MATTERS ARISING OPEN

Reply: Potential discharge, attenuation and exposure risk of SARS-CoV-2 in natural water bodies receiving treated wastewater

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The motivation of our review article¹ was to respond to emerging concerns, regarding the risk of SARS-CoV-2 in treated wastewater and natural water bodies. There had been a complete lack of quantitative risk assessment regarding presence of SARS-CoV-2 in wastewater. Since the available knowledge had been limited, the review article aimed to estimate the ceiling of possible risk based on the simplest assumptions, following the precautionary principle. The major question raised in the *Matters Arising* by Haas et al. is that the estimated risk in our review article is overestimated by lacking plausible assumptions in presence of infectious virus in water and inhalation dose in recreational water activities. For the purpose to estimate the ceiling of possible risk, the assumptions were basically made on the safety side (or the worst-case condition), which intentionally allowed potential over-estimation. The important conclusion of this review is that, even with such worst-case assumptions, the risk of SARS-CoV-2 in water bodies receiving treated wastewater is probably not a matter of serious concern unless the SARS-CoV-2 RNA in wastewater exceeds the previously detected range. Here, we explain our standpoints to the questions raised in the *Matters Arising*.

PRESENCE OF INFECTIOUS SARS-COV-2 IN TREATED WASTEWATER AND ENVIRONMENTAL WATER

The *Matters Arising* pointed out that there have been no reports that detected SARS-CoV-2 RNA in the final effluent of wastewater treatment plants (WWTPs) nor in environmental water. However, no detection of viral RNA does not mean absence of the viral RNA. Nonetheless, they are still possibly present under the detection limit. The typical detection limit of SARS-CoV-2 RNA is 10^3 – 10^4 copies/L by qPCR assay followed by the virus concentration². As reviewed in our review article, the detected range of SARS-CoV-2 RNA in raw wastewater is mostly 10^4 – 10^6 copies/L. Therefore, SARS-CoV-2 RNA is possibly present at a low concentration below the detection limit, according to the expected log removal values in WWTP, as reported in our review.

Meanwhile, the presence of viral RNA does not mean the presence of an infectious virus. As pointed in the *Matters Arising*, isolation of infectious SARS-CoV-2 had not been reported. However, possible presence of infectious SARS-CoV-2 is reported in a cohort study³, which suggested possible transmission of COVID-19 from sewage. Regarding the expected vulnerability of

enveloped viruses in water, we agree that the quantity of infectious SARS-CoV-2 in wastewater is probably much lower than viral RNA copy numbers. Hence, we did not assume in our review article that each gene copy of SARS-CoV-2 RNA is equivalent to an infectious plaque forming unit (PFU) of viable virus. Instead, we did state in the QMRA section (on p.8, left), “SARS-CoV-2 RNA in receiving water bodies does not probably exceed <100 copies/L, ... viable count of the virus, as PFU is probably smaller than the viral RNA concentration”. Accordingly, the SARS-CoV-2 concentration in example scenarios were assumed as viable count in PFU (not in viral RNA copies) from a very high-concentration case (10 PFU/L) to a low-concentration case (0.01 PFU/L) by considering the detected range of SARS-CoV-2 in wastewater, the expected removal in wastewater, and the possible dilution and decay in water bodies. This concentration range (i.e., 0.01–10 PFU/L to 100 copies/L) is not significantly inconsistent with the proportion of gene copies to viable measurement in recently published Bivins et al.⁴.

INHALATION DOSE OF SARS-COV-2 IN RECREATIONAL WATER ACTIVITIES

The dose-response model of SARS-CoV by Watanabe et al.⁵ is based on the respiratory route of infection. We agree that the introduction of ingestion dose to this dose-response model is not appropriate since ingestion dose presumes infection to the digestive system. The exposure of the respiratory system in recreational water activities possibly occurs due to the inhalation of aerosol and intrusion of water into the nasal cavity via the nostril. However, the previous knowledge about the inhalation of water aerosol in recreational water activities is limited. Hines et al.⁶ estimated the inhalation exposure of water aerosol for the QMRA of *Legionella*. In their studies, the inhalation of water aerosol was estimated at 1.7 mL at maximum (aerosolization: 2.2×10^{-3} L/m³, inhalation: 0.017 m³/min, duration: 45 min) in shower; and 0.080 mL at maximum (aerosolization: 1.1×10^{-5} L/m³, inhalation: 0.04 m³/min, duration: 181 min) in therapy pool. De Man et al.⁷ estimated the inhalation of water aerosol at 0.0494–1.55 µL/min (as 95% CI), while the ingestion volume at 5.1–279 µL/min in splash parks. These studies suggest that the inhalation volume of water aerosol is possibly up to two-log smaller than the ingestion volume in recreational activities. Water intrusion into the nasal

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Table 1. (Corresponding to Table 3 in the original article): Expected dose and probability of infection to SARS-CoV-2 for various water activities under various viral concentration scenarios.

Activity	Virus concentration in water body (PFU/L) (assumption)	Volume of water ingestion ^a (mL/event)	Expected viral dose, λ (log PFU/event)	Chance of infection per event, $P(r \lambda)$ (log)	No. of events per year (assumption)	Annual chance of infection (log)
Swimming	0.01	6.0	-4.2	-6.83 ^b	10	-5.83 ^b
	0.1		-3.2	-5.83 ^b		-4.83 ^b
	1		-2.2	-4.83 ^b		-3.83 ^b
	10		-1.2	-3.83 ^b		-2.83 ^b
Fishing	0.01	2.0	-4.7	-7.31	50	-5.61
	0.1		-3.7	-6.31		-4.61
	1		-2.7	-5.31		-3.61
	10		-1.7	-4.31		-2.61
Canoeing/ rowing	0.01	2.3	-4.6	-7.25	20	-5.95
	0.1		-3.6	-6.25		-4.95
	1		-2.6	-5.25		-3.95
	10		-1.6	-4.25		-2.95

^aMedians of water ingestion estimated by Dorevitch et al. (2011).

^bVolume of ingestion was employed as the maximum volume of exposure via the respiratory routes because inhalation is speculated smaller than ingestion.

cavity via the nostril is also speculated to be less than ingestion. Overall, it is hard to assume a reasonable volume of intruded water because no reliable data have been reported. The purpose of our review was to estimate ceiling of the possible risk with the simplest assumptions, following the precautionary principle. Therefore, the volume of ingestion was employed as the maximum volume of possible exposure via the respiratory routes. Since the inhalation dose is speculated to be smaller than the ingestion dose in actual cases, we can expect the actual risk to be lower than that.

DERIVATION OF DOSE-RESPONSE MODEL

Imposition of the Poisson distribution function is not necessary as pointed out in the *Matters Arising*. The correction in the corresponding part (in the third paragraph on p.7) is shown in the below box. Briefly, the chance of infection at the mean viral dose λ is derived from the exponential model by Watanabe et al. in Eq. (1). At very low doses $\ll 1$, chance of infection is approximately proportional to the dose as $p(r|\lambda) \approx \frac{\lambda}{k}$. This correction does not significantly affect the estimated risk values in the example scenarios as in Table 1 (corresponding to Table 3 in the original article).

When the expected viral dose per event λ is smaller than 1 PFU, chance of infection is approximately proportional to the viral dose. Equation (1) can be rearranged by using Maclaurin series:

$$p(r|\lambda) = 1 - \exp\left(-\frac{\lambda}{k}\right) = 1 - \sum_{n=0}^{\infty} \frac{(-\lambda/k)^n}{n!} = \frac{\lambda}{k} - \frac{(-\lambda/k)^2}{2} + \frac{(-\lambda/k)^3}{6} - \dots \quad (2)$$

where $p(r|\lambda)$: chance of infection per event, λ : the expected dose per event. When $-\lambda/k \ll 1$, Eq. (2) can be approximated as:

$$p(r|\lambda) \approx \frac{\lambda}{k} \quad (3)$$

PERSPECTIVES AND LIMITATIONS

The most preferable approach of QMRA is to consider the assumptions that are only supported by direct evidence. However,

reliable evidence has been lacking to estimate the mean value of the risk posed by SARS-CoV-2 in wastewater. Alternatively, our review article proposed to estimate potential risk, (i.e., the ceiling of possible risk) of SARS-CoV-2 by recreational activities in natural water bodies. Microbial risk often has a large fluctuations and uncertainty brought by variability in assumptions and level of contamination. For the purpose of decision making for risk management, the mean value of the risk is not always the primary target when the maximum risk value with sufficient safety margin is available. Especially when the risk value has very large uncertainty, the ceiling of the possible risk could be more important than the mean value of the possible risk. Accordingly, in our review article, the inhalation dose was assumed to be the worst-case scenario; thereby, the estimated risk values were overestimated. The important perspective of our review article is that the estimated risk is too low to have serious concerns, even under excessive assumptions that allow overestimation. If the inhalation dose is assumed to be two-log smaller than the original assumption, as discussed above, the estimated risk would be two-log smaller. That means recreational water activities in water bodies receiving treated wastewater are much safer. To our understanding, the *Matters Arising* and our review article agrees on this conclusion. Our review article did not claim that the estimated risk has high chance of underestimation. In our review, the dose-response model developed for SARS-CoV is employed, because the dose-response model of SARS-CoV-2 has not been developed. While all other assumptions are taken for the overestimation side, this is the only factor which possibly causes underestimation of the estimated potential risk because dose-response relationship of SARS-CoV-2 is unknown yet. Possible underestimation is stated for the purpose to clarify every uncertainty, but not to deny the possibility of overestimation. As already discussed in our original article, we agree that water bodies receiving untreated wastewater possibly cause a much higher risk for concern. Further studies are needed on the presence and infectivity of SARS-CoV-2 in environmental water affected by untreated wastewater.

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AUTHOR CONTRIBUTIONS

R.H.: writing all parts of the paper; M.K., M.A., K.K., K.D., A.H. and H.Y.: reviewing and editing all parts of the paper.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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