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Comparison of Risk Predicted by Multiple Norovirus Dose–Response Models and Implications for Quantitative Microbial Risk Assessment

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The application of quantitative microbial risk assessments (QMRAs) to understand and mitigate risks associated with norovirus is increasingly common as there is a high frequency of outbreaks worldwide. A key component of QMRA is the dose–response analysis, which is the mathematical characterization of the association between dose and outcome. For *Norovirus*, multiple dose–response models are available that assume either a disaggregated or an aggregated intake dose. This work reviewed the dose–response models currently used in QMRA, and compared predicted risks from waterborne exposures (recreational and drinking) using all available dose–response models. The results found that the majority of published QMRAs of norovirus use the ${}_1F_1$ hypergeometric dose–response model with $\alpha = 0.04$, $\beta = 0.055$. This dose–response model predicted relatively high risk estimates compared to other dose–response models for doses in the range of 1–1,000 genomic equivalent copies. The difference in predicted risk among dose–response models was largest for small doses, which has implications for drinking water QMRAs where the concentration of norovirus is low. Based on the review, a set of best practices was proposed to encourage the careful consideration and reporting of important assumptions in the selection and use of dose–response models in QMRA of norovirus. Finally, in the absence of one best norovirus dose–response model, multiple models should be used to provide a range of predicted outcomes for probability of infection.

KEY WORDS: Dose response; norovirus; QMRA; risk assessment

1. INTRODUCTION

Quantitative microbial risk assessment (QMRA) is a mathematical framework used to evaluate health risks from pathogens, which can assist in both understanding and managing microbial hazards.[1] The goal of QMRA is to gather the best available information to understand the potential effects from microbial exposures.[2] In the case of drinking water, for example, QMRA estimates the risk from what is known and can be inferred about the concentration of pathogens in the water supply and distribution system.[3] QMRA is also useful in determining which pathogens present the greatest risk when considering factors such as dose response, severity, and occurrence.[4] QMRA is increasingly being used to evaluate water and food safety as well as to inform management actions.[4–7]

Substantial interest in the development of QMRAs for noroviruses in water and food has emerged because of the high frequency of norovirus outbreaks. *Norovirus* is divided into six genogroups (GI–GVI) with GI and GII causing human

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infection and being implicated in numerous outbreaks.[8] In an etiologic assessment of ~900 outbreaks worldwide, norovirus (GI and/or GII) was implicated in 11% of waterborne outbreaks and 54% of foodborne outbreaks.[9] The numbers are similar for Sweden where norovirus (GI and GII) was the suspected cause of 18% of waterborne outbreaks and 52% of foodborne outbreaks.[10,11] In the United States, approximately 52% of the gastroenteritis cases associated with the consumption of raw or partially cooked shellfish can be attributed to norovirus, which makes them the leading cause of seafood-associated foodborne illness.[12,13]

A main component of QMRA is the dose–response model, which is a relationship between the dose and the likelihood of an outcome of illness or infection. The choice of dose–response model can be highly impactful in the overall determination of risk.[14] Several dose–response models are available for norovirus, which are summarized in Section 2.1. Yet, the majority of published QMRAs use one model that predicts high risks at low doses, which is the ${}_1F_1$ hypergeometric dose–response model. Thus, the following work identifies and describes the dose–response models currently used in QMRA and reviews the assumptions made when the models were applied. Then, using hypothetical waterborne exposure routes (recreational and drinking), the predicted risks from the numerous dose–response models are compared. Based on this work, a set of best practices is presented for the use of norovirus dose response in QMRA.

2. NOROVIRUS DOSE–RESPONSE MODELS IN QMRA

2.1. Discussion of Dose–Response Models

For norovirus, multiple dose–response models are available that assume either a disaggregated or an aggregated intake dose. Briefly, an aggregation factor ("*a*") was added to aggregated dose–response models because one human challenge inoculum was observed by electron microscopy to be "sticky."[15] The following dose–response models are used to estimate the probability of infection ($P_{\text{infection}}$).

Disaggregated dose–response models:

$$\text{Approximate beta} - \text{Poisson}: \; P_{\text{infection}} \approx 1 - \left(1 + \frac{\text{dose}}{\beta}\right)^{-\alpha}$$

$${}_1F_1 \text{hypergeometric}: P_{\text{infection}} = 1 - {}_1F_1\left(\alpha,\ \alpha+\beta,\ -\text{dose}\right).$$

Aggregated dose–response models:

$${}_2F_1 \text{hypergeometric}: P_{\text{infection}} = 1 - {}_2F_1\left(\alpha,\ \frac{\text{dose} * (1-a)}{a},\ \alpha+\beta,\ -\frac{a}{1-a}\right).$$

Pfaff Transformation: is applied when $|a/(1\text{-}a)| \geq 1$ of the ${}_2F_1$ hypergeometric:

$$P_{\text{infection}} = 1 - \left[\left({}_2F_1\left(\beta,\ \frac{\text{dose}\,(1-a)}{a};\alpha+\beta;a\right)\right)\left(\frac{1}{1-a}\right)^{\left(-\frac{\text{dose}(1-a)}{a}\right)}\right].$$

$$\text{Fractional Poisson: } P_{\text{infection}} = P * \left(1 - e^{-\text{dose}/\mu_a}\right).$$

$${}_2F_1 \text{hypergeometric with immunity}: P_{\text{infection}} = (1-\varphi) * \left[\left(1 - \theta \cdot {}_2F_1\left(\beta,\ \frac{\text{dose}\,(1-a)}{a};\alpha+\beta;a\right)\right)\right].$$

$$\theta = e^{\frac{\text{dose}(1-a)*\ln(1-a)}{a}}.$$

The dose–response parameters (α, β) are shape parameters of the beta distribution that describe variation in host susceptibility,[2,16] "*a*" is the aggregation parameter ($0 \leq a < 1$), μ_a is the mean aggregate size,[15] P is the fraction of secretor positive (Se+) individuals who are fully susceptible,[17] and φ is the fraction of Se+ individuals who are immune to norovirus.[18] The titer of the dose is estimated by reverse transcription polymerase chain reaction (RT-PCR), a molecular-based approach that estimates the concentration of pathogens but cannot differentiate between infectious and noninfectious organisms because no cell culture method is available for human norovirus. Reverse transcription polymerase chain reaction (RT-PCR) estimates the dose titer of norovirus in genomic equivalent copies (gec).[1,19–21]

These dose–response models were mathematically fit to published human challenge data to determine the parameters for infectivity (α, β) and aggregation ("*a*"). To date, the dose–response models have been fit to a variety of data sets including two multidose-level human challenge GI data sets, genotype GI.1, [15,22] and a data set that pooled multidose- and single-dose-level data obtained from human challenge studies.[17] The pooled data set included both GI.1 and GII.4 genotype data.[15,22–24] Human feeding studies with norovirus (GI.1) have demonstrated attack rates of greater than 50% with relatively low doses ($<5 \times 10^3$ gec).[22] Human challenge studies only dose healthy adults and do not consider sensitive subpopulations of the elderly,

children, and immune-compromised individuals, who may have different dose–response effects and be at increased risk.[25]

There are numerous assumptions implicit with each dose–response model that are important when selecting a model for a particular application. However, two assumptions are particularly controversial.[18] The first is related to immunity and the second to aggregation.

Innate susceptibility and acquired immunity to norovirus infection are complicated and not fully understood. Susceptibility to norovirus infection is dependent on genetic factors, such as secretor status, with individuals who express the FUT2 gene being described as secretor positive (Se+) and susceptible to norovirus infection. This genetic factor is variable depending on ancestry with approximately 20% of the North American/European Caucasian and 2% of the Meso-American/Hispanic populations not expressing the FUT2 gene,[26,27] so they are secretor negative (Se–) individuals. Thus, norovirus binding does not occur, so infection or illness is not initiated.[22,28–30] The various norovirus dose–response models were parameterized for secretor positive (Se+) individuals with some models ($_1F_1$, $_2F_1$) [15] having the implicit assumption of no immunity among Se+ individuals. However, based on the leveling off of the dose–response curves below 100% (Fig. A1), it appears that immunity is present among Se+ individuals.[18] Two dose–response models account for immunity. The fractional Poisson model assumes that Se+ individuals are fully susceptible or fully nonsusceptible and estimates the fraction of individuals who are susceptible (P) to norovirus.[17] The $_2F_1$ hypergeometric with immunity added a parameter for immunity (φ) to the aggregated exact beta-Poisson model developed by Teunis *et al.*[15] The immunity parameter accounts for the fraction of the Se+ population that demonstrates immunity to norovirus. It was argued that exclusion of the immunity parameter from the exact beta-Poisson model when the data are indicative of an immunity plateau could lead to spurious results.[18]

It should be noted that dependence on secretor status has exceptions because Se– individuals have been infected from genotypes GII.4 [23] and GI.8.[31] Furthermore, susceptibility is dependent on the binding of glycans to certain histo-blood group antigens (HBGAs), with GI.1 binding to Se+ individuals with blood groups A, O, or AB [28] and GII.4 binding to Se+ individuals with blood groups A, B, and O.[32,33] No clear evidence exists for acquired immunity but short-term, genotype-specific immunity has been observed.[27,32,34,35]

Aggregation of norovirus was assumed by Teunis *et al.* after the observation of the sticking together of viral particles as seen under electron microscopy.[15,18] Thus, a dose–response model, the $_2F_1$ hypergeometric, with an additional aggregation parameter was developed. However, Schmidt[18] demonstrated that the aggregation parameter could be a tuning parameter and non/weakly identifiable, which means that the fit might be spurious and lead to parameters that are biased. Use of this model requires the user to take either a mechanistic or empirical view of norovirus dose response. The mechanistic view believes the model form proposed by Teunis *et al.*[15] is true and describes the mechanisms of norovirus dose response correctly, including: (1) virus aggregation is log-series distributed, (2) the estimated mean aggregate size is accurate, (3) there is no immunity among secretors, and (4) the number of aggregates in the dose is Poisson distributed. A mechanistic view of dose response is advantageous because the model can be decomposed as appropriate.[18] For example, under this view, the aggregation parameter can be altered from the best-fit value of 0.9997 to 0, thus simplifying the $_2F_1$ to the $_1F_1$ hypergeometric. However, if any of the assumptions are incorrect then the $_2F_1$ hypergeometric fitted model misrepresents the mechanisms of norovirus dose response.

Alternatively, it may be better to take an empirical view of the Teunis *et al.*[15] dose–response model (thus adopting the best-fit aggregation parameter of 0.9997) with the assumption of similarity between all environmental conditions and the corresponding dose–response study conditions. Empirical interpretation of the model would not require the mechanistic assumptions and provides dose–response information for the same degree of aggregation ($a = 0.9997$). However, the specific conditions under which the empirical curve could be valid for future use, such as in risk characterization, are unknown.[18] Ultimately, more data and research are needed to determine whether a mechanistic or empirical approach to norovirus dose response must be considered.

2.2. Norovirus Dose–Response Models Currently Used in QMRA

Currently, QMRAs of exposure to a variety of environmental media make numerous assumptions related to norovirus dose response. Table I

Table I. Norovirus Dose–Response Models ($\alpha = 0.04$, $\beta = 0.055$) Currently Used in QMRA

		Dose Response (DR)			Assumptions and Differences in QMRAs							
Media	Exposure Pathway	${}_1F_1$	${}_2F_1$	"*a*"	Aggregation Parameter	Exposed Pop[a]	Secretor Status	GI/ GII	Infectious Particles	Harmonization	Software	Reference
Recreational water	Ingestion while swimming; inhalation; secondary contact	x			No assumptions about aggregation state	A, C	Limitation: everyone assumed to be Se+	GII	ND[b]	Adjustment factor of ~20 due to different RT-PCR targets used (risk vs. clinical study)	@Risk software with table of DR parameters from Prof. Teunis	Bambic *et al.*[19]
Vegetables irrigated by highly treated municipal water	Ingestion		x[c]	0.9997	No assumptions about aggregation state	A, C (exclude children <1-year old)	Se+ pop: 80–100%; Uniform (0.8, 1.0)	GI & GII	ND	ND	R software 2.12.2	Barker[38]
Direct potable reuse water	Ingestion		x[c]	0.9997	No assumptions about aggregation state	A	Se+ pop: 80–100%; Uniform (0.8, 1.0)	GI & GII	ND	ND	R software 2.12.2	Barker *et al.*[39]
GAywater irrigation of produce	Ingestion of homegrown lettuce		x[c]	0.9997	No assumptions about aggregation state	A, C (exclude children < 1-year old)	Se+ pop: 80–100%; Uniform (0.8, 1.0)	GII	All genomic copies = number of viruses	ND	R software 2.12.2	Barker *et al.*[40]
Irrigation water	Ingestion of raw produce	x[d]			ND	ND	ND	GI & GII	Ratio of total to infectious virions same as DR inoculum	ND	Mathematica v8 with table of 10,000 DR parameters from Prof. Teunis	Bouwknegt *et al.*[41]
Urban floodwater	Hand-mouth contact	x			ND	A, C	ND	GI & GII	All measured pathogens were infectious	ND	Mathematica v8	de Man *et al.*[42]
Sewage, sludge, effluent, surface water, soil, crops, drinking water	Ingestion occupational, recreational, or residential exposures	Present approximate beta-Poisson with $\alpha = 0.04$, $\beta = 0.055$, which is incorrect parameterization[e]			No assumptions about aggregation state	A, C	ND	ND	ND	ND	MATLAB 2013a	Harder *et al.*[37]
Harvested stormwater	Inhalation-ingestion via mucociliary action; ingestion of lettuce	x			Assumed monodisperse	ND	ND	GI & GII	ND	ND	MATLAB 2012a	Lim *et al.*[43]
Wastewater on food crops eaten raw	Ingestion of lettuce		x	0.9997	ND	ND	ND	ND	ND	ND	QMRA-Monte Carlo computer program[f]	Mara and Sleigh[44]

(*Continued*)

Table I. (Continued)

		Dose Response (DR)			Assumptions and Differences in QMRAs							
Media	Exposure Pathway	$_1F_1$	$_2F_1$	"*a*"	Aggregation Parameter	Exposed Pop[a]	Secretor Status	GI/ GII	Infectious Particles	Harmonization	Software	Reference
Recreational water	Ingestion while swimming; inhalation; secondary contact	x		0	Ignored aggregation	A, C	ND	GII	ND	Divide the clinical trial by 18.5 due to different RTPCR targets used (risk vs. clinical study)	@Risk software	McBride *et al.*[45]
Wastewater irrigation of vegetables	Ingestion of unwashed vegetables		x[c]	0.9997	No assumptions about aggregation state	A, C	Se+ pop: 80–100%; Uniform (0.8, 1.0)	GI	All genomic copies = number of viruses	ND	R Software 3.0.1	Mok *et al.*[46]
Drinking water from private and small community systems	Ingestion	x			No assumptions about aggregation state	A, C	ND	GI & GII	ND	ND	@Risk software	Murphy *et al.*[47]
Secondary water	Accidental ingestion from toilet and laundry; ingestion from cross-connection		x[g]	0.9997[g]	Assumed aggregated (did not indicate a value)	ND	ND	ND	ND	ND	@Risk Pro 6.01	Rygaard *et al.*[48]
Urban water	Accidental ingestion from rowing, swimming, fishing, wading, walking dog	x			No mention of aggregation parameter assumptions	A, C	ND	ND	ND	ND	R version 3.0.1	Sales-Ortells and Medema[49]
Recreational water impacted by fresh fecal contamination	Accidental ingestion		x	0.0001	Not aggregated (no information on aggregation state)	A	ND	GI & GII	Ratio of total to infectious virions same as DR inoculum	ND	R & Mathematica v5.2	Schoen and Ashbolt[50]
Recreational water	Ingestion while swimming	x			Not aggregated	A	Limitation: everyone assumed to be Se+	GI	ND	ND	R Software 2.8.1	Schoen *et al.*[51]
Recreational water and cross-connected sewage to drinking water	Accidental ingestion; ingestion of contaminated drinking water	x			Not aggregated	A, C	Limitation: everyone assumed to be Se+	ND	ND	ND	R software	Schoen *et al.*[52]

(*Continued*)

Table I. (Continued)

Media	Exposure Pathway	Dose Response (DR)			Assumptions and Differences in QMRAs							Reference
		$_1F_1$	$_2F_1$	"a"	Aggregation Parameter	Exposed Pop[a]	Secretor Status	GI/ GII	Infectious Particles	Harmonization	Software	
Source water	Ingestion of unheated drinking water	Simplified to exponential where $r = \alpha/(\alpha+\beta)$ and $\alpha = 0.04$, $\beta = 0.055$[h]			ND	ND	ND	GI & GII	All measured pathogens were infectious	ND	Mathematica 8.0	Sokolova *et al.*[36]
Recreational water	Ingestion while swimming	x			ND	A, C	Assumed everyone was Se+	GI & GII	ND	ND	MathCad 13	Soller *et al.*[53]
Recreational water	Ingestion while swimming	x			Not aggregated	A	ND	GI & GII	Ratio of total to infectious virions same w/ inoculum used in DR	ND	R and Mathematica v. 5.2	Soller *et al.*[54]
Drinking water (distribution system)	Ingestion unheated tap water	x			ND	A, C[i]	ND	GI & GII	ND	ND	Mathematica 7.0	Teunis *et al.*[55]
Freshwater impacted by agricultural animal sources	Ingestion during recreational activities	x			Not aggregated (dilute)	A, C	ND	GI & GII	ND	ND	MathCad with Microbial Risk Assessment Interface Tool (MRAIT)	USEPA[56]
Tropical coastal waters impacted by stream discharge	Ingestion while swimming	x			Not aggregated (coagulation in natural waters is negligible)	A, C	Limitation: everyone assumed to be Se+	GI & GII	ND	ND	MATLAB R2009b	Viau *et al.*[57]
Land applied biosolids	Inhalation		x	0.0001 and 0.999	Not aggregated and aggregated (account for uncertainty)	A	ND	GI & GII	ND	None (qPCR values used = in accordance with qPCR-based DR model)[j]	Matlab 7 ("hypergeom" function)	Viau *et al.*[58]
Drinking water (distribution system)	Ingestion unheated tap water	x			ND	ND	ND	GI & GII	ND	ND	Mathematica	Yang *et al.*[59]

[a]A = adults; C = children.
[b]ND = Not described in article.
[c]Authors used the Pfaff transformation of the $_2F_1$ for doses ≤33,323 gec/person/day and used the $_1F_1$ as an approximation for the $_2F_1$ at doses >33,323 gec/person/day.
[d]Authors indicate use of use of hypergeometric, but do not indicate which one. Also, present 10,000 α, β pairs obtained from Peter Teunis with most likely of $\alpha = 0.04$, $\beta = 0.055$.
[e]Authors used Teunis α, β parameters, but do not present the $_1F_1$ hypergeometric, only the approximate beta-Poisson.
[f]The computer program can be found at www.personal.leeds.ac.uk/,cen6ddm/QMRA.html.
[g]Authors specify aggregated dose response but did not specify the "a" value used.
[h]Authors state exact beta-Poisson can be simplified to exponential at low doses, but no reference provided.
[i]No difference between adults and children.
[j]qPCR (quantitative polymerase chain reactio).

summarizes how different QMRA studies have used the published norovirus dose–response models and the assumptions made about viral aggregation, the exposed population including secretor status, genogroup, fraction of infectious viral particles, the harmonization of the modeled viral dose units with the dose–response viral units, and software selection. To date, the majority of the published QMRAs use the ${}_1F_1$ or ${}_2F_1$ hypergeometric dose–response models ($\alpha = 0.04$, $\beta = 0.055$) fit to the Teunis *et al.* human challenge data set with the "a" parameter ranging from 0 (disaggregated) to 0.9997 (aggregated). Two exceptions were identified, including one QMRA that used the exponential model[36] and one that incorrectly used the approximate beta-Poisson model.[37] Following the release of a new human challenge data set, an additional dose–response model, the fractional Poisson, was also published.[17,22] However, as the ${}_1F_1$, ${}_2F_1$, and fractional Poisson models rely on questionable assumptions (i.e., with respect to host susceptibility), the appropriateness of the use of these models in QMRA has been queried.[18] Thus, QMRAs may soon use new dose–response models or multiple dose–response models to account for all the variability and uncertainty surrounding norovirus dose response.

2.3. Discussion of Assumptions for Dose–Response Model Selection

Currently, there is no standard approach to norovirus dose–response model selection, with authors choosing models without substantial justification of the assumptions made and without regard to how selected models may impact risk estimates. Thus, the assumptions for norovirus dose–response models reported in Table I are described.

2.3.1. Aggregation

All of published QMRAs assumed $\alpha = 0.04$ and $\beta = 0.055$; however, the QMRAs varied with respect to the value used for the aggregation parameter, "a" (0, 0.0001, 0.999, and 0.9997). It should be noted that by assuming the "a" parameter is close to 0, then the ${}_2F_1$ hypergeometric becomes the ${}_1F_1$. The majority of the published QMRAs (16 of 25) used the published α and β parameters of 0.04 and 0.055, but assumed the virus was not aggregated in the environmental media and so the "a" parameter was assumed to be 0.0001 or 0. It should be noted that the parameters $\alpha = 0.04$ and $\beta = 0.055$ were mathematically obtained from the ${}_2F_1$ hypergeometric function where an "a" value of 0.9997 was also estimated by maximum likelihood estimation.[15] Of these 16 QMRAs, two assumed that the dose received was not the mean but a discrete dose,[19,45] so a simplification of the ${}_1F_1$ hypergeometric was performed and the conditional dose–response function, the beta-binomial, was used.[60,61] The beta-binomial yields differences in the risk estimates at doses $<\sim 5$ gec.[19,45] It should be noted that this dose–response assumption and parameterization is not commonly used in norovirus QMRA. One QMRA compared aggregated virus ("a" = 0.999) and disaggregated virus ("a" = 0.0001) when calculating risk.[58] One QMRA designated the approximate beta-Poisson and not the ${}_1F_1$ hypergeometric as the dose–response model with the published parameters ($\alpha = 0.04$, $\beta = 0.055$).[37] This use of the approximate beta-Poisson is inappropriate because use of this model is only correct under specific circumstances ($\alpha << \beta$ and $\beta >> 1$)[62] and will lead to inaccurate risk results, which will under- or overestimate the risk burden depending on the dose. Another QMRA used the exponential model, stating that the exact beta-Poisson could be simplified to the exponential model at low doses and estimated the r from the published parameters ($\alpha = 0.04$, $\beta = 0.055$).[36] Previously published literature indicated that this simplification was valid for doses <0.1 organisms per liter and was to be used because the beta-Poisson approximation exceeded the maximum calculated risk.[63] Two QMRAs assumed aggregated virus with one QMRA assuming the same ${}_2F_1$ dose–response parameters ($\alpha = 0.04$, $\beta = 0.055$, "a" = 0.9997) that were published while the other assumed aggregated virus but did not publish the assumed "a" value.[44,48] Still other QMRAs (4 of 25) explicitly stated that no assumption about the aggregation state of the virus was made and used the Pfaff transformation of the ${}_2F_1$ hypergeometric function with the published parameters of $\alpha = 0.04$, $\beta = 0.055$, and "a" = 0.9997.[38–40,46] Overall, QMRAs tended to default to previously described dose–response models without justifying the assumptions made or adequately considering the impact of model selection on risk estimates. The predicted risk estimates for recreational and untreated drinking waters in Section 3. will further illustrate the importance of dose–response model selection on predicted risk.

2.3.2. Exposed Population

Similarly, little consideration was made of the exposed population. The majority of the QMRAs applied the norovirus dose–response model to both

adults and children, often noting as a limitation that the model was developed using a healthy adult population. Acquired immunity is currently not accounted for in QMRA because understanding is still incomplete. However, current research found that acquired immunity from one genogroup or genotype does not confer protection from all, is not permanently sustained so reinfection can occur throughout life, and can last from six months to more than four years.[27] Many of the QMRAs did not discuss secretor status or describe as a limitation that the population in the human challenge studies used for the dose–response model was only Se+ individuals. It has been observed that secretor status is important and ancestry-dependent, with Se– individuals having a decreased susceptibility to norovirus infection while Se+ display heterogeneity in infectivity from norovirus.[26,27,61] Infection was also observed in Se– individuals from genogroups GI and GII, [23,31] demonstrating the complexities in accounting for susceptibility in QMRAs. One QMRA noted that all individuals were assumed to be Se+.[53] Four QMRAs incorporated uncertainty into secretor status by assuming that 80–100% of individuals are Se+ in the population, which factors in the 20% of Caucasian people immune to norovirus GI.1 infection (Se–).[38–40,46] None of the QMRAs accounted for HBGA types, which have been demonstrated to impact secretor status susceptibility.[22,28,64–66]

2.3.3. Genogroup Differences

Most of the QMRAs calculated the dose from both GI and GII occurrence data while a few calculated the dose from only GII occurrence data. Though a GII dose–response model was developed from oyster outbreak data, it has not, as yet, been adopted for use in QMRA.[61] Thus, in the absence of a GII dose–response model developed from human challenge data, all QMRAs assumed that GI and GII are similar and used the norovirus dose–response model developed from GI human challenge data despite the fact that there could be considerable variability between strains.

2.3.4. Fraction of Infectious Particles

Most of the QMRAs did not discuss assumptions about the fraction of infectious to noninfectious viral particles. A few assumed that all measured pathogens were infectious or provided a fraction.[36,40,42,46] Two QMRAs assumed that the ratio of total to infectious particles was the same as the inoculum in the dose–response human challenge study because the ratio of total to infectious particles in the inoculum was unknown and the dose–response model parameterization is only applicable when this ratio is consistent.[41,50,54]

2.3.5. Harmonization

The majority of the QMRAs did not address harmonizing the quantitative polymerase chain reaction (qPCR) results from the environmental studies with the qPCR results from the clinical human challenge study if different target sequences were used. This is an issue because concentrations of pathogens can differ considerably between methods and in order to use a published dose–response model, the laboratory methods used to enumerate the dose in the environmental sample must be in harmony with those used to enumerate the dose in the human challenge study (clinical trial). Two QMRAs addressed this issue, indicating that RT-PCR was used for both the environmental samples and the human challenge study, but each used a different genetic target sequence, resulting in different critical thresholds for the standard curves. As a result, an adjustment factor was identified and used in the Bambic and McBride QMRAs of recreational water.[19,45] In addition, the recovery efficiency of the qPCR results from the environmental studies was rarely accounted for. This recovery may be different than the generally unknown recovery of norovirus from the clinical human challenge studies. Not harmonizing the qPCR data may result in an under- or overestimation of dose.

2.3.6. Software and Calculations

The majority of the published QMRAs used R software ($n = 9$) or Mathematica ($n = 7$) to compute the dose and dose response, while some used either MATLAB ($n = 4$), @Risk ($n = 4$), or MathCad ($n = 2$). Differences in dose–response calculations were observed with the multiple software platforms. When R software with the "gsl" package was used for the ${}_2F_1$ hypergeometric, difficulty was observed in calculating probabilities of infection resulting from large doses of norovirus. Thus, the QMRAs used the Pfaff transformation for doses <33,323 gec and the ${}_1F_1$ hypergeometric for doses >33,323 gec.[38–40,46] In contrast, Mathematica has the ability to calculate the ${}_1F_1$ and ${}_2F_1$ hypergeometric functions for the majority of doses. A comparison of

the two software platforms was done by performing risk calculations in both Mathematica without the Pfaff transformation and in R with the Pfaff transformation and the same results were observed for both (data not included). Two of four QMRAs that used @Risk, which is an add-on to MS Excel, could not directly compute the ${}_1F_1$ hypergeometric function, so the authors of two papers calculated the infection probabilities from the beta-binomial form of $P_{\text{inf}} = 1 - B(\alpha, \beta + \text{dose})/B(\alpha, \beta)$, where B is the standard Beta function with parameters α, β written in Excel as B(alpha, beta) = EXP(GAMMALN(alpha) + GAMMALN(beta) – GAMMALN(alpha + beta)).[19,45,60,67,68] The authors stated that this was a simplification of the troublesome ${}_1F_1$ hypergeometric and that use of the beta-binomial was justified because multiple individuals are exposed at each event so the mean infection risk is maintained. It should be noted that a difference in risk estimates at low doses (<5 gec) was observed, with the beta-binomial predicting a higher risk. Thus, use of this model instead of the ${}_1F_1$ hypergeometric will lead to different results.[45] The other QMRAs using @Risk did not discuss how the ${}_1F_1$ hypergeometric function was computed.[48] In general, the assumptions and parameterizations of the QMRA for the specific software platform must be described; otherwise, reproducibility of results is hindered.

3. APPLICATION OF NOROVIRUS DOSE–RESPONSE MODELS IN QMRA

Given the lack of consensus on the best norovirus dose–response model and waterborne outbreaks (drinking and recreational) being identified for norovirus, a risk assessment comparing dose–response models was conducted to estimate risk outcomes for published water concentration data.[9] For this analysis, data were collected from the literature on the concentrations of norovirus in recreational and untreated drinking water (Table II). All the drinking water sources included in Table II represent raw water that has not undergone typical drinking water treatment such as disinfection.

A deterministic exposure analysis was performed to estimate the dose of norovirus consumed. For recreational water, individuals were assumed to consume 25 mL, which is the mode volume incidentally ingested for adults (volumetric rate = 50 mL/h, duration = 0.5 hours).[19] For untreated drinking water, individuals (consumer-only adults >21 years of age) were assumed to consume 1,227 mL of water per day (mean volume).[72] No dilution or die-off in water was assumed.

Norovirus is often considered to be disaggregated in environmental waters, but as observed in Table I, QMRAs also assume that norovirus is aggregated. Thus, a range of aggregation was considered, from full disaggregation to a mean aggregate size of 1,106 viruses per aggregate, as dictated by the range considered in the reviewed dose–response models. The population exposed was Se+ adults and children. Norovirus genogroups GI and GII were analyzed and all norovirus copies (gec) were assumed to be infectious. No attempt at harmonization was performed for this analysis. A total of 19 doses were calculated and the probability of infection was estimated for each dose using available dose–response model parameters (Table III). Microsoft Excel was used to calculate the probability of infection values from the approximate beta-Poisson and fractional Poisson models while Mathematica 10 (Wolfram Research, Campaign, IL, USA) was used for the ${}_1F_1$ and ${}_2F_1$ hypergeometric models.

For the aggregated dose–response models, the "a" parameters presented and used in QMRAs are rounded to four significant figures as published in the literature, but these values are actually imprecise. The precise values, rounded to eight significant figures, are presented in the footnote. For example, the 2F1_TGIa+b has an $a = 0.9997$ that would actually yield a μ_a of 411 as opposed to the published 396 viruses per aggregate, which corresponds to an "a" of 0.99968718 calculated using the equation $\mu_a = -a/((1-a)\ln(1-a))$.[15] This discrepancy in "a" values is noted because it has a minor impact on risk estimates (see Table IV).

It is recognized that a full uncertainty analysis of the dose–response parameters is important for characterizing risk, but at this time the uncertainty analysis for models 2F1i_PGIa+bGii and 2F1_PGIa+bGII could not be done without reproducing the dose–response analysis. Still, a discussion of the differences in the best-estimate predicted risk from each model was done since the majority of reviewed QMRAs relied on the best-estimate fit. Uncertainty analysis from 10,000 published ${}_1F_1$ hypergeometric α-β pairs derived by Teunis *et al.* [41] was done for the most commonly used dose–response model, the 1F1x_TGIa+b, as well as 2F1_TGIa+b assuming no uncertainty around the "a" parameter ($a = 0.9997$). The uncertainty analysis is shown in Fig. A1 in the Appendix. Generally, the results

Table II. Concentration of Norovirus in Recreational and Untreated Drinking Water

Source	Norovirus Genogroup	Norovirus Concentration (gec/mL)				Reference
		Single Point	Mean	Median	Max	
Recreational water						
Residential SW	GII			1.00×10^{1}	1.00×10^{3}	McBride *et al.*[45]
Commercial/light industrial SW	GII			5.00×10^{1}	5.00×10^{2}	McBride *et al.*[45]
Agricultural SW	GII			1.00×10^{2}	2.00×10^{4}	McBride *et al.*[45]
CSO	GII			2.00×10^{1}	2.00×10^{3}	McBride *et al.*[45]
Forested open space SW	GII			5.00×10^{0}	5.00×10^{2}	McBride *et al.*[45]
Dry season urban runoff	GII			1.00×10^{1}	1.00×10^{2}	McBride *et al.*[45]
Tropical urban catchment	GI		9.50×10^{-2}			Rezaeinejad *et al.*[69]
Untreated drinking water						
Municipal well in community	GII	7.70×10^{-2}				Hunt *et al.*[70]
Municipal well in community	GI	1.36×10^{-2}				Hunt *et al.*[70]
Tap water, nondisinfected GW	GI		6.00×10^{-4}		1.16×10^{-1}	Borchardt *et al.*[71]
Reservoir	GI		2.30×10^{-1}			Rezaeinejad *et al.*[69]
Reservoir	GII		3.50×10^{-1}			Rezaeinejad *et al.*[69]

SW = storm water; CSO = combined sewer overflow; GW = groundwater.

demonstrate that the disaggregated dose–response models predict risks that fall outside of the 95% confidence interval predicted by the 1F1x_TGIa+b at small doses (Fig. A1(a)). For the aggregated models, the 95% confidence interval encompasses all the models at all doses, which is not surprising since the models are so similar (Fig. A1(b)). It should be noted that the analysis of the predicted 95% confidence intervals for the 1F1x_TGIa+b is only plausible if the proposed mechanistic model is assumed to be reasonable as well as true and the confidence interval derived by Teunis *et al.* (along with the 1F1x_TGIa+b fit [15]) has been called into question.[18] The predicted risks are presented in Fig. 1 and all results are presented in Table IV. The results were compared against the illness benchmark for recreational water of 0.03 (3 illnesses per 100 recreational events), which is the revised benchmark that excludes fever as a symptom of highly credible gastrointestinal illness because infection can cause illness without fever.[54,73–75] The assumption was made that if a person was infected he or she became ill. The results were also compared against the annual risk of infection guideline for drinking water of less than 1 infection per 10,000 individuals per year,[76,77] which would yield an allowable daily risk of approximately 1 in 1,000,000.[78]

The results from Table IV indicate that for a dose of <100 gec (4 of 19 doses), the highest predicted probability of infection values were estimated by FPx_PGIa+bGII followed by 1F1x_TGIa+b. At very low doses (<1 gec), all dose–response models predicted probability of infection values near 0.001 except FPx_PGIa+bGII and 1F1x_TGIa+b, which predicted 0.38 and 0.22, respectively, at a dose of 0.736 gec. The lowest predicted probability of infection values at doses <100 gec were estimated by 2F1_PGIa+bGII and FP_PGIa+bGII. For doses ranging from 100 gec to 1,000 gec (7 of 19 doses), the highest predicted probability of infection values were estimated by FPx_PGIa+bGII and the second highest by 1F1x_TGIa+b. The lowest predicted probability of infection values were estimated by 2F1_PGIa+bGII for doses 125 gec to 429 gec and BP_TGIa+b for a dose of 500. For doses ranging from 1,000 gec to 10,000 gec (3 of 19 doses), the highest predicted probability of infection values were also estimated by FPx_PGIa+bGII and the second highest by FP_TGIa+b. The lowest predicted probability of infection values were estimated by BP_TGIa+b. For doses greater than 10,000 gec (5 of 19 doses), the highest predicted probability of infection values were all estimated by 1F1_AGI while the lowest predicted probability of infection values were estimated by BP_TGIa+b.

The largest and smallest predicted probability of infection values are quite dependent on dose, as depicted in Fig. 1. At doses <100 gec, the widest range (0.00047–0.72) of predicted probability of infection values is observed. FPx_PGIa+bGII and 1F1x_TGIa+b predicted the largest probability of infection values (>0.22) while many of the other

Table III. Dose–Response Parameters

Abbreviations	Dose-Response DR Model (Data Set)	Genogroup (Type)	Dose-Response Parameters					
			α	β	a	P	μ_a	Fit or Reference
Disaggregated								
BP_TGIa+b	Approx. BP (Teunis)	GI (8fIIa+b)	0.104	32.3				MLE[a]
BP_AGI	Approx. BP (Atmar)	GI	0.349	357.1				MLE[a]
1F1x_TGIa+b	${}_1F_1$ (Teunis)	GI (8fIIa+b)	0.04	0.055				Teunis *et al.*[15]
1F1_AGI[b]	${}_1F_1$ (Atmar)	GI	0.46	1.20				McBride[25]
2F1i_PGIa+bGII	${}_2F_1$ (Pooled)	GI (8fIIa+b), GII.4	2.91	2,734	0	0.7246[c]	1	Schmidt[18]
FPx_PGIa+bGII	FP(Pooled)	GI (8fIIa+b), GII.4			0	0.72	1	Messner *et al.*[17]
Aggregated								
2F1_TGIa+b	${}_2F_1$ (Teunis)	GI (8fIIa+b)	0.04	0.055	0.9997[d]		396	Teunis *et al.*[15]
2F1_PGIa+bGII	${}_2F_1$ (Pooled)	GI (8fIIa+b), GII.4	0.0044	0.0020[e]	0.9999[d]		1,024	Messner *et al.*[17]
FP_TGIa+b	FP (Teunis)	GI (8fIIa+b)			0.9998[d]	0.686	579	Messner *et al.*[17]
FP_PGIa+bGII	FP (Pooled)	GI (8fIIa+b), GII.4			0.9999[d]	0.72	1,106	Messner *et al.*[17]

[a]Maximum likelihood estimation.
[b]The dose was converted from gec to RT-PCR units assuming 1 RT-PCR unit = 400 gec before using this dose–response model.
[c]Where $P = 1 - \varphi$ and $\varphi = 0.2754$.
[d]The "a" values are rounded and imprecise. The precise "a" values are 0.99968718, 0.99989323, 0.99979689, and 0.99990207 for 2F1_TGIa+b, 2F1_PGIa+bGII, FP_TGIa+b, and FP_PGIa+bGII, respectively.
[e]The β value reported in the Messner 2014 paper was incorrect and this is the correct β value (per communication with Michael Messner, PhD, USEPA, October 30, 2104); the published α and μ_a values were correct.

models predicted probability of infection values <0.1. At doses from 100 gec to 1,000 gec, the range of predicted probability of infection values narrows (range from 0.076 to 0.72). At a dose of ~1,000 gec, the predicted probability of infection values narrows even more with FPx_PGIa+bGII predicting the largest probability of infection (0.72 for all doses) and BP_TGIa+b predicting the lowest (between 0.32 and 0.36). At a dose of >10,000 gec, the predicted probability of infection values narrow further with 1F1_AGI predicting the largest probability of infection (from 0.8 to 0.96) and BP_TGIa+b predicting the lowest (0.46–0.63).

The difference between the maximum and minimum predicted probability of infection values across dose levels ranged from 0.33 to 0.71, demonstrating the impact the choice of dose–response model can have on QMRA results. The impact was greatest at low doses (<100 gec) with the difference in risk estimates ranging from 0.37 to 0.71 and ranging from 0.47 to 0.64 for doses from 100 gec to 1,000 gec. The impact was the least for doses from 1,000 gec to 10,000 gec, ranging from 0.36 to 0.40, as well as at the largest doses (>10,000 gec), ranging from 0.33 to 0.36. The differences in the risk estimates have important implications for risk assessment because for low doses (<10 gec) there is approximately a 400-fold (2.6 orders of magnitude) to 800-fold (2.9 orders of magnitude) difference in risk estimates for <10 gec and <1 gec, respectively, depending on which model is used. This difference diminishes to <1 order of magnitude for doses above 100 gec. Thus, the FPx_PGIa+bGII and 1F1x_TGIa+b may overpredict risk in relatively safe drinking water by nearly three orders of magnitude, which was also noted by others.[18]

Another observation for doses ≤50,000 gec was that the largest probability of infection values were all estimated from disaggregated dose–response models (FPx_TGIa+bGII and 1F1_AGI) while the smallest probability of infection values were estimated from both disaggregated (BP_TGIa+b) and aggregated (2F1_PGIa+bGII with a rounded "a" value) dose–response models. None of the models predicting the highest and lowest risk values have been used in QMRA. The dose–response model that predicted the second highest probability of infection values was the 1F1x_TGIa+b at doses below ~1,000 gec, which is the dose–response model often used in

Table IV. Point Estimates of Risk (Maximum) from Water Concentration Analysis

	Recreational Water							Untreated Drinking Water				
Parameter/ DR Model	Residential SW	Commercial/ Light Industrial SW	Ag SW	CSO	Forested Open Space SW	Dry Season Urban Runoff	Tropical Urban Catchment	Municipal Well	Community Well	Tap Water, Nondisinfected GW	Reservoir	Reservoir
Dose (gec)	2.50×10^{2} (2.50×10^{4})	1.25×10^{3} (1.25×10^{4})	2.50×10^{3} (5.00×10^{5})	5.00×10^{2} (5.00×10^{4})	1.25×10^{2} (1.25×10^{4})	2.50×10^{2} (2.50×10^{3})	2.38×10^{0}	9.45×10^{1}	1.67×10^{1}	7.36×10^{-1} (1.42×10^{2})	2.82×10^{2}	4.29×10^{2}
Pinf with disaggregated dose												
BP_TGIa+b	2.02×10^{-1} (4.99×10^{-1})	3.18×10^{-1} (4.62×10^{-1})	3.65×10^{-1} (6.33×10^{-1})	2.53×10^{-1} (5.34×10^{-1})	1.52×10^{-1} (4.62×10^{-1})	2.02×10^{-1} (3.65×10^{-1})	7.37×10^{-3}	1.33×10^{-1}	4.24×10^{-2}	2.34×10^{-3} (1.61×10^{-1})	2.11×10^{-1}	2.42×10^{-1}
BP_AGI	1.69×10^{-1} (7.74×10^{-1})	4.08×10^{-1} (7.14×10^{-1})	5.16×10^{-1} (9.20×10^{-1})	2.63×10^{-1} (8.22×10^{-1})	9.94×10^{-2} (7.14×10^{-1})	1.69×10^{-1} (5.16×10^{-1})	2.32×10^{-3}	7.87×10^{-2}	1.58×10^{-2}	7.18×10^{-4} (1.10×10^{-1})	1.84×10^{-1}	2.41×10^{-1}
1F1x_TGIa+b	5.44×10^{-1} (6.21×10^{-1})	5.73×10^{-1} (6.10×10^{-1})	5.84×10^{-1} (6.64×10^{-1})	5.57×10^{-1} (6.31×10^{-1})	5.31×10^{-1} (6.10×10^{-1})	5.44×10^{-1} (5.84×10^{-1})	4.03×10^{-1}	5.26×10^{-1}	4.91×10^{-1}	2.23×10^{-1} (5.34×10^{-1})	5.46×10^{-1}	5.54×10^{-1}
1F1_AGI	1.47×10^{-1} (8.54×10^{-1})	4.45×10^{-1} (7.99×10^{-1})	5.84×10^{-1} (9.63×10^{-1})	2.54×10^{-1} (8.94×10^{-1})	7.97×10^{-2} (7.99×10^{-1})	1.47×10^{-1} (5.84×10^{-1})	1.65×10^{-3}	6.14×10^{-2}	1.14×10^{-2}	5.10×10^{-4} (8.95×10^{-2})	1.63×10^{-1}	2.27×10^{-1}
2F1i_PGIa+bGII	1.63×10^{-1} (7.24×10^{-1})	4.82×10^{-1} (7.20×10^{-1})	6.15×10^{-1} (7.25×10^{-1})	2.80×10^{-1} (7.24×10^{-1})	8.84×10^{-2} (7.20×10^{-1})	1.63×10^{-1} (6.15×10^{-1})	1.83×10^{-3}	6.82×10^{-2}	1.27×10^{-2}	5.67×10^{-4} (9.92×10^{-2})	1.80×10^{-1}	2.50×10^{-1}
FPx_PGIa+bGII	7.20×10^{-1} (7.20×10^{-1})	7.20×10^{-1} (7.20×10^{-1})	7.20×10^{-1} (7.20×10^{-1})	7.20×10^{-1} (7.20×10^{-1})	7.20×10^{-1} (7.20×10^{-1})	7.20×10^{-1} (7.20×10^{-1})	6.53×10^{-1}	7.20×10^{-1}	7.20×10^{-1}	3.75×10^{-1} (7.20×10^{-1})	7.20×10^{-1}	7.20×10^{-1}
Pinf with aggregated dose												
2F1_TGIa+b	2.34×10^{-1} (6.20×10^{-1})	5.21×10^{-1} (6.08×10^{-1})	5.69×10^{-1} (6.64×10^{-1})	3.68×10^{-1} (6.31×10^{-1})	1.33×10^{-1} (6.08×10^{-1})	2.34×10^{-1} (5.69×10^{-1})	2.91×10^{-3}	1.04×10^{-1}	2.01×10^{-2}	9.01×10^{-4} (1.49×10^{-1})	2.55×10^{-1}	3.37×10^{-1}
2F1_TGIa+b w/precise a^{*}	2.40×10^{-1} (6.20×10^{-1})	5.24×10^{-1} (6.08×10^{-1})	5.70×10^{-1} (6.64×10^{-1})	3.75×10^{-1} (6.31×10^{-1})	1.38×10^{-1} (6.08×10^{-1})	2.40×10^{-1} (5.70×10^{-1})	3.01×10^{-3}	1.08×10^{-1}	2.08×10^{-2}	9.34×10^{-4} (1.53×10^{-1})	2.62×10^{-1}	3.44×10^{-1}
2F1_PGIa+bGII	1.43×10^{-1} (7.02×10^{-1})	4.75×10^{-1} (7.00×10^{-1})	6.27×10^{-1} (7.06×10^{-1})	2.56×10^{-1} (7.03×10^{-1})	7.55×10^{-2} (7.00×10^{-1})	1.43×10^{-1} (6.27×10^{-1})	1.52×10^{-3}	5.78×10^{-2}	1.06×10^{-2}	4.70×10^{-4} (8.51×10^{-2})	1.59×10^{-1}	2.27×10^{-1}
2F1_ PGIa+bGII w/precise a^{*}	1.50×10^{-1} (7.02×10^{-1})	4.90×10^{-1} (7.00×10^{-1})	6.36×10^{-1} (7.06×10^{-1})	2.68×10^{-1} (7.03×10^{-1})	7.97×10^{-2} (7.00×10^{-1})	1.50×10^{-1} (6.36×10^{-1})	1.61×10^{-3}	6.12×10^{-2}	1.12×10^{-2}	4.98×10^{-4} (8.98×10^{-2})	1.67×10^{-1}	2.38×10^{-1}
FP_TGIa+b	2.41×10^{-1} (6.86×10^{-1})	6.07×10^{-1} (6.86×10^{-1})	6.77×10^{-1} (6.86×10^{-1})	3.97×10^{-1} (6.86×10^{-1})	1.33×10^{-1} (6.86×10^{-1})	2.41×10^{-1} (6.77×10^{-1})	2.81×10^{-3}	1.03×10^{-1}	1.95×10^{-2}	8.71×10^{-4} (1.49×10^{-1})	2.64×10^{-1}	3.59×10^{-1}
FP_PGIa+bGII	1.46×10^{-1} (7.20×10^{-1})	4.87×10^{-1} (7.20×10^{-1})	6.45×10^{-1} (7.20×10^{-1})	2.62×10^{-1} (7.20×10^{-1})	7.69×10^{-2} (7.20×10^{-1})	1.46×10^{-1} (6.45×10^{-1})	1.55×10^{-3}	5.90×10^{-2}	1.08×10^{-2}	4.79×10^{-4} (8.68×10^{-2})	1.62×10^{-1}	2.31×10^{-1}

*These models were also calculated with aggregation parameter (a) values precise to 8 decimal places.

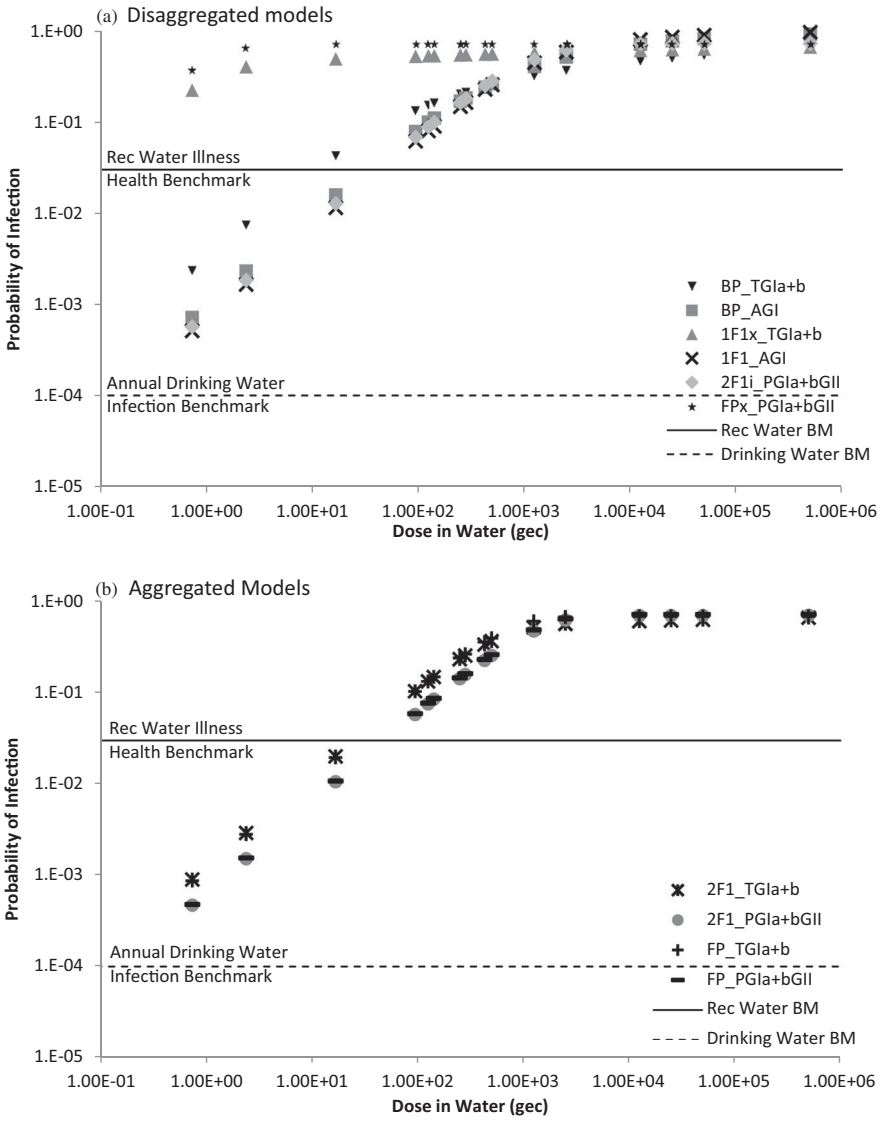

Fig. 1. Comparative risk analysis for norovirus in water for (a) disaggregated and (b) aggregated models.

QMRA (Table I). It should be noted that the larger probability of infection values were all estimated from disaggregated dose–response models, including two models that were fit with an aggregation parameter, but then assumed a disaggregated dose with an "*a*" parameter of 0 (1F1_AGI, 1F1x_TGIa+b, and FPx_TGIa+bGII).

Overall, the difference between the maximum and minimum predicted risk estimates varies more or less widely depending on whether the QMRA application is for untreated drinking water or recreational water. In untreated drinking water, the concentration of norovirus was quite low, as Table II demonstrates, with concentration values ranging from 77 gec/L to 350 gec/L. At this low concentration range, 1F1x_TGIa+b, which is the dose–response model most often used in QMRA, as well as FPx_PGIa+bGII estimate the highest probability of infection values. For recreational water, the norovirus concentrations are higher (up to 20,000 gec/mL), often yielding doses >1,000 gec, which is where the dose–response models predict more similar risk estimates. Thus, it is very important which dose–response model is selected for untreated drinking water because the resulting estimated risk values are quite different, with the most conservative

models being either FPx_PGIa+bGII or 1F1x_TGIa+b. For recreational water, the 10 dose–response models predicted similar probability of infection values, so the choice of model is less critical.

The importance of dose–response model selection is further highlighted when comparing the results to the health benchmarks for drinking and recreational water. The health benchmark for drinking water is an annual risk, which is very low ($<10^{-4}$ infections/person/year), so when used for the untreated drinking water scenarios all dose–response models at all doses exceed the annual health benchmark. However, for recreational water, at doses <10 gec, all dose–response models predict risk values less than 0.03 except FPx_PGIa+bGII and 1F1x_TGIa+b, which predict exceedance of the benchmark. When the doses range from 10 gec to 100 gec, less than half of the predicted risk values are lower than the health benchmark (0.03). Although the comparison of the predicted probability of infection values to the recreational illness benchmark is conservative since a theoretical corresponding infection benchmark would be higher, the results highlight the difference in norovirus dose (and, ultimately, level of contamination) that corresponds to exceedance or not of the recreational health benchmark among dose–response models.

4. CONCLUSIONS AND BEST PRACTICES

4.1. Conclusions

Multiple dose–response models have been proposed for use in QMRA, but a single model has not been agreed upon as the "best" or most accurate. However, some conclusions about the application of norovirus dose–response models in QMRA can be made.

(1) Currently, the majority of published QMRAs of norovirus in a variety of media use the ${}_1F_1$ or ${}_2F_1$ hypergeometric dose–response model obtained from the Teunis data set ($\alpha = 0.04$, $\beta = 0.055$) with a variety of aggregation assumptions.
(2) From the comparative risk estimates of norovirus in water, the dose–response model that estimated the highest or lowest risk was dependent on the ingested dose and varied between disaggregated and aggregated dose–response models. This has implications for drinking water QMRAs where the concentration of norovirus is low (<1,000 gec). In this case, the use of the ${}_1F_1$ hypergeometric fit to the Teunis data with the assumption of no aggregation ($a = 0$), which is commonly used in QMRA, would provide the most conservative risk estimates.
(3) Moreover, from the comparative water risk analysis, at a dose <1 gec (untreated drinking water), an 800-fold difference (2.9 orders of magnitude) in calculated probability of infection values among the dose–response models was observed. This difference reduced to <1 order of magnitude for doses above 100 gec, indicating some models (the fractional Poisson fit to the pooled data set assuming disaggregation and the ${}_1F_1$ hypergeometric fit to the Teunis data set assuming disaggregation) may overpredict risk in relatively safe drinking water by nearly three orders of magnitude.
(4) For untreated drinking water with low norovirus concentrations, the annual health benchmark was exceeded with all dose–response models and at all doses. For recreational water at doses <10 gec, all dose–response models predicted risks that did not exceed the health benchmark except the fractional Poisson fit to the pooled data set and the ${}_1F_1$ hypergeometric fit to the Teunis data assuming disaggregation. At higher recreational water doses (>100 gec), all of the dose–response models exceeded the health benchmark. The recreational water results highlight the importance of dose–response model selection at low doses (<10 gec) because two models predicted exceedance of the benchmark while with the other models predicted no exceedance. At higher doses, the choice of dose–response model was less important.

4.2. Best Practices for Assumptions in Dose–Response Models Used in QMRA

As no one norovirus dose–response model is best, some best practices can be used when selecting and applying a model for QMRA.

4.2.1. Aggregation

Some of the dose–response models are parametrized to characterize the aggregation state of norovirus. The assumption that the risk assessor

makes about the aggregation state when using these specially parameterized models affects the predicted risk. As the aggregation moves from highly aggregated to disaggregated ("a" $\rightarrow$ 0), the ID_{50} value is lowered and the predicted risk estimates increase, as observed in Fig. A1 where at a dose of 100 gec, the aggregated 2F1_TGIa+b ($a = 0.9997$) predicts a probability of infection of 0.11 while the 1F1x_TGIa+b ($a = 0.0001$) predicts a much higher 0.53.

Considerations for viral aggregation include (a) environmental media, (b) pH and isoelectric point (IEP) because if pH > IEP then aggregation is less likely, and (c) the likelihood of viral aggregates having greater than 1,000 viruses per aggregate. Currently, most QMRAs assume that norovirus is disaggregated in environmental media, such as water.[45,50] In this case, the disaggregated assumption is supported because the pH of environmental water is typically greater than the IEP of norovirus, which ranges from 5.9 to 6.0 for GI.[79,80] However, it has been observed that small changes in the pH of interstitial water may strongly influence the electrostatic character and, ultimately, aggregation for Norwalk virus.[81] Finally, some dose–response models estimated ~1,000 viruses per aggregate, but aggregates in environmental media are likely not this large.

Best Practice: Selection of a disaggregated or aggregated model must be based on sound assumptions about how norovirus behaves in the identified media. Without concrete knowledge on how norovirus behaves in either the inocula or environmental media with respect to aggregation, the best approach for QMRA is to use both described disaggregated and aggregated dose–response models to express the full range of uncertainty for this parameter.

4.2.2. Exposed Population

Secretor status should be considered in QMRA because the exposed population in the human challenge experiments only includes Se+ individuals. Typically, the Se– portion of the population is ignored in QMRA. However, assuming 100% of the exposed population is Se+ and excluding Se– individuals from the QMRA could result in an overestimation of the risk. Accounting for susceptibility is difficult because the percentage of Se– individuals varies by ancestry with only 2% of people from Hispanic ethnicity[26] and 20% of Caucasian individuals being Se– (i.e., not susceptible to norovirus infection).[27,29] Further, secretor status susceptibility by blood group has demonstrated variable results and may be dependent on genogroup.[23,28,64,65] Other considerations are that only a small portion of healthy adults are used in human challenge experiments, which does not represent susceptible subpopulations such as the elderly, children, or immunocompromised. Finally, the shape of the aggregated dose–response models ($_2F_1$ hypergeometric and fractional Poisson), where the probability of infection values level off at high doses, indicates that a portion of the susceptible Se+ population may be immune to norovirus infection. Currently, acquired immunity is not accounted for in QMRA.

Best Practice: The most conservative assumption is to assume all individuals are Se+, which could result in up to a 20% overestimation of risk in QMRAs. With the new information on ancestry-dependent susceptibility and if the population exposed is diverse, then this is the best approach. An alternate approach if the population is Caucasian is to randomly sample from a uniform distribution (0.80–1.00) to adequately account for the Se– portion of this population.[46]

4.2.3. Genogroup Differences

The published dose–response models are only for GI, which constrains QMRA to consider both GI and GII genogroups to be equally infectious.[45] Not enough data are available to fully ascertain whether it is appropriate to assume both genogroups display the same infectivity in the population, particularly at low doses. Also, not enough GII data are available to mathematically fit a new dose–response model specific to this genogroup. Ultimately, more GII data are needed.

Best Practice: In the absence of another dose–response model, the GI model must be used in all norovirus QMRAs. However, incorporation of variability into dose response should be considered, such as running multiple dose–response models, toggling between dose–response models, or selecting a dose–response model that factors in GII data.[17]

4.2.4. Infectious and Noninfectious Particles

The fraction of infectious to total viral particles cannot be accurately considered because no cell culture method currently exists to estimate the infectivity of norovirus; thus, determining the true infectious dose is difficult. It is important in QMRA

to make accurate assumptions about the infectious dose of norovirus particles because the validity of comparisons of infectivity is dependent on how many ingested virus particles are infectious.[15] Also, the ratio is not known but has the potential to impact risk estimates, particularly when a disinfection step is included.

Best Practice: The conservative assumption for QMRA is that all viral particles are infectious. However, the conservative assumption is unlikely for all viruses especially in environmental samples and may inflate risk estimates by overestimating the infectious dose. Thus, explicit assumptions regarding the infectivity should be clearly stated and the impact of the assumptions on the estimated risk should be discussed.[15,22,82]

4.2.5. Harmonization

Accounting for differences between PCR targets used in environmental sample analysis versus clinical samples may be an important consideration that could lead to different risk estimates. Additionally, differences in the matrix effect (i.e., environmental inhibition of RT-PCR assays) can be a significant factor and should be expressly considered.

Best Practice: Harmonization of methods should be considered in QMRA, if possible, recognizing that not enough data may be available to harmonize the doses.

4.2.6. Software

As described, the different software programs vary in their limitations, such as the absence of a convenient way for @Risk or MATLAB to compute the ${}_2F_1$ hypergeometric function or the failure of downloadable packages in the R software at certain doses.

Best Practice: Risk assessors should indicate the software and extra software packages used to model dose response as well as describe any limitations of that software to increase the reproducibility and confidence in the results. Risk assessors should also specify all assumptions and parameterizations used in the software. Furthermore, care should be taken when using software packages built by other authors to ensure accurate results.

4.2.7. Selection of Dose–Response Model

Model selection is very important and should be carefully considered with respect to the dose as well as the exposure scenario, specifically aggregation of the virus, the environmental media, and the genogroup/type. As described, the numerous norovirus dose–response models make different assumptions, are fit to different data sets, and yield very different risk estimates, especially at low doses. This was observed in the results of the comparative water risk analysis (Section 3). Thus, no one dose–response model can be identified as being best for use in QMRA.

Best Practice: With no clear dose–response model found to be best for use in QMRA, risk assessors must carefully consider the exposure scenario as well the uncertainty when determining which dose–response models to use. Specifically, the risk assessor should evaluate the exposure scenario to make accurate assumptions about aggregation. Also, in the absence of one best norovirus dose–response model, multiple models should be used to provide a range of predicted outcomes for probability of infection. These models should encompass the extreme values as well as a central tendency to provide a complete picture of the risk and to describe the uncertainty in dose–response modeling.

In conclusion, no clear norovirus dose–response model can be identified as "best"; however, this work was an important first step in analyzing the differences in the proposed norovirus dose–response models in terms of predicted risk. The presented best practices for norovirus dose–response, demonstrated in the QMRAs within, encourage careful consideration about the applicability of each dose–response model in relation to the exposure scenario and the assumptions affecting the calculation of dose and, ultimately, the predicted risk.

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APPENDIX

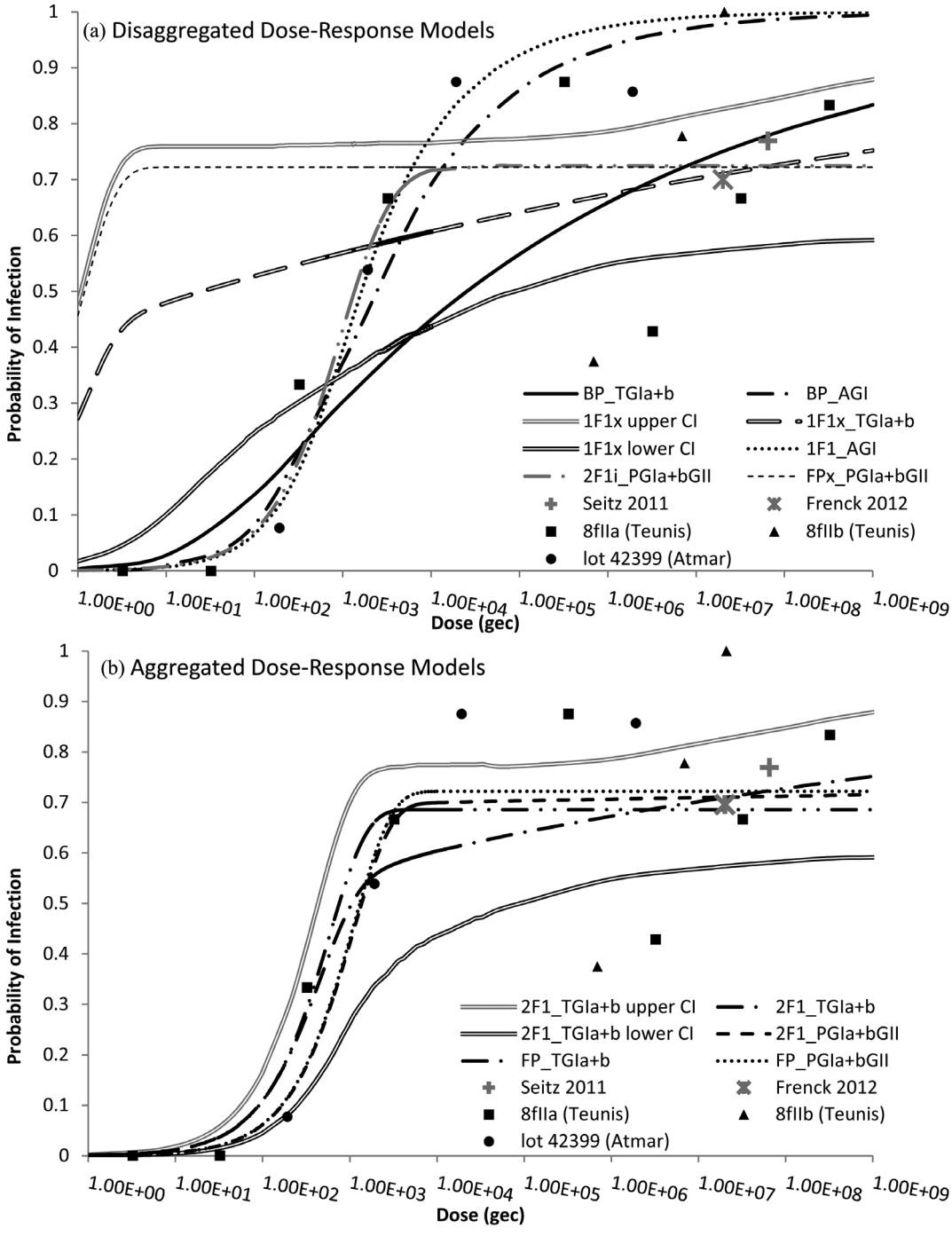

Fig. A1. Uncertainty analysis in the form of confidence intervals (CI) for (a) disaggregated and (b) aggregated dose–response models.

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