Abschlussbericht TransMiT

Teil B

B 3.1
Material und Methoden – Analyse/Analytik

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Kurzbeschreibung des Einzelkapitels

Ein besonderer Schwerpunkt innerhalb des TransMIT-Projektes war die Untersuchung der Qualität des Regenwassers im Hinblick auf Hygieneparameter/Pathogene, um ggf. vorliegende Risiken einer Nutzung zu erkennen und durch baulichen und betrieblichen Anpassungen zu begegnen. Methodisch wurde hierzu die QMRA – quantitative mikrobielle Risikoanalyse eingeführt und angewendet.
In dem folgenden Kapitel werden die Materialien und Methoden beschrieben, welche innerhalb des Untersuchungspakets verwendet wurden.

Die Ergebnisse wurden im Detail auch veröffentlicht unter https://doi.org/10.1111/risa.14145

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1 Health risk assessment

The quantitative microbial risk assessment (QMRA) approach was used in the present study to assess the risks posed by microbial agents and obtain the statistical probability of an

adverse health outcome. This approach for risk assessment integrates a wide scientific knowledge about microorganisms, such as their concentrations and behavior in water, the routes and amounts of exposure to humans and the probable health outcomes from exposure. With this information a single assessment is performed, allowing a proper management of the risk (CAMRA, 2020a; WHO, 2016).

The main structure of the QMRA adopted in the present study consisted of four steps. The first step is hazard identification (section 2.4.1), where the bacteria of interest that could be present in the system are identified, as well as the illnesses caused by them, the transmission routes, and probable health outcomes. Second, in the exposure assessment step (section 2.4.2), the bacterial numbers present in a single exposure event are quantified. For the third step, health effects assessment (section 2.4.3), the corresponding dose-response models are applied to each bacterium depending on the sought outcome (infection, illness, or death) and in the last step, risk characterization (section 2.4.4), an estimate of the risk is obtained (Ortells Sales, 2015; WHO, 2016). A summary of the steps carried out for the QMRA study, their outcome, and the sections where each step is further described is presented in Fig. 3.

Given that most of the exposed population in the studied locations are children, the present study assesses the health risk posed by ornamental fountains to children.

1.1 Hazard identification

The QMRA was performed using as reference bacteria E. coli, Enterococci, and Salmonella non-typhoid to account for the probability of gastrointestinal illnesses and P. aeruginosa to assess the probability of infection due to dermal exposure. Vibrio cholerae, Listeria monocytogenes, and Campylobacter spp. were not included in the risk assessment study because the concentrations found from lab analysis were always below the limit of detection of the cultivation method.

The concentrations of E. coli, Enterococci, Salmonella, and P. aeruginosa were evaluated to identify the probability distribution that best represents the data obtained from each bacterium. The probability distributions, Weibull, Gamma, Beta and Log-normal were tested using maximum likelihood estimation (MLE) in RStudio software (2020) and the goodness-of-fit parameters for all the distributions were determined using the Loglikelihood and Akaike criteria (AIC). The selected probability distribution describing the concentration of each bacterium was used later as input for the dose-response model (section 2.4.3).

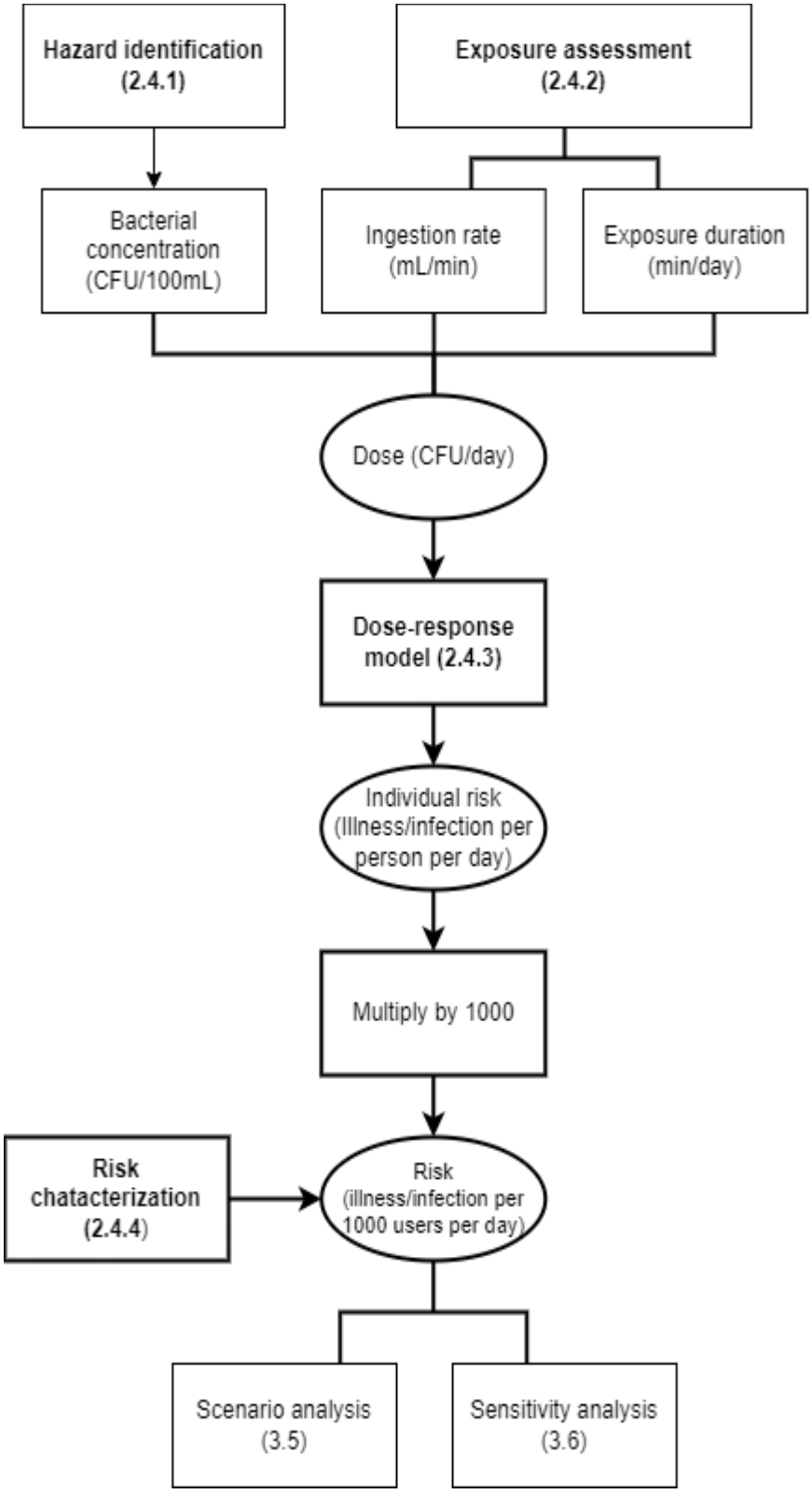

Figure 1: Flow chart of the calculation of illnesses/infections per 1000 users per day. In brackets are the number of the section detailing each step (Sunger and Haas, 2015).

1.2 Exposure assessment

In the following paragraphs we describe the procedure used to estimate the ingestion rate (QI), the rate of water in contact with the skin of the hands (QH), and time of exposure (t).

For the ingestion exposure route associated with gastrointestinal illnesses, two main pathways were analyzed for people having direct water contact: ingestion due to hand-to-mouth contact and ingestion of water droplets during splashing when playing with water. The ingestion rates for each pathway and the total ingested rate of water were estimated using equations 1 - 3.

Ingestion rate per minute due to hand-to-mouth contact (QHM)

QHM (µL/ min) = h (mm) x A (mm2) x fHM (n/ min) (1)

Ingestion rate per minute due to water droplets (QD)

QD (µL/ min) = VD (µL) x fD (n/ min) (2)

The total ingestion rate per minute was calculated as follows (Q):

QI (µL/ min) = QHM (µL/ min) + QD (µL/ min) (3)

Given that no field observations were made at the inner courtyard to identify the interaction of the residents with the water of the blue elements and the exposure duration, the probability distributions of the exposure parameters were taken from the health risk assessment study for splash parks that use rainwater as source water, done in the Netherlands by Man et al. (2014a), as it is considers similar exposures to the ones in the present study (Table 1).

For the dermal exposure route, the rate of water in contact with the skin of the hands (QH) was estimated using equation 4 considering the parameters: 1. film thickness of water on hands (h), 2. surface area of the hands (AH), and 3. frequency of having hands immersed in water (fH). The value for fH was obtained from the field observations done for the previous study for ornamental fountains by the same authors of this study.

QH (µL/ min) = h (mm/ min) x AH (mm2) x fH (n/ min) (4)

For the ingestion exposure route associated with gastrointestinal illnesses, three main pathways were analyzed for people having direct water contact: ingestion due to hand-to-mouth contact, ingestion of water droplets during splashing, and ingestion of mouthfuls of water. The ingestion rates for each pathway and the total ingested rate of water were estimated using equations 1 - 4.

Ingestion rate per minute due to hand-to-mouth contact (QHM)

QHM (µL/ min) = h (mm) x A (mm^2) x fHM (n/ min) (1)

Ingestion rate per minute due to water droplets (QD)

QD (µL/ min) = VD (µL) x fD (n/ min) (2)

Ingestion rate per minute due to drinking mouthfuls of water (QM)

$$QM\ (\mu L/\ min) = VM\ (\mu L) \times fM\ (n/\ min) \quad (3)$$

The total ingestion rate per minute was calculated as follows (Q):

$$Q\ (\mu L/\ min) = QHM\ (\mu L/\ min) + QD\ (\mu L/\ min) + QM\ (\mu L/\ min) \quad (4)$$

Table 1:Exposure parameters used for ingestion and dermal exposure routes.

Parameter	Distribution of values*	Source
A, surface area of the hand that is mouthed (mm^2)	U (100, 2000)	USEPA, 2019
A_H, surface area of the hand (mm^2)	U ($15x10^3$, $72x10^3$)	USEPA, 2019
f_D, frequency of ingesting water droplets (n/min)	G (2.1, 0.17)	Man et al., 2014a
f_H, frequency of hand immersion	G (2.3, 0.65)	Field observations
f_{HM}, frequency of hand to mouth contact (n/min)	G (1.3, 0.8)	Field observations
f_M, frequency of taking a mouthful of water (n/min)	G (0.5, 1.4)	Field observations
h, film thickness of water on hands (mm)	U ($1.97x10^{-2}$, $2.34x10^{-2}$)	USEPA, 2019
V_D, volume of a water droplet (µL)	U (0.5, 524)	Man et al., 2014a
V_M, volume of a mouthful of water (µL)	G (4.72, $5.3x10^3$)	USEPA, 2019

*U-Uniform probability distribution (min, max); G-Gamma probability distribution (shape, scale)

Values and probability distributions of the parameters VD, fD, h, A, and VM were taken from the health risk assessment study for splash parks in the Netherlands done by Man et al. (2014a), given the similarities with our study (Table I). The values of the parameters fHM, fM, and fH were obtained by pooling the data from our field observations of all the fountains and fitting it to a gamma distribution, which is used to describe the waiting time between events (Table I). The parameter QM was used as input only for the dose-response model of the Körtingbrunnen because drinking mouthfuls of water was observed only at this fountain.

$$QH\ (\mu L/\ min) = h\ (mm/\ min) \times AH\ (mm^2) \times fH\ (n/\ min) \quad (5)$$

For each pathway, different parameters and statistical distributions were used as input for the dose-response model, as presented in Table 1.

Based on the field observations regarding recreational activities involving direct water contact described in section 2.3, the time of exposure (t) at each fountain was obtained and fitted to a Beta probability distribution with RStudio (2020) as it describes data falling within a specific interval, in this case the duration of the field observations.

1.3 Dose response models

The dose of exposure (d) to the several bacterial hazards was calculated by multiplying the concentration of the bacteria in water (C), the rate of water ingested or in contact with the

skin during exposure (Q), depending on the exposure route, and the time of exposure (t) of one recreational event (equation 5) (Haas et al., 2014).

d (MPN/ CFU) = C (MPN/ mL) x Q (mL/ min) x t (min) (5)

The risk of GI illness per 1000 users per day (Pill) due to ingestion of E. coli, Enterococci, and Salmonella non-typhoid was estimated according to the β-Poisson dose-response model (equation 6).

The analytical methods used in our study to quantify E. coli and Enterococci are not specific for pathogenic strains. Thus, a "worst case scenario" was assumed for the calculation of Pill that considers the presence of the pathogenic E. coli strain ETEC O111 (CAMRA, 2020) and pathogenic Enterococci (Haas et al., 2014) in the water samples. This overestimation of the risk was assumed because there is no regulation that obliges water quality monitoring in private individual water systems using RHRW and the studied system does not have a regular maintenance scheme.

Pill = 1 - [1 + d x ((2(1/α) - 1)/ N50)]-α (6)

Additionally, the risk of dermal infection per 1000 users per day (Pinf) due to exposure to P. aeruginosa was estimated with the exponential dose-response model (equation 7) (Roser et al., 2015).

Pinf = 1 – exp –k x d (7)

The values for the parameters α, N50 and k were taken from the literature and are presented in Table 2.

Table 2: Dose response models used for the selected bacteria.

Bacteria	Dose response model	Parameters	Source
E. coli (ETEC O111)	$P_{ill} = 1 - [1 + d \times ((2^{(1/\alpha)} - 1)/ N_{50})]^{-\alpha}$	$\alpha = 2.63 \times 10^{-1}$ $N_{50} = 3.56 \times 10^{6}$	*(CAMRA, 2020)*
Enterococci	$P_{ill} = 1 - [1 + d \times ((2^{(1/\alpha)} - 1)/ N_{50})]^{-\alpha}$	$\alpha = 1.6 \times 10^{-1}$ $N_{50} = 59.9 \times 10^{3}$	*(Sunger and Haas, 2015)*
Salmonella non-typhoid	$P_{ill} = 1 - [1 + d \times ((2^{(1/\alpha)} - 1)/ N_{50})]^{-\alpha}$	$\alpha = 31.26 \times 10^{-2}$ $N_{50} = 23.6 \times 10^{3}$	*(WHO, 2001)*
P. aeruginosa	$P_{inf} = 1 - \exp^{-k \times d}$	$k = 4.3 \times 10^{-7}$	*(Roser et al., 2015)*

The dose of exposure (d) to the several bacterial hazards (equation 6) was calculated by multiplying the concentration of the bacteria in water (C), the rate of water ingested or in contact with the skin during exposure (Q), depending on the exposure route, and the time of exposure (t) of one recreational event (Haas et al., 2014).

D (MPN/ CFU) = C (MPN/ mL) x Q (mL/ min) x t (min) (6)

The bacterial concentrations were determined as described in sample processing and analysis (section 2.4). The exposure rates were calculated using equations 4 - 5 (section 2.4.2), and the time of exposure was quantified during the field observations (section 2.3). The risk of GI illness per 1000 users per day (Pill) due to ingestion of *E. coli*, Enterococci,

and *Salmonella non-typhoid* was estimated according to the β-Poisson dose-response model (equation 7).

The analytical methods used in our study to quantify *E. coli* and Enterococci are not specific for pathogenic strains. Thus, a "worst case scenario" was assumed for the calculation of P_{ill} that considers the presence of the pathogenic *E. coli* strain ETEC O111 (CAMRA, 2020) and pathogenic Enterococci (Haas et al., 2014) in the water samples. This overestimation of the risk was assumed because there is no regulation that obliges water quality monitoring in ornamental fountains and in most cases the water is continuously recirculated without treatment during summer. The values for the parameters α and N50 were taken from the literature as presented in Table 2.

$$P_{ill} = 1 - [\, 1 + d \times ((2^{(1/\alpha)} - 1)/ N50)]^{-\alpha} \quad (7)$$

Additionally, the risk of dermal infection per 1000 users per day (P_{inf}) due to exposure to *P. aeruginosa* was estimated with the exponential dose-response model (equation 8) (Roser et al., 2015).

$$P_{inf} = 1 - \exp^{-k \times d} \quad (8)$$

1.4 Risk characterization

To account for uncertainty in the exposure parameters and time of exposure (t), Monte Carlo simulations were performed in MATLAB (2020), the software generated 10.000 combinations of the parameters of the dose-response model to estimate the risk of illness/ infection. For this study, all the model parameters were assumed to be independent.

Moreover, given the wide range of possible bacterial concentrations in water, a scenario analysis was carried out with different concentrations in the range from $1 \times 10^1 - 1 \times 10^4$ MPN/ 100 mL. Afterwards, a sensitivity analysis was done to quantify the contribution of each parameter in percentage to the final risk of illness/infection. This analysis was executed using the Microsoft Excel Add-In Oracle Crystal Ball (2020) which computes the contribution to variance of each parameter by squaring the rank correlation coefficients and normalizing them to 100 %.

1.5 USEPA mean illness rate

The USEPA carried out the National Epidemiological and Environmental Assessment of Recreational Water study (NEEAR study) published in 2003, in which a broader definition of gastrointestinal illness than the one considered in the guidelines of 1986 was used. NEEAR-Gastrointestinal Illnesses (NGI) definition includes diarrhea, stomachache, or nausea without the requirement of fever. This was included in the USEPA guidelines for Recreational Water Quality Criteria (RWQC) of 2012, which established an estimate illness rate of 32NGI/1000 or 36NGI/1000, depending on the targeted water quality. Thus, to verify if the current water quality of the sampled blue elements complies with the RWQC of 2012, the calculated illness and infection estimates were compared with an estimate illness rate of 36 NGI/1000 users (USEPA, 2012). It is important to note that there is no estimate illness or infection rate for dermal infections established in the USEPA RWQC 2012; however, the NEEAR study also found that other waterborne illnesses occur at lower rates than GI illnesses. Therefore, protecting public health against GI illnesses will also prevent most

types of illnesses related to recreational activities in water, which is why we used the same benchmark to compare the risk of skin infections (USEPA, 2012).

The risk estimate for GI illness per 1000 users was calculated in the present study as follows; first, the dose was calculated by running Monte Carlo simulations from the bacterial concentration distribution, ingestion rate distribution and exposure duration; the obtained values were then replaced in the appropriate dose-response model to obtain the individual probability of a person getting ill/infected. Finally, these individual risk estimates were then multiplied by 1000 to estimate the risk of illness/ infection per 1000 users.

1.6 USEPA means illness rate

Thus, to verify if the current water quality of the ornamental fountains complies with the RWQC of 2012, the calculated illness and infection estimates were compared with an estimate illness rate of 36 NGI/1000 users (USEPA, 2012).

The risk estimate for GI illness per 1000 users was calculated in the present study as follows; first, the dose was calculated by running Monte Carlo simulations from the bacterial concentration distribution, ingestion rate distribution and exposure duration distribution identified for each fountain in previous steps, the obtained values were then replaced in the appropriate dose-response model to obtain the individual probability of a person getting ill/infected, these individual risk estimates were then multiplied by 1000 to estimate the risk of illness per 1000 users.