Quantifying the impact of runoff events on microbiological contaminant concentrations entering surface drinking source waters

R. S. Signor, D. J. Roser, N. J. Ashbolt and J. E. Ball

ABSTRACT

Concentrations of microbiological contaminants in streams increase during rainfall-induced higher flow 'event' periods as compared to 'baseflow' conditions. If the stream feeds a drinking water reservoir, such periods of heightened pathogen loads may pose a challenge to the water treatment plant and subsequently a health concern to water consumers downstream. In order to manage this risk, it is desirable to first quantify the differences in surface water quality between baseflow and event conditions. The Event Mean Concentration (EMC) is a flow-weighted average concentration of a contaminant over the duration of a single event, proposed here as a standard parameter for quantifying the net effect of events on microbial water quality. Application of the EMC concept was assessed using flow and quality data for several events from an urbanised catchment. Expected mean EMCs were significantly larger than expected mean baseflow concentrations (*p-value*$\leq$0.012) for three microbial agents - *Escherichia coli* (13,000 *[n = 7]* v. 610 *[n = 16]* mpn/100 ml), *Cryptosporidium* (234 *[n = 6]* v. 51 *[n = 16]* oocysts/10 litres) and *Campylobacter* (48 *[n = 5]* v. 2.1 *[n = 16]* mpn/100 ml). These parameter estimates were complemented by estimating data *variability* and *uncertainty* in the form of second-order random variables. As such the results are in a format appropriate for potential use as components in probabilistic risk assessments evaluating the effect runoff events have on drinking water quality.

Key words | *Campylobacter*, *Cryptosporidium*, drinking water, Event Mean Concentration, *E. coli*, pathogen

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INTRODUCTION

The World Health Organisation's third edition of the *Drinking Water Quality Guidelines* promotes the concept of Water Safety Plans (WSPs), encouraging water supply utilities to set up a proactive framework for monitoring and controlling the quality of the water product before it reaches consumers (WHO 2004). Two components of any WSP are: (i) identification of occurrences in the water supply system that could introduce, or increase, the risk of infection to a water consumer from a waterborne pathogen – otherwise termed a 'hazardous event', and (ii) some quantification of the effect the hazardous event has on consumer risk levels.

Quantitative Microbial Risk Assessment (QMRA) (Haas *et al.* 1999) is an evolving tool used to quantify risks of infection and illness to consumers (e.g. Rose *et al.* 1991; Glicker & Edwards 1991; Teunis *et al.* 1997; Crabtree *et al.* 1997; Barbeau & Payment 2000; Petterson *et al.* 2001; Teunis & Havelaar 2002; Pouillot *et al.* 2004). To date, water supply QMRA applications have rarely explored the effects of hazardous events, such as periods of peak source water contamination or treatment failures, though there has been some recent work (e.g. Westrell *et al.* 2003).

Current QMRA techniques are reliant on obtaining knowledge of the variation and central tendency in

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microbiological quality of the raw source water (Teunis & Havelaar 1999). It is generally acknowledged that concentrations of microbial pathogens and faecal contamination indicators in streams and rivers are higher during periods of contribution from rainfall-induced runoff compared to those seen simply during dry weather baseflow conditions (e.g. Atherholt *et al.* 1998; LeChevallier *et al.* 2002; Ashbolt & Roser 2003). In these circumstances it is thought that rainfall mobilises and transports non-point source microbial particles via runoff, though in some cases it is possible that the increased flow also leads to re-suspension of contaminants in the sediment (Nagels *et al.* 2002). Regardless, when the stream or river is a component of a drinking water supply system, elevated pathogen and faecal indicator loadings provide a challenge to the downstream water treatment system. Studies have shown significant correlations between periods of extreme precipitation and waterborne disease outbreaks attributable to microbial pathogens (e.g. Rose *et al.* 2000; Curriero *et al.* 2001). Other studies have attributed specific waterborne disease outbreaks and contamination incidents to adverse consequences that have resulted from preceding rainfall events, e.g. the Montana giardiasis outbreak (Weninger *et al.* 1983), and the 1993 Milwaukee cryptosporidiosis outbreak (Mackenzie *et al.* 1994). Consequently, rainfall-induced runoff can be classified as a potentially hazardous event worthy of attention in a WSP.

Modelling the extent to which runoff events affect pathogen concentrations and loadings in surface waters is complex, and the many contributing and controlling factors will vary considerably both within and between events. Ferguson *et al.* (2003) provide a discussion of knowledge gaps in current microbial transport and fate models, citing factors such as pathogen host prevalence and the ability of the environment to buffer pathogen transport into waterways among others. Previous studies have measured and compared pathogen concentrations in streams during event and baseflow periods (e.g. Atherholt *et al.* 1998; LeChevallier *et al.* 2002), but have not considered the effect of fluctuating concentrations within an event. The latter work has confirmed that rainfall periods can correlate with elevated pathogen concentrations in streams, however, by neglecting the likelihood of significant concentration variation during events, such an approach may misrepresent the overall effect.

The Event Mean Concentration (EMC) is a concept used extensively in urban stormwater engineering to quantify the effect that high flow event conditions have on contaminant and pollutant loads within flowing water (Huber 1993). Calculating the EMC is reliant on collecting a representative series of flowrate and contaminant concentration pairs during a single event that account for fluctuations in flow and concentration levels with time to estimate an overall mean effect.

Given a series of events in a single catchment, the EMC of each will vary. Likewise, dry weather baseflow concentrations will also vary over time – such is the nature of environmental systems. Measuring and describing this variation is important in formulating appropriate inputs for a stochastic QMRA model. However the collection of samples to measure microbiological water quality, particularly to directly detect pathogens, is difficult, time consuming, and expensive compared with methods to measure other water quality parameters. As such, one is typically challenged when analysing microbiological data by having to deal with small (and often censored) datasets. This produces a need to describe firstly the *variability* in the data, and secondly, the *uncertainty* in any inferences made about the data. Recognising these two concepts is essential for evaluating the results from probabilistic QMRAs and interpreting the implications for risk management (Haas *et al.* 1999).

Considering periods of rainfall-induced runoff as hazardous events, this paper applies the EMC principle to quantify differences in microbiological water quality between dry weather and event conditions in a small urban catchment draining to a drinking source water reservoir in southern Australia. Three microbiological agents were considered: two pathogens (*Cryptosporidium* and *Campylobacter* spp.) and one faecal indicator (*E. coli*). The *variation* in baseflow concentrations and calculated EMCs between sampling occasions were each described by means of separate probability distributions (and estimated parameters). Additionally, *uncertainties* in the statistical parameters were derived by the application of maximum-likelihood techniques.

MATERIALS AND METHODS

The study area (8.4 km^2) is an urbanised partly sewered catchment located in the Adelaide Hills of southern

Australia. The catchment has an average annual rainfall of approximately 1045 mm (daily rainfall recorded from 1990–2002). The stream itself is intermittent (flowrate $>0\,m^3/s$ recorded on 82% of days from 1994–2002), with the vast majority of the 'zero flow' periods occurring in the warmer southern hemisphere summer months, and an average annual flow of approximately 2050 ML (for the period from 1994–2002). There were no known point sources polluting the stream feeding the drinking water reservoir, thus it may be assumed that all microbiological contaminants in the stream were of an urban non-point source.

'Dry weather' (or 'baseflow') periods were defined as periods when there had been no rainfall exceeding 2 mm for at least 48 hours over the catchment prior to sampling, and no identifiable event driven runoff was contributing to the stream flow. Water samples during dry weather (baseflow) periods were collected on 16 independent occasions between 27th November 2000 and 3rd June 2002, and analysed for a range of parameters including *E. coli, Cryptosporidium, Giardia,* and *Campylobacter* spp.

'Event' sampling was conducted between 8th September 2001 and 25th November 2002 during seven separate rainfall-induced runoff events. Event samples were collected by an 'event sampler' that was triggered by a rapid marked water level increase in the stream that could be attributed to rainfall (rise >0.2 metres within a 4-hour period), and efforts were made to take samples that represented the water quality over the rise, peak, and fall of the hydrograph. Sampling intervals were preset to increase from 30 to 60 minutes over the course of the event in line with typical historic hydrograph behaviour at the site. On each occasion the event sampler collected 12 samples over a period of generally five hours, the typical duration of the runoff hydrograph for a single peak event. Each sample was analysed for microbial indicators including *E. coli.* In addition, from each full event four samples were selected, representing water quality conditions during the rise (1), peak (1) and fall (2) of the event hydrograph, and were analysed for *Cryptosporidium, Giardia* and *Campylobacter* spp. (Roser *et al.* 2002*b*).

Samples were collected by two modified ISCO 3700 units (Lincon, NE, USA) operating in parallel and slaved to a master controller (Datataker 50, Datataker Pty. Ltd. Rowville, Vic., Australia (Figure 1)). One was used to collect up to twenty four 10 litre unrefrigerated samples for *Cryptosporidium* (ambient temperature 8–20°C). The other was mounted on a 12 V refrigerator (4°C) and used to collect twenty-four 1 litre refrigerated samples for bacteriological analyses. Samples were transported to the laboratory for collection within 24 hours of event sampler triggering.

During the design and commissioning phase, simulated contaminated river water (200 litres of river water amended with 20% settled raw sewage) was prepared and sampled overnight (6 samples every 3 hours) using the refrigerated system to 'prime' the collection system and lines, in order to evaluate the extent of carryover. The sampling hose was then transferred to 200 litres of sterile deionised water without flushing and two further samples collected. The procedure was undertaken twice and showed that carryover for the model analytes (*E. coli*, total coliforms, *Clostridium perfringens,* total phosphorous, and turbidity) was *ca* 2% per sampling cycle.

To check if spills or aerosol contamination was a concern 'collection blanks' were analysed (sterile water poured at the point of general collection into unfilled containers exposed to the same conditions as the normal samples). Contamination was found to be negligible. Of 47 assays for four main microbial indicator groups (*E. coli*, enterococci, coliphage, clostridia) only two samples yielded false positives out of twelve and measurements were all at the limit of detection. Further, no false positives were detected in the five *Cryptosporidium* event sampler collection blanks though ColorSeed™ were recovered in samples concurrently inoculated (12–48% recovery).

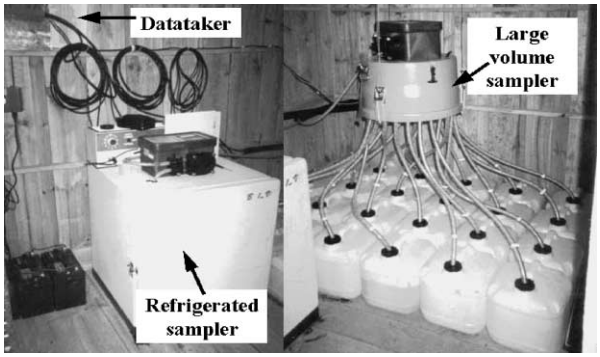

Figure 1 | Event sampler sample collection systems, control unit (on wall), large sample bottles, deep cycle batteries and working space.

All water quality analyses were undertaken by the Australian Water Quality Centre's Analytical Laboratory, Bolivar, South Australia using their standard operating procedures for *E. coli, Cryptosporidium*, *Giardia*, and *Campylobacter* spp. (AWQC methods 2246-19/2247-50/2248-50, 88–01 and 81–07 respectively). *E. coli* was analysed using Colilert™. Protozoa were concentrated by flocculation of 10 litre samples, immunomagnetic separation and centrifugation, and then counted using the USEPA method 1623 (USEPA 1999). *Campylobacter* spp. were assayed by enrichment in 3 × 3 MPN tubes followed by PCR based confirmation.

A fluorescently labelled internal control 'ColorSeed™' of known concentration was incorporated into each of the protozoan samples, allowing an estimation of the 'recovery fraction' for each sample (Roser *et al.* 2002*a*), i.e. the ratio of *the number of spiked organisms counted* using the detection method to *the number of spiked organisms that were in the sample*. Data reported here are the total numbers of organisms adjusted for recovery by multiplying the sample organism count by the inverse of that sample's recovery fraction. *Giardia* data are not reported further as the number of samples with countable native cysts was too few to provide a satisfactory data set despite 'good' recoveries [$n = 35$, average = 34%, range 17–62%]. *Cryptosporidium* recovery fractions ranged from 11–69% [$n = 37$, average = 41%]. Forty percent of all samples analysed in the study (not counting ColorSeed™) were controls (collection blanks, replicates, and spikes).

In cases where duplicate samples were taken, the reported concentration value was the arithmetic mean of the two. Baseflow concentrations were matched with daily stream flow data. Event concentration data were matched with high resolution (5 minute intervals) stream flow data that enabled investigation of flow and concentration fluctuations within each event. All flow data was collected approximately 0.5 km upstream of the water quality site by staff of the South Australian Department of Water Resources.

Data analysis aims and methods

The data analysis logic that follows stems from the following: (i) a need to quantify an overall effect of rainfall-induced runoff event periods on microbial water quality (accounting for within event flowrate and concentration fluctuations), such that the results may be compared to baseflow concentrations, (ii) a need to describe the *variability* and *uncertainty* in the data, as is becoming common practice in data analyses and such that it may be in a form suitable for probabilistic QMRA applications, and (iii) a desire to display the data analysis results in a concise visual form.

Quantifying overall event water quality

By quantifying an overall effect from each event, and reasonably assuming independence between them, then the quantified overall effects can be considered a set of random independent variables. Overall event water quality has been conveniently summarised in the form of an Event Mean Concentration (EMC) (Huber 1993). The EMC is the flow-weighted average of the concentration of each constituent for each event, derived as (Huber 1993):

$$EMC_j = \int_0^T Q_j C_j \cdot dt \Big/ \int_0^T Q_j \cdot dt \approx \sum_{i=1}^{n} Q_{ij} C_{ij} \Big/ \sum_{i=1}^{n} Q_{ij} \qquad (1)$$

where EMC_j is the EMC of the j^{th} event that lasts from time $t = 0$ to $t = T$, and Q_{ij} and C_{ij} are the i^{th} flowrate and concentration pair that was measured during the j^{th} event. The summation on the right hand side of *Equation 1* is appropriate only where concentrations and flowrates are measured as instantaneous pairs and in this notation a total of n flow and concentration pairs were taken during the j^{th} event. The summation is easier to undertake than the integration, and it is probable that differences in EMC_j values calculated by either the integration or summation techniques are negligible when compared to other measurement uncertainties (Huber 1993). The EMC improves on the concept of taking single water quality samples per event and more realistically characterises an overall change in pathogen and indicator concentrations in the flowing water. In addition, localised instantaneous measurements can be put into context by adjacent measurements.

Variability and uncertainty

Risk assessors commonly distinguish between *variability* – the intrinsic variation of a parameter that occurs naturally

and will always exist, and *uncertainty* – the lack of knowledge about data, parameters and models. One key distinction between the two is that under these definitions, *uncertainty* can be reduced (and theoretically eliminated) through further research and data collection, while *variability* will always exist (Enhealth 2002).

Water quality *variability* has been most often described by way of lognormal probability distributions (Ott 1995). Further, *variability* in EMCs specifically, for a variety of pollutants, has also commonly been described as lognormal (Huber 1993; Van Buren *et al.* 1997), though it is conceded that the majority of studies have focussed on non-microbiological contamination. The lognormal distribution has been adopted as the model to describe the variability among EMCs and baseflow concentrations respectively. The lognormal probability distribution function is defined as:

$$X \sim Lognormal(x; \mu, \sigma) = \frac{1}{\sqrt{2\pi}\sigma x} \cdot e^{-[\ln(x)-\mu]^2/2\sigma^2}, x \quad 0 \qquad (2)$$

where μ is the mean and σ the standard deviation of the natural logarithm of the random variable X. The mean $E(X)$ and standard deviation $SD(X)$ of the lognormally distributed random variable X in real space is:

$$E(X) = e^{\mu+(1/2)\sigma^2} \qquad (3)$$

$$SD(X) = \sqrt{e^{2\mu+\sigma^2}(e^{\sigma^2} - 1)} \qquad (4)$$

How best to estimate statistical parameters that describe *variability* of small censored datasets, with several measurements that are 'below a detection limit' (BDL), is a contentious issue (e.g. Gleit 1985; El-Shaarawi 1989; Travis & Land 1990; Cohen 1991; Akritas *et al.* 1994). Often a method of substitution is employed, where the BDL reading is replaced by a value of half the detection limit though this approach has obvious problems, regularly over or underestimating data standard deviations (Travis & Land 1990). As water quality data is often assumed to follow a lognormal probability distribution (Ott 1995), parametric maximum likelihood techniques may be conveniently employed, though they may be biased when used with small censored datasets (Akritas *et al.* 1994:225). El-Shaarawi (1989) has presented a nonparametric modified log-probability regression model and found that it was superior to maximum likelihood methods for datasets that are assumed lognormal. Gleit (1985) developed an iterative maximum likelihood-based 'fill-in with expected values' technique that is suitable when assuming lognormality of the data, and, after a simulative comparison with other methods, claimed it to be the best estimator when dealing with small datasets. The latter two methods were both employed to analyse the censored data, enabling comparisons between the results borne from each method, while standard maximum likelihood methods were employed for our data that had no BDL measurements. As a final word on *variability*, it is essential that any assumptions (such as that of lognormality) be tested by applying and interpreting the results of appropriate statistical hypothesis tests.

Uncertainty is inherent in any statistical analysis. Due to difficulties and costs involved in obtaining microbiological datasets they are frequently 'small' (defined as $n < 30$ observations). Estimators of parameter values of the lognormal distribution for each dataset are expected to be *uncertain* due to small sample sizes (Frey & Burmaster 1999). Second-order random variables are a means of quantifying the *uncertainty* about a set of estimated parameters (that represent the variability of another random variable) (Burmaster & Wilson 1996). Thus, when the *variability* of second-order random variable $\boldsymbol{X}$ is described by the probability distribution function f having k parameters:

$$\boldsymbol{X} \sim f(x; \Theta_1, \Theta_2, \ldots, \Theta_i, \ldots, \Theta_k) \quad i = 1, 2, \ldots, k \qquad (5)$$

then each element of the vector ($\Theta_1, \Theta_2, \ldots, \Theta_i, \ldots, \Theta_k$) are also random variables that have their *uncertainty* described by means of a series of probability distribution functions h_i, each having m parameters, where:

$$\Theta_i \sim h_i(\theta_i, \theta_{i1}, \theta_{i2}, \ldots, \theta_{ij}, \ldots, \theta_{im}) \quad i = 1, 2, \ldots k \quad j = 1, 2 \ldots, m \qquad (6)$$

Burmaster & Thompson (1998) describe an approach for fitting second-order parametric distributions to data using maximum likelihood-based methods. It is assumed that the uncertainty about the estimated variability parameters μ and σ from lognormal distributions in Equation 2 can each

be represented by normal distributions[1] where $M \sim Normal(\mu;\ \mu_\mu,\ \sigma_\mu)$ and $\Sigma \sim Normal(\sigma;\ \mu_\sigma,\ \sigma_\sigma)$ with the constraint that $\Sigma > 0$. The method makes use of the likelihood function to produce joint confidence regions with apt correlations among the parameters that are being estimated.

Displaying data analysis results

Exceedance Probability (EP) charts, with confidence bands about the EP plot, integrate and allow both the *variability* and *uncertainty* of the data to be visualised and read directly from the chart. Here, the term *Exceedance Probability* refers to the probability that, either under event or baseflow conditions, the microbial concentration will be greater than a defined value at any time. Confidence intervals about the maximum likelihood EP plot can be derived as described by Burmaster & Wilson (1996) who demonstrate that the isopleths for the confidence intervals on the lognormal probability plot can be estimated from the second-order random variable outputs as:

$$\ln(X)_{z_u|z_v} \approx \mu_\mu + z_v \cdot \mu_\sigma + z_u \cdot \sqrt{\sigma_\mu^2 + (z_v \cdot \sigma_\sigma)^2} \quad (7)$$

which denotes the point value of the natural logarithm of X at the z_u^{th} percentile of *uncertainty* conditional on the z_v^{th} percentile of *variability*. The values of z_u and z_v in the equation are the normalised z-scores of the respective percentiles, noting there is a prior assumption of normally distributed uncertainties.

RESULTS AND DISCUSSION

Dry weather (baseflow) water quality data

E. coli were detected on all occasions that sampling was undertaken. One measurement out of the 16 daily samples resulted in a non-detect (<1 counted oocyst/10 litres) with respect to *Cryptosporidium*, however there were a significant number (7) of non-detects (<0.3 mpn/100 ml) associated with the *Campylobacter* spp. data.

The modified log-regression method (El-Shaarawi 1989) and the 'fill-in with expected values' technique (Gleit 1985) were used to estimate point values of the below detection limit *Campylobacter* spp. concentrations in the stream baseflow. As only one *Cryptosporidium* measurement was below the detection limit, the method of substitution was employed, replacing the sole BDL measurement with a value of half the detection limit (i.e. 0.5 oocysts/10litres), which was then adjusted by multiplying it by the inverse of that sample's recovery fraction (Table 1).

The register of 'zero flow' as the minimum recorded baseflow (Table 1) is an example of the uncertainties surrounding stream flowrate estimation, especially when estimating very low (or very high flows) from stage-discharge relationships (Pelletier 1988). Although the study stream is intermittent, on two sampling occasions the daily flowrate was officially recorded as 0 m^3/s despite the existence of a very low flow that was witnessed and sampled by the data collection team.

Event water quality data (EMCs)

A typical event hydrograph and pollutograph (for *E. coli*) is included as Figure 2. Water samples were taken at several times over the course of each event as shown and previously described. Generally 12 *E. coli* samples and four *Campylobacter* spp. and *Cryptosporidium* measurements were taken per event, though there were variations (Table 2). *E. coli* were measured during each of the seven events. *Cryptosporidium* and *Campylobacter* spp. samples were taken for six and five events respectively.

EMCs (Table 2) were calculated using the summation technique and also by the integration technique in Equation 1 (assuming concentrations varied linearly as a function of time between instantaneous readings, i.e. interpolating a straight-line between each adjacent concentration measurement). A comparison of the arithmetic mean of the EMCs estimated by the summation technique with those estimated by the integration technique showed little difference in the results for *E. coli* (11,000 v. 12,000 mpn/100 ml), *Cryptosporidium* (241 v. 213 oocysts/10 litres) and *Campylobacter* spp. (24 v. 23 mpn/100-ml). Further, the differences were

[1]A random variable X is normally distributed according to the normal probability distribution function where μ and σ are the mean and standard deviation of real X:

$$X \sim Normal(x;\mu,\sigma) = \frac{1}{\sqrt{2\pi}\sigma} e^{-(x-\mu)^2/(2\sigma^2)}$$

Table 1 | Summary of dry weather baseflow water quality and flowrates

	E. coli (mpn/100 ml)	*Cryptosporidium* (oocysts/ 10 litres)	*Campylobacter* spp. (mpn/ 100 ml)
Arithmetic mean	600	41	1.4[a] / 1.5[b]
Normal st. dev.	490	44	2.4[a] / 2.5[b]
Geometric mean	450	22	0.31[a] / 0.56[b]
# samples[1]	16	16	16
# BDL[1]	0	1	7
% detected[2]	100	94	56
Maximum	2,000	129	9.3
Minimum	86	<2.4	<0.3
Average Flow[3] (m^3/s)		0.167	
Max. Flow[3] (m^3/s)		0.497	
Min. Flow[3] (m^3/s)		0.000	

BDL values estimated using:
[a]modified log-regression model (El-Shaarawi 1989).
[b]'fill-in with expected values' technique (Gleit 1985).
[1]Count (unitless).
[2]Percent of samples that were above the detection limit.
[3]On days samples were taken.

exacerbated by rounding to the appropriate number of significant figures, perhaps suggesting the extra computational effort in employing the integration technique was unwarranted. No individual sample counts were below the detection limit for any of the three contaminants.

Concentrations of each contaminant fluctuated markedly over the duration of the events (Figure 2, Table 2) demonstrating the danger in relying on single grab samples to describe the overall water quality for the duration of the event. In most cases there is at least one order of magnitude difference between the minimum and maximum concentrations measured for the one event. The EMC accounted for these (and flow) fluctuations to summarise the data in a form suited to allow a more realistic estimate of the total load of contaminant that enters the reservoir during the event period.

Each of the events characterised were a result of rainfall bursts that had a noticeable impact on the stream hydrograph. A basic analysis of daily rainfall data measured within the catchment from 1990–2002 showed that rainfall events of similar magnitudes (within a 24-hour period) could be expected several times per year (Table 2). The range of events depicted has not allowed observation of the effect of 'large' rainfall events that may occur more infrequently, such as the 10, 20, or 50 years average recurrence interval rainfall event. For all three

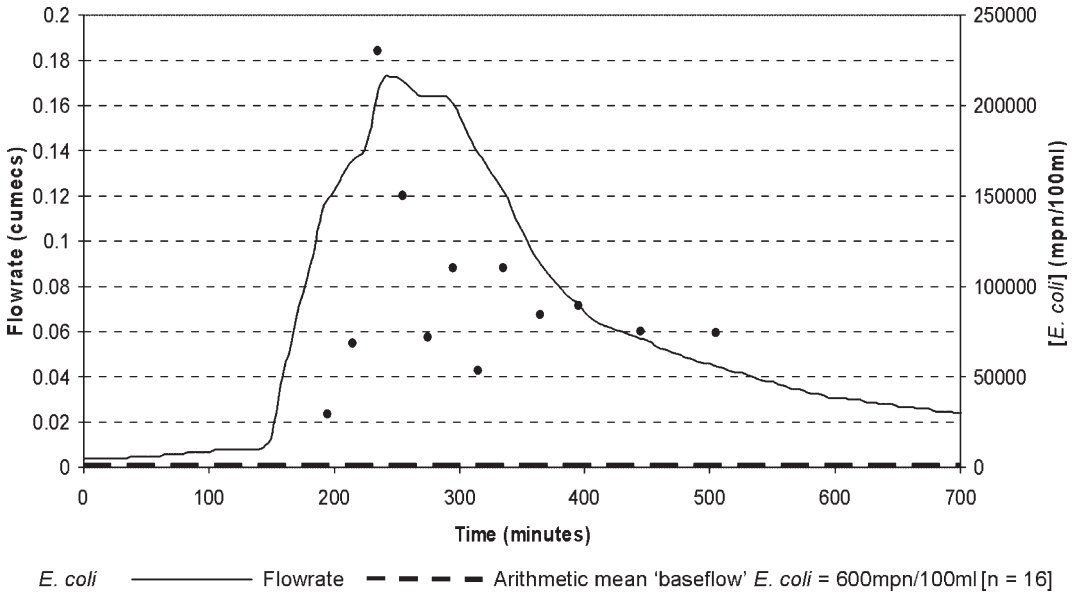

Figure 2 | A rainfall-induced runoff event hydrograph and fluctuating *E. coli* concentrations.

Table 2 | Event data and EMCs

	Event statistics			EMC [# samples taken during event]			Minimum[4]: Maximum[4]		
Event	24-hr Rainfall[1] [no. days p.a.][2] (mm)	Peak Flow[3] (m^3/s)	Sampling duration (hrs)	*E. coli* (mpn/100 mL)	*Cryptosporidium* (oocysts/10 L)	*Campylobacter* spp. (mpn/100 mL)	*E. coli* (mpn/100 mL)	*Cryptosporidium* (oocysts/10 L)	*Campylobacter* spp. (mpn/100 mL)
1	18.0 [16]	1.241	5.0	18,000 [7]	157 [7]	–	6,800: 39,000	44: 340	–
2	15.4 [20]	1.545	3.3	1,700 [12]	–	–	870: 3,100	–	–
3	27.0 [8]	1.752	4.8	14,000 [12]	742 [4]	0.35 [4]	1,800: 20,000	10: 1273	0.3: 1.1
4	17.8 [16]	1.171	5.2	3,700 [12]	140 [4]	26 [4]	1,300: 7,300	50: 400	1.5: 46
5	8.8 [39]	0.660	5.2	3,500 [12]	187 [4]	7.6 [3]	2,400: 6,200	48: 241	2.3: 46
6	10.0 [34]	0.173	5.8	10,000 [12]	134 [4]	60 [4]	5,300: 23,000	36: 210	1.6: 165
7	27.6 [8]	0.514	5.2	34,000 [12]	133 [5]	21 [4]	11,000: 130,000	16: 238	1.1: 110
Arithmetic mean				12,000	213	23			
Normal standard deviation				11,000	240	23			
Geometric mean				7,900	195	9.7			

[1]Total rainfall in 24 hrs prior to the last water quality sample being taken.
[2]Average number of days per year that have experienced 24-hour rainfall events of the same or greater magnitude than that of the corresponding recorded event. Based on daily rainfall data for the catchment from 1990–2002 collected by the Australian Bureau of Meteorology.
[3]During water quality sampling period.
[4]Minimum and maximum concentrations measured during the indicated event, for comparison with EMC.

contaminants, scatter plots (not shown) of the instantaneous concentrations against flowrates (considering each event separately) displayed a common trend in that no consistent functional relationships were evident. No pattern emerged from these events to support the hypothesis that either event peak contaminant concentrations or EMCs may be directly estimated by simply measuring and considering recent rainfall or current flowrates (this result is similar to the findings of an early study of the response of other microbiological contaminants in urban stormwater to rainfall events by Qureshi & Dukta 1979). However, increases in microbial contamination of surface waters during such events, of a significant magnitude, are likely (Table 2). The collection of more EMC data, emanating from a diverse range of hydrologic event magnitudes, is desirable to examine the possibilities that (i) there is some minimum rainfall or flow intensity that is required to mobilise non-point source pathogens, as is the case for other contaminants (Ball *et al.* 1998) and (ii) that there is a finite budget of non-point source pathogens, such that during 'large' events there is perhaps either a 'first-flush' effect or a dilution of the microbiological contamination in the surface water, neither of which were evident from this data.

The issue of what actually constituted an event posed problems due to the sporadic nature of rainfall bursts and the varied response of natural stream and river system catchments. Further complicating the issue is that a single rainfall-induced runoff event may be the result of several separate rainfall bursts, resulting in multiple peak flows in the runoff hydrograph. The sampling strategy adopted did not investigate the effect of sequential rainfall bursts on microbial water quality, and focussed on the initial rainfall burst period that yielded the first hydrograph peak and subsequent drop in flowrate.

Event v. baseflow microbial water quality

For all three constituents, a one-tailed t-test assuming unequal variances (Devore 2000:366) was employed to test the null hypothesis that the normal population means of the natural log-transformed EMC and baseflow data were equal, against the alternative hypothesis that the mean EMCs were greater than the mean baseflow concentrations of the transformed data[2]. In all three cases the expected mean EMCs were higher than the expected mean baseflow concentrations (Figure 3), and t-test results support the alternative hypothesis that they arose from different statistical populations ($p\text{-}value = 6.6 \times 10^{-5}$ for *E. coli*, $p\text{-}value = 4.3 \times 10^{-5}$ for *Cryptosporidium*, $p\text{-}value = 6.1 \times 10^{-3}$ for *Campylobacter* spp. when BDL data is analysed using El-Shaarawi's (1989) method, and $p\text{-}value = 0.012$ for *Campylobacter* spp. when BDL data is analysed using Gleit's (1985) method).

Variability in water quality

Maximum likelihood methods (Devore 2000:267) were used to estimate *E(X)* and *SD(X)* of each dry weather and EMC dataset (Table 3). Using these estimated parameters Kolmogorov-Smirnov (Conover 1999) 'goodness-of-fit' test results did not provide evidence to reject the hypothesis that the data came from a lognormal distribution for *Cryptosporidium* and *E. coli* (under both baseflow and event conditions), and *Campylobacter* spp. (EMCs only) [$\alpha > 0.2$]. Due to the occurrence of left-censored *Campylobacter* spp. baseflow concentrations, a different test was necessary that makes use of the Kaplan-Meier estimator (Hollander & Proschan, 1979). The hypothesis of lognormally distributed data was not rejected (5% significance level) regardless of which method was used to estimate the probability distribution parameters.

It is of interest to note the influence of the choice of methodology in dealing with the dry weather *Campylobacter* spp. BDL data. Gleit's (1985) method produces an estimate of *E(X)* broadly similar to that produced by El-Shaarawi's (1989) nonparametric approach (approximately 1.5 mpn/100 ml v. 2.1 mpn/100 ml). However the *SD(X)* estimates differ substantially – the nonparametric method yielded an estimate approximately four times greater. Where management or risk assessment decisions rely on these analyses, the nonparametric log-regression method proposed by El-Shaarawi (1989) appears to be preferable, having produced a probability distribution

[2]The assumption of lognormality has been described previously, and is verified in the following section.

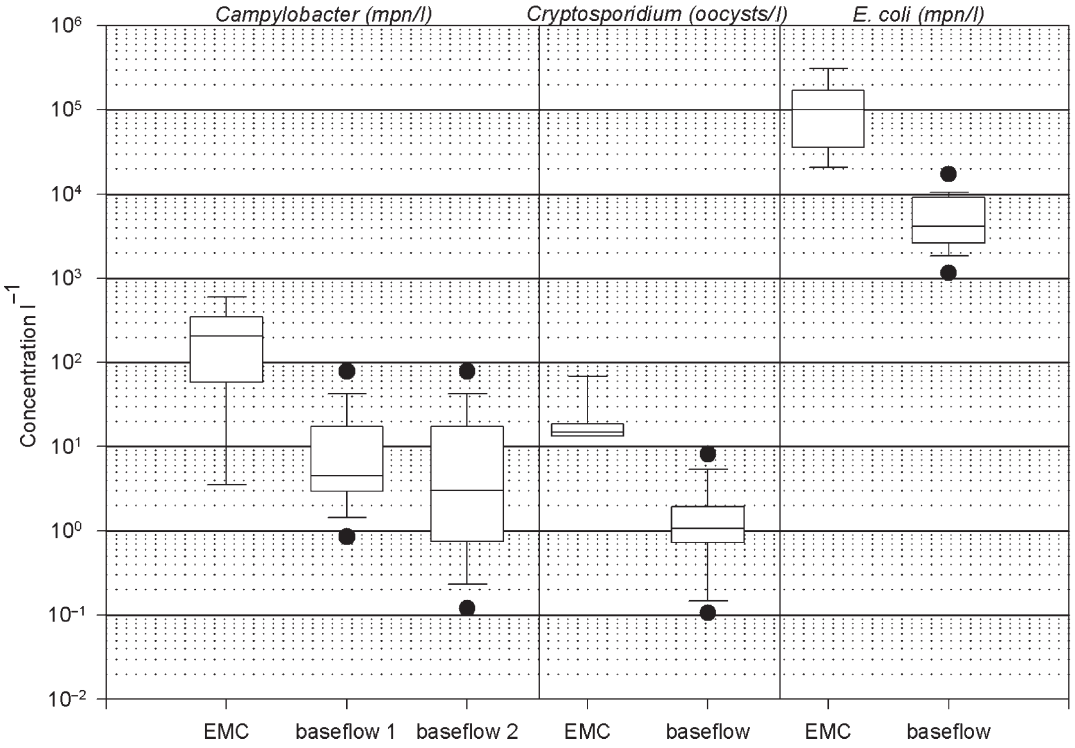

Figure 3 | Box plots showing differences in baseflow concentrations and EMCs. Circles are (bottom) 5th and (top) 95th percentiles for this data. NB: for Campylobacter spp., 'baseflow 1' and 'baseflow 2' uses modified log-regression (El-Shaarawi 1989) and the 'fill-in with expected values' technique (Gleit 1985) respectively to estimate the BDL values. All concentrations converted to concentrations per litre for comparative purposes.

Table 3 | Maximum likelihood estimates of mean (μ) and standard deviation (σ) of log-transformed data for EMC and baseflow data

	E. coli **(mpn/100 ml)**		***Cryptosporidium*** **(oocysts/10 litres)**		***Campylobacter*** **spp. (mpn/100 ml)**	
	Baseflow	**EMC**	**Baseflow**	**EMC**	**Baseflow**	**EMC**
μ	6.10	8.97	3.11	5.27	-1.16^{a} / -0.58^{b}	2.27
σ	0.80	0.98	1.28	0.61	1.96^{a} / 1.42^{b}	1.79
E(X)	610	13,000	51	234	2.1^{a} /1.5^{b}	48
SD(X)	580	16,000	104	157	15^{a} / 3.9^{b}	230

BDL values estimated using:
[a]modified log-regression model (El-Shaarawi 1989).
[b]'fill-in with expected values' technique (Gleit 1985).

function with more conservative values in the upper tail than Gleit's (1985) method. However, such logic could result in excessive rather than conservative estimates, especially given our small dataset, and a risk assessor must make an educated judgment on which *variability* analysis results are the more appropriate.

Uncertainty analysis

The maximum-likelihood based method (Burmaster & Thompson 1998) was used to derive second-order random variables from our datasets (Table 4). In each case the correlations between the estimated parameters $\{\mu_\mu, \sigma_\mu\}$ and $\{\mu_\sigma, \sigma_\sigma\}$ approached zero.

Table 4 | Parameter uncertainty analysis results using second-order random variables derived using maximum likelihood-based method described by Burmaster & Wilson (1996)

	Mean[1], $M \sim$	Standard Deviation[2], $\Sigma \sim$
Baseflow		
E. coli (mpn/100 ml)	*Normal* (6.10,0.20)	*Normal* (0.80,0.14)
Cryptosporidium (oocysts/10 litres)	*Normal* (3.11,0.32)	*Normal* (1.28,0.23)
Campylobacter[a] spp. (mpn/100 ml)	*Normal* (-1.16,0.49)	*Normal* (1.96,0.35)
Campylobacter[b] spp. (mpn/100 ml)	*Normal* (-0.58,0.36)	*Normal* (1.42,0.25)
EMC		
E. coli (mpn/100mL)	*Normal* (8.97,0.37)	*Normal* (0.98,0.26)
Cryptosporidium (oocysts/10 litres)	*Normal* (5.27,0.25)	*Normal* (0.61,0.18)
Campylobacter spp. (mpn/100 ml)	*Normal* (2.27,0.80)	*Normal* (1.79,0.57)

BDL values estimated using:
[a]modified log-regression model (El-Shaarawi 1989).
[b]'fill-in with expected values' technique (Gleit 1985).
[1]*where* $X \sim Lognormal(x;\mu,\sigma)$, $M \sim Normal(\mu;\mu_\mu,\sigma_\mu)$.
[2]*where* $X \sim Lognormal(x;\mu,\sigma)$, $\Sigma \sim Normal(\sigma;\mu_\sigma,\sigma_\sigma)$, $\Sigma > 0$.

Many other sources of *uncertainty* in the data and analysis were not acknowledged. The choice of probability distribution and parameter estimation method could also affect risk analysis applications, especially if decisions are based on the results in the tails of the distribution (Haas 1997; Mumpower & McClelland 2002), and as seen with the BDL *Campylobacter* spp. dry weather concentration variability analysis in the previous section. Possible errors in measurements of microbial concentrations, pathogen recovery fractions and flowrates, due to a combination of instrumental, methodological or human aspects, can also impact on the quality of data and results. The degree to which a single grab sample represents the water quality across a whole flow cross-section at any point in time is another uncertainty.

Exceedance probability (EP) charts and confidence intervals

EP plots (Figures 4 and 5) visually depict the data *variability* (based on the outcomes in Table 3) and *uncertainty* (based on the outcomes in Table 4) estimates.

The plotting positions of the data points in Figures 4 and 5 were estimated by ranking the data and using standard probability plot methods (Helsel & Cohn 1988). All data fell within or very near the 95% confidence bands, which were estimated using *Equation 7* with the 2.5th ($z_u = 1.96$) and 97.5th ($z_u = -1.96$) percentiles of *uncertainty*.

The statistical methods applied to generate the EPs and confidence bands do not exhaust the range of analyses that might be applied to describe the *variability* and *uncertainty* of our data. Other methods of *variability* analysis include nonparametric methods (Conover 1999) and parametric moment-based parameter estimation (Devore 2000:265), while other methods of *uncertainty* analysis include bootstrapping techniques (Efron & Tibshirani 1993) and Bayesian applications (Gelman *et al.* 1995).

SUMMARY AND CONCLUSION

Microbiological contaminant concentrations significantly increased and quite markedly fluctuated over the course of rainfall-induced runoff events within the study catchment.

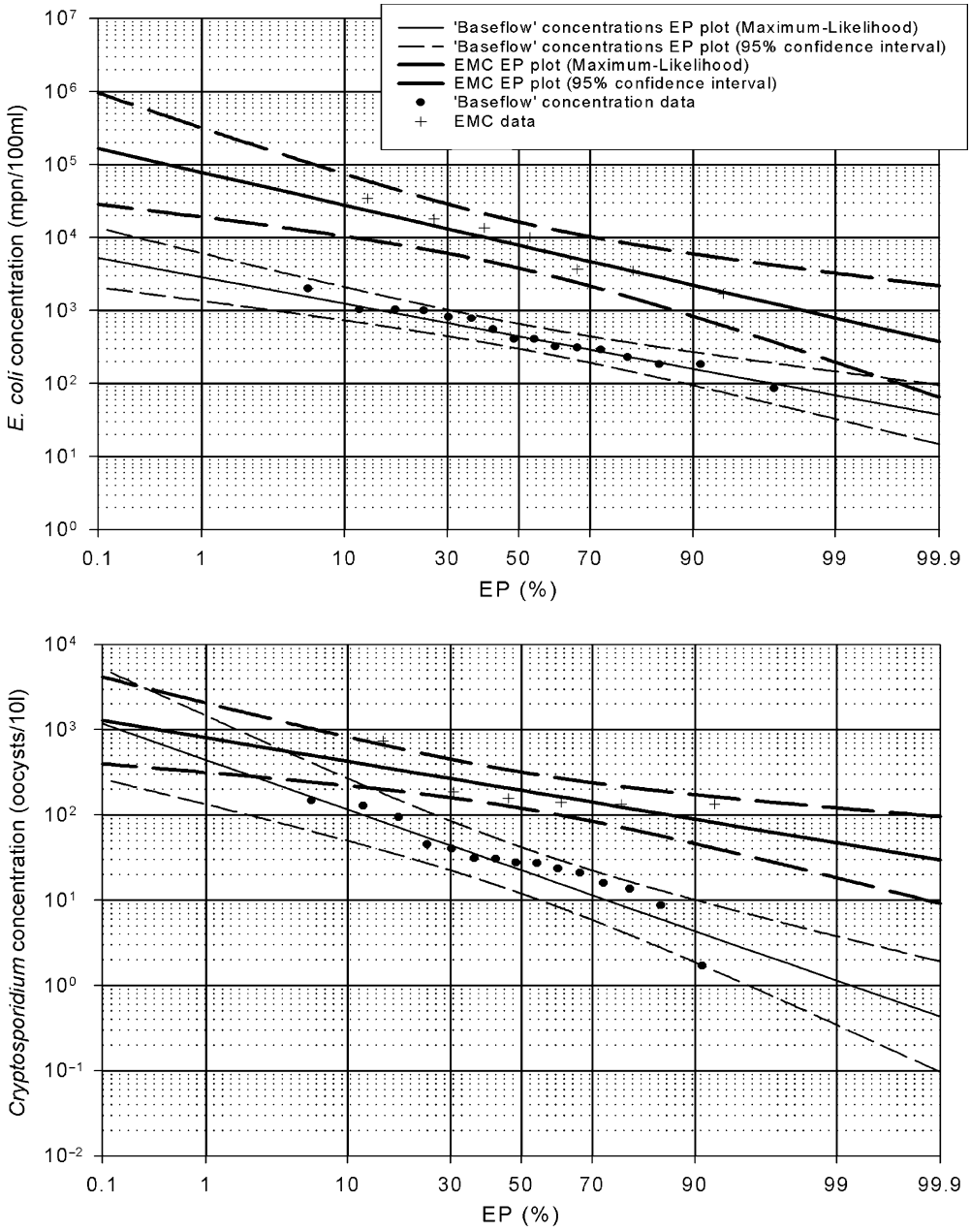

Figure 4 | EP plots for *E. coli* (top) and *Cryptosporidium*.

The practice then of relying on single samples taken during events to estimate water quality for the event duration is questionable. The Event Mean Concentration (EMC) has been investigated and successfully applied as a simple method accounting for the fluctuation of microbiological concentrations within an event to describe overall event water quality, and to act as a suitable summary parameter for comparison with baseflow microbiological water quality. From the data *variability* analysis the expected mean values of EMCs were significantly higher than the expected mean during baseflow conditions (Table 3) for the two pathogens (*Cryptosporidium* and *Campylobacter* spp.) and the faecal indicator (*E. coli*). The data analyses presented confirms that the microbial water quality of the stream feeding

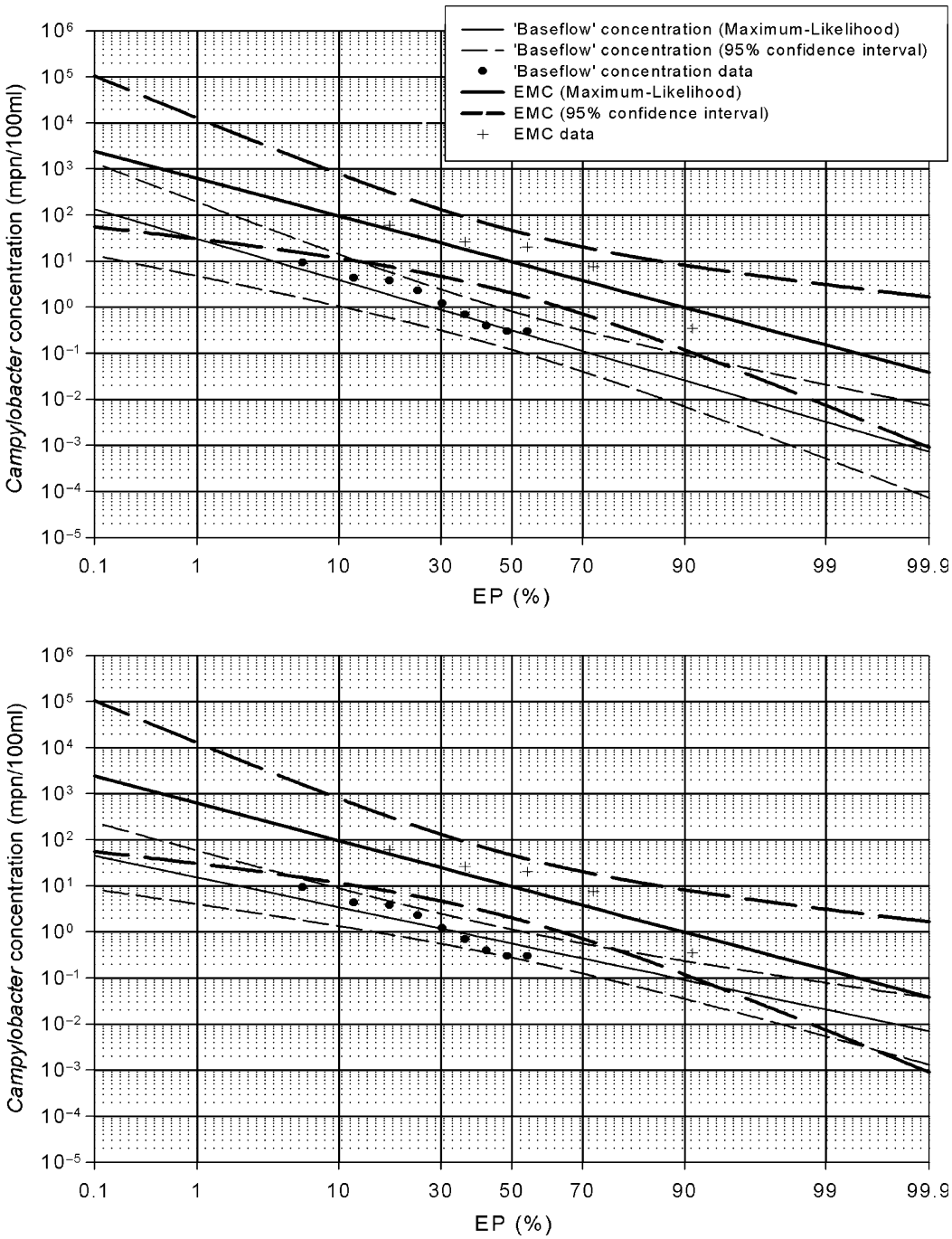

Figure 5 | EP plots for *Campylobacter* spp. demonstrating discrepancies in the distribution tails when 'baseflow' BDL data values are estimated by El–Shaarawi's (1989) 'Modified log-regression' model (top) and Gleit's (1985) 'Fill-in with expected values' method. NB: BDL data not plotted.

the drinking water reservoir deteriorates during rainfall-induced runoff event periods, supporting studies that have yielded similar results in other catchments (e.g. Atherholt *et al.* 1998; LeChevallier *et al.* 2002). Such a phenomenon will have microbial risk implications on drinking water systems that draw source waters either from river off-takes or from reservoirs fed by streams or rivers.

Describing the differences between EMCs and dry weather baseflow concentrations is a first step in describing the effect of runoff events on microbiological risk levels. In terms of risk analysis, this EMC approach may well be as informative as more detailed mechanistic and empirical models, given the complexity of modelling catchment systems and processes, and current knowledge gaps (Ferguson *et al.* 2003). The EMC has the added advantage of being a potential input for mass-balance modelling of surface water reservoirs receiving water of fluctuating quality.

Separately defining and describing data *variability* and *uncertainty* may enable the influence of both to be investigated in 'two-dimensional' QMRAs, which are becoming common in many microbial risk analysis applications (e.g. Pouillot *et al.* 2004). We have described *uncertainties* in our analysis by deploying a maximum likelihood-based approach that deals with only the uncertainties in statistical parameter estimations due to our small datasets, noting that this has special importance when dealing with microbiological datasets, and acknowledging that other methods may have been employed. The *uncertainty* involved when dealing with censored data was also investigated, specifically by adopting two different methods: a nonparametric modified log-probability regression model (El-Shaarawi 1989) and an iterative maximum likelihood-based 'fill-in with expected values' technique (Gleit 1985) to estimate the values of 'baseflow' *Campylobacter* spp. that were below the enumeration method detection limit (of 0.3 mpn/100 ml). While each method yielded similar mean values, the estimated standard deviations differed substantially, which will have implications in risk assessments where the rare occurrences (in the tails of the probability distribution functions) may heavily dictate overall risk estimates. Risk assessors must use judgment to determine how to best incorporate such knowledge uncertainties into their analyses.

Finally, the results of this study provide a platform for more detailed modelling of the effect rainfall-induced runoff has on microbiological contaminant concentrations in flowing water bodies in the study (and similar) catchment(s). Future studies aiming to build on these results may: (i) investigate methods to account for fluctuations in microbial concentrations for the duration of an event that has multiple peaks in the hydrograph, (ii) explore the relationships between pathogen and pollutant indicator EMCs that may aid in on-line water quality management, (iii) investigate ways to incorporate the effects of data measurement *uncertainty* into the presented analyses, (iv) investigate the existence of temporal or seasonal patterns in both EMC and baseflow concentration *variability* for a variety of catchment types, (v) collect and analyse more microbiological EMC data, and (vi) further explore and develop microbial fate and transport models. It is envisaged that a detailed catchment hydrology analysis, combined with our water quality analysis (or similar), will allow the stochastic estimation of the magnitude and frequency of microbiological loads that a water supply system may expect to be challenged with. Such information could aid decision-makers to set priorities in areas such as catchment management, hazardous event and risk management, and water treatment system design.

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