban-Azar 2020). Yet depending on the level of treatment there may still be waterborne pathogens or pharmaceuticals and personal care products (PPCPs) present in reclaimed wastewater used for irrigation. In the fit-for-purpose treatment approach, various engineered and agricultural controls can be utilized to reduce the potential risk to users irrigating with reclaimed wastewater (Mohr et al. 2020). For example, irrigation methods can be selected to limit risk to consumers by either limiting the edible portion of the crops exposed to irrigation water (i.e., drip or subsurface irrigation of lettuce) or only irrigating crops that are cooked prior to eating (i.e., potatoes) (van Ginneken & Oron 2000).

Depending on the level of treatment of reclaimed wastewater, numerous waterborne pathogens have been reported to be present including various bacteria (e.g., Escherichia coli, enterococci, Staphylococci, Salmonella) (Goldstein et al. 2014b; Santiago et al. 2018), protozoans (e.g., Giardia and Cryptosporidium) (Ryu et al. 2007; Domenech et al. 2018) and viruses (e.g., adenovirus, norovirus) (Ijema et al. 2010; Gonzales-Gustavson et al. 2019). Yet the risk to human health from exposure to these pathogens in reclaimed wastewater used for irrigation is not always clear. This uncertainty arises from (1) lack of analysis of pathogens themselves and reliance instead on fecal coliforms which do not always correlate with pathogen abundance (Wu et al. 2011); (2) lack of information on the viability of the pathogens due to reliance on nucleic acid-based techniques rather than culturing (Whiley & Taylor 2016); (3) lack of studies on persistence of pathogens after irrigation and (4) left-censored datasets of pathogen concentrations below detection limits (Kato et al. 2013). Furthermore, there are a limited number of risk assessments of vegetable crops irrigated with reclaimed wastewater where the crops themselves were evaluated for pathogens. Instead, many quantitative microbial risk assessments (QMRAs) for irrigation of foods are based on measuring coliforms or pathogens in water that are presumed to be retained on crops (van Ginneken & Oron 2000; Hamilton et al. 2006; Al-Sa’ed 2007; Ryu et al. 2007; Verbyla et al. 2016; Gonzales-Gustavson et al. 2019).

In addition to risks posed by pathogens, PPCPs in reclaimed wastewater used for irrigation may also present human health risks. There are several review articles on the impacts of PCPPs on vegetable crops irrigated with reclaimed wastewater (Qin...
Fourteen types of vegetables and fruits (Supplementary Table S1) were collected from
Vegetable and soil sample collection and handling
analysis were returned to the laboratory within 4 h and stored at 4 °C. The water samples for PPCP
microbial analysis, samples were stored on ice during transport to the laboratory. All water samples for microbial analysis
samples collected and dates of collection are shown in Supplementary Table S1. After aseptic collection of samples for
All water samples were collected in separate sterile 4-L amber glass bottles for microbial and PPCP analysis. Details of the
Water supply. The volume of reclaimed wastewater is not suf-
and L3), only surface water (L6, L7 and reservoir at the end of season) or a mixture of reclaimed wastewater and surface
water from the irrigation reservoir. Therefore, different parts of the city receive either only reclaimed wastewater (L1, L2
and L3), only surface water (L6, L7 and reservoir at the end of season) or a mixture of reclaimed wastewater and surface
water (L4 and L5) throughout the irrigation season.

Water sample collection and handling
All water samples were collected in separate sterile 4-L amber glass bottles for microbial and PPCP analysis. Details of the
samples collected and dates of collection are shown in Supplementary Table S1. After aseptic collection of samples for microbial analysis, samples were stored on ice during transport to the laboratory. All water samples for microbial analysis were stored at 4 °C upon arrival and were processed within 12 h of arrival at the laboratory. The water samples for PPCP analysis were returned to the laboratory within 4 h and stored at 4 °C.

Vegetable and soil sample collection and handling
Fourteen types of vegetables and fruits (Supplementary Table S1) were collected from five household gardens that were located close to the WWTP (Figure 1, locations G1 to G5). Vegetables and fruits that are eaten raw, with the exception of
...
potatoes, were sampled and analyzed along with soil co-located with the plants. Vegetation and soil collection and processing methods are detailed in the Supplementary material and Table S1.

**Pathogen detection methods**

Pathogens in water, soil and vegetables were quantified by both culture and qPCR. Culturing of *E. coli* and enterococci from samples was performed following EPA Methods 1603 and 1600, respectively (USEPA 2002a, 2002b). Modified MTEC and MEI agar (Hardy Diagnostics, USA) were prepared based on the manufacturer’s instructions. Water samples were also filtered through sterile mixed cellulose ester 0.45 and 0.22 μm filters (Fisher Scientific Inc., USA), as necessary. Nucleic acids were extracted from the filters using the methods described in the Supplementary material. Quantitative PCR was used to determine the abundance of the *uidA* gene of *E. coli*, the 23S rRNA gene of *Enterococcus* spp. (enterococci), the *invA* gene of *Salmonella enterica*, the *mip* gene of *Legionella pneumophila*, the β-giardin gene of *Giardia intestinalis*, the polymerase-capsid junction (ORF1-ORF2) of norovirus genotype I and the hexon gene for serotypes human adenovirus serotype 40 and 41. Standard curves were made using a nine-fold serial dilution of plasmids containing *uidA*, *invA* or 18S genes or stabilized Ultramer® DNA oligonucleotides (Integrated DNA Technologies, USA) for *L. pneumophila*, *G. intestinalis*, Norovirus GI and Human Adenovirus 40/41. The thermocycler conditions, primer and probe concentrations and mastermix used for qPCR are presented in Supplementary Table S2. The abundance of pathogens in samples determined by qPCR in gene copies/mL water or gene copies/g vegetable or soil were converted to cells per mL or g by dividing by the number of gene loci per organism. It was assumed that *E. coli* had five copies of *uidA* (Metcalf & Wanner 1993); enterococci have nine copies of 23S rRNA (Chakravorty *et al.* 2007); *Salmonella* spp. have five copies of *invA* (González-Escalona *et al.* 2009) and *Giardia intestinalis* have two copies of β-Giardin P241 (Alonso *et al.* 2010). Norovirus, adenovirus and *Legionella* were assumed to have one copy of the gene targeted by qPCR.

**Figure 1** | Sample collection locations and distribution line for reclaimed wastewater in Hyrum, UT. L#, distribution line sampling locations; G#, garden sampling locations.
Antibiotic resistance profiling

The nucleic acids extracted from the water distribution system lines (n=12), irrigation reservoir (n=2), treatment plant effluent (n=2) and crops (n=5 each of cherry tomato, cucumber, lettuce and zucchini) were analyzed for antibiotic resistance genes at the University of Minnesota. Details of the microfluidic qPCR assay targeting 47 antibiotic resistance genes were described previously (Ahmed et al. 2018). The resulting concentrations of ARGs were normalized by the mass of DNA extracted from each sample as measured by a Qubit fluorometer.

Quantitative microbial risk assessment methods

All pathogen concentrations for vegetables and water were aggregated and a Bayesian analysis of the left-centered data was performed to generate distributions for each exposure route. Details of the Bayesian analysis, R code, distributions of raw data and model fit parameters are included in Supplementary Figures S1 and S2. The two exposure routes considered for home gardeners were: (1) direct ingestion through drinking from a hose and (2) eating raw produce irrigated with the reclaimed wastewater with and without rinsing. The exposure routes were selected based on surveys of the community where 3.7% of respondents indicated drinking from the hose containing reclaimed wastewater and 92% of respondents used the reclaimed water with and without rinsing. The exposure routes were selected based on surveys of the community where 3.7% of respondents indicated drinking from the hose containing reclaimed wastewater and 92% of respondents used the reclaimed wastewater to irrigate vegetables (Flint & Koci 2020). The daily dose of pathogen ingested through eating vegetables was estimated by Dosevegetables = Ci* Mvegetables Mbody. Furthermore, as many QMRA studies evaluate contaminated water retained on vegetables as a surrogate for testing vegetables themselves, we also estimated the daily dose of pathogen ingested through reclaimed wastewater retained on vegetables by Dosewater on vegetables = Ci* Mvegetables Mbody Vwater retained. The daily dose of pathogen ingested during drinking from a reclaimed wastewater hose was estimated by Dossedrinking water ingestion = Ci* Vdrinking water. Where Ci is drawn from 10,000 resamplings of the Bayesian transformed distribution for each pathogen in reclaimed wastewater or vegetables measured by culture and/or qPCR methods; Mvegetables is the mass of vegetables consumed per unit body weight; the Mbody is the body weight of an average adult in the United States; Vwater retained is the volume of reclaimed wastewater retained on lettuce after irrigation and Vdrinking water is the volume of water consumed from a hose. Furthermore, the Vdrinking water from a hose was assumed to be the volume of cold tap water consumed at home by pregnant women employed part time or less. While this assumption is less conservative than assuming the total volume of water consumed in a day comes from the garden hose, it is likely still over estimating the volume of reclaimed wastewater that is consumed per exposure event from a hose (USEPA 2019).

The annual probability of infection after eating vegetables or drinking water contaminated with E. coli (Powell et al. 2000) or Salmonella (Ahmed et al. 2010) were estimated using the beta-Poisson model, with enterococci (Tseng & Jiang 2012) and adenovirus (Teunis et al. 2016) by two different exponential models, and with norovirus (Messner et al. 2014) by the fractional Poisson model. Details of the exposure models, parameters and assumptions for each organism are shown in Supplementary Tables S3 and S4. The probability of illness, Pi11, was determined by modifying the probability of infection, Pinf, by the model Pi11 = Pinf * Pi11inf. The probability of illness given infection, Pinf, for each organism is shown in Supplementary Table S4. The annual risk of disease, Pann ill, was calculated by Pann ill = 1 - (1 - Pi11) ^ n, where n represents the number of exposure events per year (Seidu et al. 2013). In this study, it was assumed that people ate vegetables irrigated with reclaimed wastewater either 15, 30 or 90 days a year and drank reclaimed wastewater from the garden hose once or twice per week during the growing season or 12–36 days per year. The models did not account for the decay of pathogens on produce after deposition.

The estimated annual probability of illness was compared against two benchmarks. First, residents drinking reclaimed wastewater from the homes were compared against the acceptable probability of illness benchmark from the USEPA of a one-time infection per 10,000 individuals in a given year (abbreviated as 10^-4 per person per year or pppy) (Rose & Gerba 1991). Previous QMRA studies for vegetables irrigated with reclaimed wastewater have commonly applied the drinking water 10^-4 probability of infection per year benchmark (Hamilton et al. 2006). This benchmark is highly conservative. Second, data from the CDC on annual illness originating from pathogen contaminated vegetables reports 9,388,075 annual illnesses from 1998 to 2008 for an average population of 298 million US citizens in 2006 (Scallan et al. 2011). This results in a 3.15 x 10^-2 pppy or 315 in 10,000 individuals in a given year falling ill from pathogens on produce.

The QMRA distribution fitting, calculations and random sampling for the risk study were conducted with SAS (ver. 9.4; SAS Institute, Inc., Cary, NC) using PROC UNIVARIATE, PROC COOR and the RAND function. All input parameters were drawn from 10,000 random samplings of their probability distribution functions (Supplementary Tables S3 and S4). The sensitivity of the estimated probability of infection due to variability in the input parameters was assessed by evaluating the Pearson correlation between the Pann inf and input parameters (e.g., pathogen concentration, volume of water retained).
This method was chosen due to its ease of implementation and ability to show nonlinear correlations between parameters as reported previously (Hamilton et al. 2006; Haas et al. 2014; Verbyla et al. 2016).

**PPCP analysis**

The analytes used in this study included pharmaceuticals (acetaminophen, carbamazepine, gemfibrozil, sulfamethoxazole and fluoxetine), hormones (estrone, progesterone and β-estradiol), personal care products (N,N-diethyl-metatoluamide (DEET) and triclosan), a fire-retardant (tris-2-chloroethyl phosphate) and caffeine. These compounds were selected due to their wide usage and varying physical/chemical properties providing representative chemicals of the many the PPCPs commonly used. Samples were analyzed for PPCPs using a modified USEPA Method 1694. The selected PPCPs were quantified using an Agilent 1290 Infinity LC system with an Agilent 6490 Triple Quadrupole MS. Details of sample processing and analytical methods are given in the Supplementary material.

**PPCP risk assessment methods**

The two PPCP exposure routes considered for home gardeners were the same as those considered for the microbial risk. None of the PPCPs evaluated in this study are reported carcinogens, and quantitative risks associated with chemical PPCP exposure due to the use of reclaimed wastewater was estimated by using the following clinical dose or toxicity endpoints: clinical dose of pharmaceuticals, mg/d; toxic dose low (TDLO), mg/kg and average daily intake (ADI), mg/kg, for 70 kg adult and/or 7 kg infant; no-observed-adverse-effect level (NOAEL), mg/d and the margin of exposure (MOE)=NOAEL/Calculated Dose based on 1 L/d water or 356 g/d vegetable intake, which is recommended to be >10 (Coordinators 2017). The values of these clinical doses or toxicity endpoints available for the PPCPs monitored in this study are shown in Supplementary Table S5. If a clinical dose was available for a compound, the volume of water, Volwater, or mass of vegetable matter, Massvegetable, that would need to be consumed to reach this clinical dose was calculated by Volwater=Clinical Dose/Cw or Massvegetable=Clinical Dose/Cv, where Cw and Cv are the highest concentration of the compound measured in the paired garden spigot water (mg/L) and the household garden vegetable samples (mg/g), respectively, from samples collected in 2018. If a TDLO or an ADI were available, the lowest Volwater or Massvegetable, required to reach these doses were calculated by Volwater=TDLO or ADI*70 kg/(Cw) for adult exposure [or 7 kg/(Cw) for infant exposure] or Massvegetable=TDLO or ADI*70 kg/Cv for adult exposure [or 7 kg/(Cv) for infant exposure]. If a NOAEL for a compound was available, an MOE was calculated based on the ratio of the calculated dose expected from exposure to the highest concentrations for that PPCP detected in a spigot or vegetable sample collected during the study, and standard 1 L/d water or 356 g/d vegetable intake values. The MOE was then calculated by MOE=NOAEL/[(Cw)*1 (L/d)] or NOAEL/[(Cv)*336 (g/d)].

**RESULTS AND DISCUSSION**

**Seasonal PPCP concentrations in treatment plant effluent and distribution line reuse wastewater**

The PPCPs included in this study, with the exception of β-estradiol and estrone, were consistently detected in the wastewater effluent and in the irrigation reservoir (Figure 2). The concentrations of most PPCPs in the effluent were similar to literature values (Munoz et al. 2010; Goldstein et al. 2014a; Malchi et al. 2014); however, the concentration of sulfamethoxazole was consistently an order of magnitude higher (Figure 2) than reported in other studies (Wang & Gardinali 2014; Chitescu et al. 2015). The entire distribution system was initially influenced by the WWTP effluent (Figure 2, spring plots) as effluent is used to fill the reclaimed water distribution system and irrigation reservoir prior to the availability of surface water supplied by canal in the spring. However, by the end of the irrigation season, the influence of the WWTP effluent is found primarily in the northwest sector of the reclaimed wastewater system (Figure 2, line locations L2 and L3) as the wastewater treatment plant effluent supplies only a portion (≈14%) of the total irrigation demand during the warmer portion of the summer.

**Microbial quality of treatment plant effluent and distribution line reclaimed wastewater**

Pathogens were detected by qPCR and culture-based methods in the treatment plant effluent and water from the reservoir and distribution system (Figure 3, blue bars). Viable cells estimated by culture-based methods for E. coli and enterococci (data not shown) were typically lower than those estimated by qPCR presented in Figure 3. The range of E. coli and enterococci determined by culture-based methods in the treatment plant effluent were below typical permitted discharge limits at 0.55±0.07 and 2.4±2.6 CFU/100 mL (average ± standard deviation), respectively. The coliforms estimated by qPCR were higher,
E. coli and enterococci were 0.66 ± 0.83 and 1.5 ± 1.1 log cells/100 mL (after accounting for numbers of uidA and 23S gene copies per cell). In contrast, the distribution lines had higher culture-based concentrations (199 ± 581 E. coli and 127 ± 216 enterococci CFU/100 mL) as compared to qPCR-based methods (20.9 ± 49.6 and 33.7 ± 134 gene copies/100 mL for E. coli and enterococci). The geometric mean of E. coli (23 CFU/100 mL) was below the Food Safety Modernization Act irrigation water limit (126 CFU/100 mL) (FDA 2016). The mean concentrations of pathogens in the reservoir were typically lower than in the treatment plant effluent and the distribution system (Figure 3). Legionella was detected infrequently (9 of 29 water samples and quantifiable in 2 samples) and had low concentrations (8.0 ± 15 gene copies/100 mL when quantifiable). Similarly, Giardia was detected infrequently (1 of 19 water samples). Therefore, Legionella and Giardia were not tested for in vegetable and soil samples. The antibiotic resistance profiles of the reclaimed wastewater effluent, reservoir and distribution system indicated the presence of eight different families of drug resistance (Supplementary Figure S3) and included aminoglycosides, beta-lactamase, chloramphenicol, sulfonamide, tetracycline, macrolides, metal resistance genes and multi-drug resistance. Typically, the water in the reclaimed wastewater distribution system contained a larger percentage of multi-drug resistant genes than the reservoir water or the WWTP effluent. It is suspected that the biofilms in the distribution system are harboring the antibiotic resistance genes and serving as a location for horizontal gene transfer as reported by others (Garner et al. 2018). Given the persistence of the antibiotics in the reclaimed wastewater (Figure 2), there is selective pressure to maintain these antibiotic resistance genes within the biofilm population.

### Distribution of pathogens in vegetable and soil samples

Pathogen genomic material was frequently detected on home garden produce and in soils (Figure 3). There was a wide variability in the pathogen detection frequency with the uidA gene of E. coli (87%) and the 23S gene of enterococci (88%) being
found most frequently, while invA gene of Salmonella (40%), adenovirus 40/41 (42%) and norovirus G1 (17%) were detected less frequently. The highest pathogen concentrations observed were for enterococci ranging from $3.4 \pm 2.1$ log gene copies/g, then the uidA gene of E. coli at $2.1 \pm 1.2$ log gene copies/g, invA gene of Salmonella spp. at $1.5 \pm 1.7$ log gene copies/g, adenovirus 40/41 at $23 \pm 69$ gene copies/g and norovirus at $15 \pm 66$ gene copies/g. In all cases, the pathogen genomic material was in higher concentration in stomached samples as compared to the corresponding vegetable rinse water (Figure 3).
Culturable enterococci were found more frequently in the stomached vegetable samples (23 of 37 samples) than in the rinse water (20 of 40 samples) from the vegetables. In contrast, fewer culturable *E. coli* were detected in stomached vegetable samples (12 of 37 samples) than in the rinse water (17 of 40 samples). Culturable enterococci (5 of 12 composite samples) and *E. coli* (1 of 12 composite samples) were detected in the garden soils, although at low levels (3.4 ± 6.3 enterococci CFU/g and 0.08 ± 0.29 *E. coli* CFU/g). Pathogen genomic material was in higher concentration in soils compared to culture-based results (Figure 3) and ranged from 20 gene copies/g soil to 4 log gene copies/g soil.

Eight antibiotic resistance families were detected in lettuce, cucumber, zucchini and cherry tomatoes (Supplementary Figure S3). In general, the cherry tomatoes and lettuce were found to have higher abundances of antibiotic resistance genes than the cucumbers and zucchini. These findings support the studies by others that suggested antibiotic resistance genes decreased in concentration along the continuum from soil, rhizosphere, roots, leaves and fruits (Cerqueira et al. 2019). In this study, sulfonamide resistance genes made up 7–28% of the antibiotic resistance genes detected in the crops in which sulfamethoxazole was quantifiable (Table 1 and Supplementary Figure S3), i.e., lettuce, zucchini and cucumber. Relatively few studies have reported on field trials evaluating the plant uptake of antibiotics from reclaimed water irrigation (Malchir et al. 2014; Wu et al. 2014; Prosser & Sibley 2015; Christou et al. 2017a). In contrast to the results herein, some studies did not detect the accumulation of sulfamethoxazole in tissues of vegetables irrigated with water containing 0.28–250 ng/L of the antibiotic (Goldstein et al. 2014a; Wu et al. 2014), yet others reported accumulation in select crops (Malchir et al. 2014; Franklin et al. 2016).

### Distribution of PPCPs in vegetable and soil samples

The frequency of detection of PPCPs, as well as the maximum concentration detected in various samples collected during the field study, is shown in Table 1. Samples included home garden spigots, home garden vegetables and soils that were irrigated with reclaimed wastewater and corresponding samples from the control locations. The spigot water from the Salt Lake City

| Compound* | Spigot (ng/L) | Crops (ng/g) | Soils (ng/g) | MOE* | Quantity of Spigot water consumed to reach risk thresholdb | Quantity of vegetables consumed to reach risk thresholdb |
|-----------|--------------|--------------|--------------|------|----------------------------------------------------------|----------------------------------------------------------|
| ACE       | 0 of 15      | 1 of 66 (33.7) | 1 of 20 (4.0) | 278 (Plant) | NA                                                      | 98 Ton/d                                                  |
| CAFF      | 7 of 15 (4.3) | 4 of 66 (23.9) | 3 of 20 (11.2) | 21,792 (Plant) | 24.6 Mgal/d                                             | 18 Ton/d                                                  |
| CARB      | 14 of 15 (13.5) | 18 of 66 (10.9) | 10 of 20 (2.3) | 31.3 Mgal/d | 162 Ton/d                                               |
| DEET      | 15 of 15 (104.8) | 4 of 66 (27.3) | 0 of 20                  | 10.902 (Plant) | 0.53 Mgal (TDLo) | 0.85 (TDLo) Ton |
| FLUO      | 9 of 15 (13.6) | 34 of 66 (63.5) | 8 of 20 (5.5) | 1.2 Mgal/d | 1.0 T/d                                                  |
| GEMF      | 0 of 15      | 0 of 66                  | 0 of 20                  | NA | NA                                                      | NA                                                      |
| PROG      | 8 of 15 (24.8) | 33 of 66 (25.0) | 4 of 20 (6.0) | 238 (Plant) | 1.1 Mgal/d                                               | 4.4 T/d                                                  |
| SULF      | 15 of 15 (284.4) | 12 of 66 (44.5) | 0 of 20                  | 10.4 Mgal (TDLo) | 277 T                                                  |
| TRIC      | 1 of 15 (11.3) | 0 of 66                  | 0 of 20                  | 23,800 gal/d | NA                                                      | NA                                                      |
| TRIS      | 5 of 15 (20.4) | 0 of 66                  | 0 of 20                  | 444 (Infant) gal/d | NA                                                      | NA                                                      |

*aACE, Acetaminophen; CAFF, Caffeine; CARB, Carbamazepine; DEET, N,N-diethyl-metatoluamide; FLUO, Fluoxetine; GEMF, Gemfibrozil; PROG, Progesterone; SULF, Sulfamethoxazole; TRIC, Triclosan; TRIS, Tris-(2-chloroethyl) Phosphate.*

*bClinical dose or toxicity endpoint tabulated in Supplementary Table S5.

The distribution of PPCPs in vegetable and soil samples is shown in Table 1. Samples included home garden spigots, home garden vegetables and soils that were irrigated with reclaimed wastewater and corresponding samples from the control locations. The spigot water from the Salt Lake City...
control site had detectable concentrations of carbamazepine and DEET in all samples, while progesterone and sulfamethoxazole were detected in one of the three samples collected (Supplementary Table S6).

PPCP detection was generally more frequent and at higher concentrations in the Hyrum household samples compared to the control location samples (Table 1 and Supplementary Table S6), indicating that the Hyrum WWTP effluent did impact PPCP concentrations in home garden samples collected in this study (Table 1). All PPCPs that were found in household spigot water, except triclosan and tris-(2-chloroethyl), were found in fruits and vegetables irrigated with this reclaimed wastewater. Triclosan (Wu et al. 2013) has been reported to accumulate in plant roots although roots were not analyzed in this study. Triclosan also biodegrades in soil (Durán-Álvarez et al. 2015) supporting the lack of detection of this compound in both the irrigated and control soils. In contrast to the results herein, the flame retardant tris-(2-chlorethyl) phosphate was reported by others to accumulate in strawberries and lettuce up to 200 ng/g dry weight (Hyland et al. 2015a). It was shown that the accumulation and translocation of tris-(2-chlorethyl) phosphate was primarily due to transport from the roots to shoots (Hyland et al. 2015b). Therefore, although this flame retardant was detected in 30% of the spigot water samples, its lack of detection in soils, likely due to biological transformation of the compound in soils (Zhang et al. 2021), resulted in a lack of accumulation of the compound in vegetables in this study.

DEET and sulfamethoxazole accumulated in the edible portion of plants but were not detected in the soil (Table 1). Acetaminophen, caffeine, carbamazepine, fluoxetine and progesterone also accumulated in the edible portion of plants in concentrations 2–15 times greater than found in the adjacent soil (Table 1). Fluoxetine as a cation, sorbs to soil limiting plant uptake (Wu et al. 2010, 2014) and has limited translocation within plants (Wu et al. 2013). But in this study fluoxetine was one of the more frequently detected PPCP in vegetable samples (52%) and was found in all vegetable types (cucumber, tomato, squash, peppers, apples, potato, strawberry) (Supplementary Figure S4). Progesterone was also present in 50% of the tested vegetables (mean of 10.16 ± 4.57 ng/g) as well as 53% of the spigot water (4.07 ± 5.84 ng/L) and in 20% of the tested soil samples (mean of 3.84 ± 1.51 ng/g) (Supplementary Figure S4). Progesterone, however, has been reported to be naturally present in plant tissue including tomato, apples, potato, peas and beans and functions as a natural plant growth regulator (Janeczko 2012). Detection of progesterone in control plant samples in this study, including tomatoes, onion and potatoes, occurred at a lower frequency (33%) and at a lower average (8.48 ± 3.22 ng/g) but not statistically different concentration than plants irrigated with reclaimed wastewater. Gemfibrozil was not detected in spigot, plant nor soil samples despite being consistently detected in the WWTP effluent and distribution line samples (Figure 2 and Table 1).

Quantitative microbial risk assessment
The annual probability of illness from eating vegetables irrigated with this reclaimed wastewater exceeded the United States average rate of foodborne illness (Figure 4(a)) when residents ate raw home-grown vegetables more than 90 days of the year. In particular, the risk of gastroenteritis was estimated to be highest from adenoivirus 40/41 and enterococci. Estimates of the gastroenteritis risk from pathogens measured in water were always lower than the risk estimated from pathogens on vegetables not removed by rinsing (i.e., processed by stomaching where the whole vegetable is mashed). The risk estimated from qPCR-based detection of E. coli and enterococci was always one or two-log greater than the risk estimated from culture-based concentrations of pathogens. Individuals who drink water from the reclaimed wastewater line had an unacceptable risk of gastroenteritis (i.e., exceeding 1 in 10,000 risk) regardless of how frequently they drank from the garden hose (Figure 4(b)). The QMRA estimated annual probability of illness model from exposure to pathogens on food was found to be most sensitive to pathogen concentration (Pearson’s correlation, $r=0.2–0.82$, $P<0.0001$, $n=10,000$. Supplementary Table S7). In contrast, the volume of water consumed tended to be equally as important as the pathogen concentration for the drinking water exposure route (Supplementary Table S7).

As with all risk assessments, there are uncertainties in the input variables that should be considered when interpreting the results. The QMRA likely overestimated the risk to consumers as the dose of pathogens was estimated from qPCR results which include both live and dead organisms. Furthermore, the risk may be overestimated as the decay of pathogens on the vegetables after irrigation was not considered. Other inputs into the QMRA that could result in over or underestimation of risk include: the mass of the vegetable consumed, the volume of reclaimed wastewater retained on the vegetable, the individual bodyweight, exposure frequency, the volume of reclaimed wastewater consumed from a garden hose and the potential for population immunity. The role of population immunity in the risk of illness was not considered herein, but it is likely that
sustained exposure to the pathogens in the reclaimed wastewater used for irrigation may result in fewer annual illnesses in this exposed population.

**PPCP risk assessment**

The chemical risk assessment based on clinical dose or toxicity endpoints available for the PPCPs monitored in this study is shown in Table 1 and is considered negligible for all PPCPs evaluated. The calculated MOE for water or vegetable consumption values were based on the highest concentrations of a given PPCP detected in a spigot or vegetable sample collected during the study (Table 1), and the volume of water or mass of vegetable matter that would have to be consumed to reach the NOAEL for that PPCP. The ADI via ingestion by exposure pathway (contaminated food and water) and route (drinking or eating contaminated food or water) is listed as the water volume (gallons per day) or mass (tons per day of produce) that would be required to be consumed to reach either the clinical dose, the lowest toxic threshold dose TDLO or the recommended ADI, whichever was lower. The lowest MOE was determined to be 238 based on a daily consumption of 336 g/d of green beans grown in the garden location with the maximum progesterone concentration of 25 ng/g (Table 1), compared to the recommended value for the MOE of 100 or more. Risks associated with clinical dose, toxic dose or ADI-related impacts were found to be even lower based on the excessive daily quantities of water (444 gal/d for infants exposed to tris-(2-chloroethyl phosphate) to 31.3 MGD for adults exposed to carbamazepine) or vegetables (0.85 T for adult DEET

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**Figure 4** | Log annual probability of illness from pathogens on (a) vegetables irrigated with reclaimed water and (b) from drinking irrigation water from garden hoses. EC uidA, uidA gene of E. coli; ENT 23S, 23S gene of enterococcus; CFU, colony forming units; ADE, adenovirus; NOR, norovirus; SAL, Salmonella spp.; S, pathogen abundance measured on the vegetables by the stomacher method; W, pathogen abundance assumed to be in reclaimed wastewater retained on vegetables, n=15 or 90 assumes 15 or 90 days of eating raw vegetables during the summer growing season. Vertical dashed lines indicate the benchmark foodborne illness from produce (15 in 1,000 annual probability of illness) and from drinking water (1 in 10,000 annual probability of illness). The symbols indicate the mean probability of illness by organism and exposure frequency and the gray horizontal lines indicate the range of 5th and 95th percentiles of the Monte Carlo simulations.
exposure to 118 T/d for adult exposure to carbamazepine) that are required to be consumed to reach these levels of human health concern.

The PPCPs in the irrigation water all represent insignificant risks to the public under worst-case exposure assumptions of direct ingestion of either reclaimed wastewater itself, or raw foods irrigated with reclaimed wastewater containing low concentrations of these PPCPs. The required harmful human threshold consumption rates of water and produce from the private gardens sampled in this study are not physically possible, which confirms findings from other researchers (Prosser & Sibley 2015; Christou et al. 2017b) that human health risks associated with PPCPs in reclaimed wastewater appear to be de minimis, although this study did not evaluate degradation products of various PPCPs that may pose higher risk than the parent compound. Risk from PPCPs in reclaimed water via direct water ingestion or ingestion of raw foods irrigated with reclaimed wastewater does not contribute directly to the overall risk of reclaimed wastewater use in Hyrum, the bulk of which comes from health risks associated with pathogen exposure.

Limitations in the PPCP risk assessment primarily arise from the small subset of the potential organic chemicals present in the wastewater treatment system that were identified and quantified in this study. The compounds that were studied were selected based on their presence in other reclaimed wastewater sources, the range of physical/chemical/biological properties they represent and the authors’ experience with these compounds in prior and on-going studies. There may be other compounds, i.e., PFAS compounds for example, that were not quantified in this study that could represent a greater human health risk than those that were studied. In addition, as Malchi et al. (2014) have suggested, metabolites could potentially represent greater risk than their parent compounds, and these issues warrant further study. Furthermore, lifetime exposure to PPCPs in home garden vegetables and reclaimed wastewater at low-doses observed in this study could present potential risks not captured by the clinical dose values available for these compounds.

CONCLUSION
The results presented herein are one of the few studies looking at uptake of PPCPs, pathogens and antibiotic resistance genes in food crops at a field scale. Overall, the results suggest that after irrigation with reclaimed wastewater: (1) the risks from pathogens on crops eaten raw exceed benchmark levels of gastroenteritis in the US (Scallan et al. 2011), (2) the risks from exposure to PPCPs taken into crops or through direct ingestion of reclaimed wastewater is de minimis, but (3) multiple families of antibiotic resistance genes are present on food crops which may be associated with the presence of antibiotics in this reclaimed wastewater. Therefore, while the direct chemical risk from exposure to PPCPs is low, antibiotics in reclaimed water may exert selective pressure on microorganisms on food crops to acquire or maintain antibiotic resistance. When partnered with surveys of community members using the reclaimed wastewater (Flint & Koci 2021), this work suggests that increased outreach to the public is required to ensure community members are not drinking the nonpotable water and that they are properly washing harvested fruits and vegetables before consumption. Furthermore, the inclusion of a post-chlorination step should be considered for the reclaimed wastewater that is distributed for irrigation to minimize the growth and accumulation of biofilms and subsequent antibiotic resistance genes within the secondary water distribution system. Finally, QMRAs evaluating risk to consumers from irrigation with reclaimed wastewater should consider also sampling vegetation rather than just water to more accurately reflect the true health risk associated with reclaimed wastewater reuse.

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DATA AVAILABILITY STATEMENT
All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST
The authors declare there is no conflict.
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