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Epidemiological Determination of Disease Risks from Bathing
Epidemiologische Erfassung des Erkrankungsrisikos beim Baden

von

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DAS WASSER FINDET SEINEN WEG

(K. Hoffmann)

Dedicated to Konrad Botzenhart,

Director of the Institute of General and Environmental Hygiene
of the University of Tübingen,
on the occasion of his 65th birthday,
with gratitude for his supreme scientific support,
his lasting personal engagement,
and the outstanding working conditions he has provided to us.

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1 Executive Summary

1.1 Aim:

Five epidemiological studies were performed following a randomised, controlled study design in order to evaluate the risk of contracting infectious diseases from bathing in fresh water.

1.2 Materials and Methods:

Study locations were situated in the north, east, southwest and southeast of Germany: four sites on lakes and one site on a river. All sites had complied with current EU regulations for at least the three previous years. The sources of faecal contamination included raw and treated municipal sewage, agricultural and municipal run-off after rainfall and contamination by water fowl.

A total of 2196 participants were recruited from the local population. Two to three days before exposure the participants were interviewed in person, and all participants underwent a short medical examination. On the exposure day participants had to register at the beach, where they were randomised into equal-sized groups of bathers and non-bathers. They received a lunch package and were interviewed a second time. Non-bathers stayed within roped-off areas on the beach but away from water. Bathers were exposed under individual supervision in four roped-off areas each divided into a swimmers' and a non-swimmers' zone. They were instructed to stay in the water for exactly ten minutes and to immerse their head at least three times. After exposure they were asked whether they had swallowed water.

For the entire time the participants were being exposed water samples were collected every twenty minutes from the centres of the swimmers' and non-swimmers' zones in all four areas. These samples were analysed in a nearby mobile laboratory for six microbiological parameters: *Escherichia coli* (EC), intestinal enterococci (IE), *Clostridium perfringens* (CP), somatic coliphages (SOMCP), aeromonads (AE) and *Pseudomonas aeruginosa* (PA).

One week after exposure all the participants were interviewed again in person and underwent a medical examination. Individual exposure concentrations for all microbiological parameters were assigned to each bather by minute-wise interpolation between the monitoring results and by calculation of the arithmetic mean concentration of the ten individual exposure minutes. Threshold-of-effect concentrations (NOAEL's) were defined as the exposure concentrations which revealed the most significant difference between the incidence rates among bathers below and above these concentrations in a Pearson's Chi Square test, provided that the incidence rates of bathers below the threshold concentration were not significantly lower than the incidence rates of non-bathers, and provided that no expected cell value was less than five. Potential threshold concentrations were tested against a variety of possible confounding variables including age, gender, study location, weather conditions, previous diseases, diseases in household members, consumption of prescription drugs, nutritional factors, travel history, socio-economic status, risk perception and additional water contact, using a multiple logistic regression procedure (Effect Likelihood Ratio Test).

1.3 Results:

The follow-up rate was 91.9%. Statistically significant and unbiased dose-response effects ($p<0.05$) could be demonstrated for three different definitions of gastroenteritis (GE) and all four faecal indicator parameters (EC, IE, CP and SOMCP). Skin ailments were partially associated with the concentration of aeromonads. No other significant and unbiased association could be observed between any of the six microbiological parameters and any of the other evaluated disease definitions (acute febrile respiratory infections, common cold, ear infections, eye infections, urinary tract infections).

The relative GE risks of bathers above threshold concentrations of EC or IE compared to non-bathers ranged between 1.9 [95% CI: 1.3; 2.8] and 3.6 [95% CI: 1.8; 7.0], depending on the definition of GE. Higher relative risks were detected with the more stringent definitions of GE (e.g. GE = vomiting or diarrhoea with three or more

bowel movements per day), which indicates that the majority of bathing-associated cases of GE were not only mild forms. Bathers who reported having swallowed water were at a higher risk of acquiring GE, particularly when they were exposed to above-threshold concentrations.

Taking into consideration the results from all combinations of indicator organisms and disease definitions, as well as the results from classifying participants into concentration classes defined by quartiles and quintiles and by equal arithmetic concentration ranges, the following threshold concentrations for increased risk of GE from 10 minutes of bathing and three or more head immersions are proposed: 100 EC/100ml, 25 IE/100ml, 10 CP/100ml and 10 SOMCP/100ml.

The excess risks of GE among bathers above threshold concentrations in this cohort never exceeded 5%, even though exposure concentrations reached maximum levels of up to 4600 EC/100ml and up to 1200 IE/100ml. The concept of deriving standards by limiting the maximum acceptable excess risk to 5%, as suggested in the current draft version for the revision of the European bathing water directive (Commission 2002) and in WHO publications (Havelaar et al., 2001; WHO 2003) is thus shown to be inappropriate. If this concept were applied, it would be impossible to derive standards for fresh water in Germany or other parts of the world, because in practice the maximum detectable excess risk rate never exceeded 5%.

1.4 Policy Implications:

(1) Introduce regulations to make the monitoring of *Escherichia coli* and intestinal enterococci in European bathing waters mandatory at two-week intervals by application of methods ISO 9308-3 and ISO 7899-1 for three years.

(2) Introduce a mandatory European external quality assessment scheme for these methods.

(3) Introduce an area-integrated sampling technique according to ISO 5667-4 to provide better estimates of the mean water quality on the sampling day.

(4) Collect all raw monitoring data (concentrations and sampling dates) from all member states for three years.

(5) Introduce a risk-related and time-integrated interpretation system for the monitoring results based on the estimation of the percentage of indicator concentrations exceeding the threshold-of-effect levels (NOAEL's) determined in the European randomised controlled trials in sea and fresh water.

(6) Introduce a risk-related classification system for bathing waters based on rates of compliance with the thresholds of effect (e.g "Time-integrated Quality Scores") rather than on disease burden estimates, since disease burden estimates are more variable and depend on the susceptibility of cohorts (ca. 20% excess risk in the UK studies, vs. <5% in the German studies and in studies in other parts of the world).

(7) Set guidelines and imperative values based on a political decision on the desirable and feasible levels of health protection.

2 Zusammenfassung und Empfehlungen

2.1 Ziel:

Um Infektionsrisiken beim Baden in natürlichen Binnengewässern zu erfassen, wurde eine epidemiologische Untersuchung mit fünf Teilprojekten nach dem Prinzip einer randomisierten kontrollierten Studie durchgeführt.

2.2 Material und Methoden:

Die fünf Studienorte lagen im Norden, Osten, Südwesten und Südosten Deutschlands. Vier Badestellen lagen an Seen, eine an einem Fluss. Alle Badestellen hatten mindestens in den drei Jahren vor Durchführung der Studie die Anforderungen der EU-Badegewässerrichtlinie erfüllt. Quellen fäkaler Verunreinigungen waren geklärte und ungeklärte kommunale Abwässer, Abschwemmungen aus Siedlungsgebieten und landwirtschaftlichen Nutzflächen oder Einträge durch Wasservögel.

Insgesamt 2196 Studienteilnehmer wurden aus der örtlichen Bevölkerung angeworben. Zwei bis drei Tage vor der Exposition wurden persönliche Interviews durchgeführt, und alle Teilnehmer wurden einer kurzen ärztlichen Untersuchung unterzogen. Am Expositionstag wurden alle Teilnehmer am Strand registriert und nach dem Zufallsprinzip in jeweils gleich große Gruppen von Badenden und Nicht-Badenden eingeteilt. Alle erhielten ein einheitliches Lunch-Paket und wurden ein zweites Mal interviewt. Die Nicht-Badenden hielten sich in abgegrenzten Strandarealen auf, wo sie nicht mit dem Wasser in Berührung kamen. Die Badenden wurden unter individueller Beobachtung in vier abgegrenzten Wasserarealen exponiert. Jedes der Areale war zusätzlich in einen Schwimmer- und einen Nicht-Schwimmerbereich unterteilt. Die Badenden wurden angewiesen, genau zehn Minuten im Wasser zu bleiben und dabei mindestens dreimal den Kopf unter Wasser zu tauchen. Nach Ablauf der Expositionszeit wurden sie gefragt, ob sie beim Baden Wasser geschluckt hatten.

Parallel zu den Badeaktivitäten wurden in der Mitte der Schwimmer- und Nicht-Schwimmerbereiche der vier Badeareale alle 20 Minuten Wasserproben gezogen, die in

einem nahe gelegenen mobilen Laboratorium auf insgesamt sechs verschiedene mikrobiologische Parameter hin untersucht wurden: *Escherichia coli* (EC), intestinale Enterokokken (IE, syn.: Darmenterokokken), *Clostridium perfringens* (CP), somatische Coliphagen (SOMCP), Aeromonaden (AE) and *Pseudomonas aeruginosa* (PA).

Eine Woche nach Exposition wurden alle Teilnehmer erneut persönlich interviewt und anschließend einer kurzen ärztlichen Untersuchung unterzogen. Jedem Badenden wurde seine individuelle Expositionskonzentration für alle sechs mikrobiologischen Parameter zugeordnet, indem zwischen den einzelnen Messwerten minutenweise interpoliert und das arithmetische Mittel der Konzentrationen in den zehn individuellen Expositionsminuten berechnet wurde. Als Schwellenkonzentration für ein erhöhtes Erkrankungsrisiko (engl.: threshold of effect, NOAEL) wurden diejenigen Konzentrationen gewertet, die im Pearson's Chi Quadrat Test den signifikantesten Unterschied zwischen der Inzidenzrate der Badenden unterhalb und oberhalb der jeweiligen Konzentration ergaben, vorausgesetzt die Inzidenzrate unterhalb des Schwellenwerts war nicht signifikant niedriger als die Inzidenzrate der Nicht-Badenden und vorausgesetzt, dass sich im Vier-Felder-Test in keinem der Felder ein Erwartungswert von kleiner fünf ergab. Potentielle Schwellenwerte wurden mit Hilfe eines Verfahrens der multiplen logistischen Regression (Effect Likelihood Ratio Test) gegen eine Vielzahl möglicher Störfaktoren getestet, darunter Alter und Geschlecht, Studienort, Witterungsbedingungen, vorausgegangene Erkrankungen, Erkrankungen von Haushaltsmitgliedern, Medikamenteneinnahme, Ernährungsfaktoren, Reiseanamnese, sozio-ökonomische Bedingungen, Risikowahrnehmung und zusätzlicher Wasserkontakt.

2.3 Ergebnisse:

Die follow-up Rate betrug 91.9%. Statistisch signifikante und nicht durch Störfaktoren verfälschte Dosis-Wirkungs-Effekte ($p<0.05$) ergaben sich für alle drei getesteten Definitionen der Gastroenteritis (GE) und alle vier Fäkalindikator-Parameter (EC, IE, CP und SOMCP). Hautbeschwerden waren partiell mit der Konzentration von Aeromonaden assoziiert. Weitere signifikante und nicht durch Störfaktoren verfälschte

Assoziationen zwischen den mikrobiologischen Parametern und anderen untersuchten Krankheitsbildern (akute fieberhafte Atemwegsinfekte, Erkältungskrankheiten, Ohrinfektionen, Augeninfektionen, Harnwegsinfekte) konnten in dieser Studie nicht festgestellt werden.

Das relative GE-Risiko von Badenden, die oberhalb der berechneten Schwellenwerte für EC bzw. IE exponiert waren, betrug im Vergleich zu den Nicht-Badenden in Abhängigkeit von der GE-Definition zwischen 1.9 [95% KI: 1.3; 2.8] und 3.6 [95% KI: 1.8; 7.0]. Die höheren relativen Risiken ergaben sich für die stringenteren GE-Definitionen (z. B. GE = Erbrechen oder Durchfall mit mindestens drei Stuhlgängen pro Tag). Dies deutet darauf hin, dass die Mehrzahl der badebedingten Gastroenteritiden nicht nur milde Verlaufsformen waren. Badende, die angaben, Wasser geschluckt zu haben, hatten ein höheres GE-Risiko als Badende, die angaben, kein Wasser geschluckt zu haben. Dieser Effekt zeigte sich besonders deutlich bei Badenden, die oberhalb der Schwellenwerte exponiert waren.

Unter Berücksichtigung der Ergebnisse aller Kombinationen der unterschiedlichen GE-Definitionen und der vier Fäkalindikator-Parameter, und unter Berücksichtigung der Ergebnisse der Einteilung der Badenden entsprechend ihrer Expositionskonzentration in Quartilen, Quintilen und in gleich große Konzentrationsbereiche können folgende Schwellenwerte für ein erhöhtes GE-Risiko bei zehnminütigem Baden mit mindestens dreimaligem Kopf-Untertauchen angenommen werden: 100 EC/100ml, 25 IE/100ml, 10 CP/100ml and 10 SOMCP/100ml.

Das attributierbare GE-Risiko (Zusatzrisiko) der Badenden oberhalb der Schwellenwerte überstieg in der untersuchten Kohorte nie die 5%-Marke, obwohl z. T. hohe Expositionskonzentrationen von bis zu 4600 EC/100ml und bis zu 1200 IE/100ml aufgetreten waren. Das im derzeitigen Entwurf der Novelle der EU-Badegewässerrichtlinie (Commission 2002) und in WHO-Veröffentlichungen (Havelaar et al. 2001; WHO 2003) zur Ableitung des Grenzwerts für IE angewandte Konzept, den Grenzwert so festzulegen, dass das maximal tolerierte Zusatzrisiko beim Baden auf 5% beschränkt wird, ist daher unzulänglich. Bei Anwendung dieses Konzepts könnten für deutsche Binnengewässer und für andere Regionen der Welt, in denen bei

epidemiologischen Untersuchungen die maximalen Zusatzrisiken ebenfalls unterhalb der 5%-Marke blieben, überhaupt keine Standards erlassen werden.

2.4 Handlungsempfehlungen:

(1) Einführung einer Untersuchungspflicht für die beiden Parameter *Escherichia coli* und intestinale Enterokokken (Darmenterokokken) in europäischen Badegewässern in 14-tägigen Abständen unter einheitlicher Anwendung der Methoden ISO 9308-3 und ISO 7899-1 für einen Zeitraum von drei Jahren.

(2) Einführung eines für alle Prüflaboratorien obligatorischen europäischen externen Qualitätskontrollsystems für diese Methoden.

(3) Einführung einer flächenintegrierten Probenahme-Technik nach ISO 5667-4 um präzisere Schätzwerte für die mittlere Wasserqualität einer Badestelle am Tag der Probenahme zu erhalten.

(4) Einführung einer Meldepflicht an die EU für die Rohdaten (Messwerte incl. Probenahmedatum).

(5) Einführung eines risiko-orientierten Interpretationssystems für die Ergebnisse des Untersuchungsprogramms mit zeitlicher Gewichtung der Einzelergebnisse ("Time-integrated Quality Score"). Dieses System sollte auf der Schätzung des Anteils mikrobieller Konzentrationen beruhen, die an einer Badestelle über den Schwellenwerten (NOAEL's) liegen, die in den beiden europäischen randomisierten und kontrollierten Studien ermittelt wurden.

(6) Einführung eines risiko-orientierten Klassifizierungssystems für Badestellen. Dieses System sollte eher auf dem Ausmaß der Überschreitung der epidemiologisch begründbaren Schwellenwerte basieren und weniger auf der Abschätzung der badebedingten Erkrankungszahlen, da diese sehr viel variabler sind und von der Immunität der untersuchten Kohorte abhängen (ca. 20% Zusatzrisiko in den britischen Studien, <5% in den deutschen Studien und in anderen Teilen der Welt).

(7) Entscheidung über Grenz- und Richtwerte, sobald eine politische Entscheidung darüber vorliegt, welches Ausmaß an Gesundheitsschutz an den Badestellen wünschenswert und zu verwirklichen ist.

3 Introduction

3.1 Aim of the study

In order to evaluate the infectious disease risks from bathing in fresh water, five epidemiological studies were carried out following the same "randomised controlled study" design which had already been applied at Northern European coastal bathing sites according to WHO recommendations (Kay et al., 1994).

4 Materials and Methods

4.1 Study sites and potential sources of contamination

Two of the five study sites were located in the southwest, one in the southeast, one in the east and one in the north of Germany. All study sites had been officially registered and monitored according to European law for many years and had complied with European standards for at least the three previous bathing seasons (Annex 1). Three lakes were primarily ground water lakes with little or no surface water input, one of them located in a rural environment with adjacent conservation areas and sparse natural populations of water fowl, and the remaining two in an urban environment with moderate to high overpopulation with ducks, geese and gulls. One site was located on Germany's biggest natural inland lake with the Rhine River running through and hundreds of smaller rivers and streams running into it. These carry discharge from tertiary treated sewage as well as from sporadic sewage treatment plant and combined sewer overflows and from agricultural run-off. The lake could also be influenced by illegal discharge from boats and by faecal deposits from urban and natural populations of water fowl with varying densities. One bathing site was located at the shore of a blind arm of a stream running through a major town with sporadic contamination from combined sewer overflows and urban run-off after heavy rain falls and with permanent but spatially scattered contamination, mainly from gulls, ducks and swans.

4.2 Weather conditions

Weather conditions which may have contributed to elevated concentrations of faecal indicators during the studies did in fact occur shortly before two of the studies. The weather conditions during the trials were rainy and cool in one case, cool and windy in two other cases, and warm and sunny in the remaining ones.

4.3 Recruitment of volunteers and inclusion and exclusion criteria

With support from the local media (Annex 37, Annex 38), study volunteers were recruited from the local population in the three weeks before each trial (Annex 39, Annex 41). For the pilot trial only volunteers above 18 years of age were accepted for participation. After additional ethical clearance with the ethics commission of the University of Tübingen the lower age limit was set to 4 years for the remaining four trials. Children below the age of fourteen had to be accompanied by one of their parents. Teenagers between 14 and 18 were accepted with the written, signed consent of their parents (Annex 40). Children below the age of four were excluded because they were considered to be a potential source of faecal accidents during the trials which might have biased the results. No upper age limit was set, as bathing was considered to be a recreational activity with no such generally accepted limit. Two or three days before the trials the volunteers were invited for a structured interview on their personal details and their health conditions which was carried out by specially instructed interviewers. The questionnaires (Annex 42) were subsequently checked by professional physicians. After an additional inspection of the volunteers' throat, ears and eyes and electronic temperature measurement in the ear the physicians decided whether the volunteers appeared to be physically fit for participation in the study trials. Reasons for exclusion were serious acute infectious diseases or fever or health conditions where bathing might have presented a serious or life-threatening risk.

4.4 Environmental exposure

Two or three days after the first interview, the participants came to the beach at a designated time and registered at an information desk, where they were randomised into equally sized groups of bathers and non-bathers using a block-randomisation procedure with blocks of ten. Children who were accompanied by their parents were asked whether they would like to stay with their father or their mother or be on their own before their parents received their randomisation result. Each participant received a standardised lunch package consisting of two fresh rolls, sterilised cheese, canned sausage, a banana, a package of wheat biscuits and a bottle of mineral water suitable for baby food preparation, i .e. with guaranteed heterotrophic plate counts of less than 5/ml. Five percent but no more than 20 of the lunch packages per trial were microbiologically analysed and monitored for food pathogens in a state food safety laboratory. The bathers were assigned to one of four roped-off bathing areas of approximately 10m width and 20m length which were distributed across the beach and each divided into a non-swimmers' and a swimmers' zone. The non-bathers were directed to a roped-off area of the beach with lawn or sand grounds where they could not come into contact with the water (Annex 2). When the participants arrived at their destination they were interviewed again by one of the project helpers. In this second interview (Annex 43) the questions focused on symptoms having occurred after the first interview and on nutritional details of the past two or three days. Participants who reported any new symptoms were sent to one of the physicians on site, who decided whether these symptoms were a reason for exclusion or not. After the interview the participants were free to have their lunch and non-bathers were free to stay in the non-bathers' area for normal beach activities like sun-bathing or playing, depending on the weather conditions. Party-style facilities (tables, benches and tents) were available on the beach both in the bathing and the non-bathing areas to provide an opportunity for eating and to protect participants and interviewers from sunshine or rain if required. Non-bathers were not urged to put on bathing clothes if the weather conditions were inappropriate.

Participants who had been randomised for bathing entered the water under the individual supervision of their interviewers. Bathing duration was limited to exactly 10 minutes, and participants were asked to stay inside the roped-off areas and to stay or swim around balloons which had been placed like buoys in the centre of the areas. They were also instructed to completely immerse their heads at least three times during the 10 minutes. The supervisors individually recorded the start time and the end of the bathing period and the number of head immersions performed in each of the ten minutes. After leaving the water the participants were asked whether they had swallowed water (http://www.badegewaesserstudie.de).

4.5 Monitoring of the microbiological water quality

For the duration of the trials water samples were collected from the centre of the swimmers' and non-swimmers' zones of all of the four bathing areas at twenty-minute intervals. They were delivered to a nearby mobile lab within 30 minutes in thermo-insulated boxes with cooling elements. After arrival at the lab the samples were immediately analysed by a team of professional technicians or stored in a refrigerator for no longer than eight hours before the beginning of the analyses. All samples were analysed for the following microbiological parameters according to international or national standards or national recommendations (methods and specifications or modifications in brackets): *Escherichia coli* (ISO 9308-3, MUG hydrolysis, microtiter plate method, 3 plates per sample, 192 wells with a 1:2 dilution, 96 wells with a 1:10 dilution), intestinal enterococci (ISO 7899-1, MUD hydrolysis and formazan formation, 3 plates per sample, 288 wells with a 1:2 dilution), *Clostridium perfringens* (Council of the European Union, 1998: membrane filtration, 100 and 10 ml on m-CP agar), somatic coliphages (ISO 10705-2, 10 x 1 ml, sample pre-filtration with beef-extract-saturated 0.45 µm membrane filters, double agar layer method), aeromonads (Schulze 1996, membrane filtration, 100 and 10 ml on ampicillin-dextrine-agar) and *Pseudomonas aeruginosa* (ISO 12780, membrane filtration, 10 and 1 ml on cetrimide agar; only pyocyanine-positive strains were counted). The values of the method-specific lower

detection limits were assigned to samples with analytical results below the detection limit. Upper detection limits were not reached in any of the samples.

In addition to the parameters, which were monitored every 20 minutes, grab samples were collected once per study location in each of the four exposure areas. These samples were analysed for F-specific coliphages (10 x 1 ml Double Agar Layer Method according to amended ISO 10705-1, February 1997, host strain *Salmonella typhimurium* WG49), *Bacteroides fragilis* phages (10 x 1ml, Double Agar Layer Method according to ISO/CD 10705-4, January 1999, host strain *Bacteroides fragilis* RYC2056), enterovirus (10 litre grab sample, AlCl3 flocculation, glass-wool filtration, elution with skim-milk buffer, precipitation at pH 4.5, resolution in Na2HPO4 at pH 7.5, membrane filtration 0.2µm, BGM-Flow monolayer plaque assay), EHEC (membrane filtration of 100 ml, incubation on Endo-Agar, PCR detection of the stx1 and stx2 genes; performed by Landesuntersuchungsamt für das Gesundheitswesen Südbayern), and for *Cryptosporidium* oocysts and *Giardia* cysts (20 litre grab sample, compressed foam filtration, immunomagnetic separation, DAPI staining, direct immunofluorescent assay with FITC-MAB).

4.6 Quality control for the microbiological procedures

Quality control procedures included positive and negative media controls for target organisms. For *E. coli* and intestinal enterococci quantitative reference materials were also applied which had been evaluated in earlier international round robin trials funded by European Union research projects ("reference lenticules K", donated by Institute Pasteur de Lille, European Community Contract SMT4-CT95-1603; DG12-RSMT). In addition the organisers' main lab provided many years of professional experience in microbiological water analysis (routine and research) with continued participation in national and international external quality assessment schemes (Niedersächsisches Landesgesundheitsamt, Außenstelle Aurich, Germany, for E. coli and total coliforms; PHLS, UK, external quality assessment schemes for

Cryptosporidium, *Giardia* and viruses in water). ΦX174 and MS-2 coliphages and B56-1 *Bacteroides fragilis* phages were used as internal quality controls.

4.7 Retrospective molecular analysis of frozen samples

Aliquots of all water samples were frozen at -20°C on the sampling day. Glycerol was used as a preservative. These samples were retrospectively analysed for *Escherichia coli* and intestinal enterococci by quantitative real time PCR (LightCycler™-PCR). Details of the methods are described in Annex 46.

4.8 Follow-up procedures

One week after the trials all participants were invited again for a third interview (Annex 44) and a subsequent inspection of the throat, eyes and ears. Interviewers and doctors were unaware of the exposure status of the participants, and had been instructed not to ask the participants about their exposure status. Participants who reported symptoms of gastroenteritis were asked to collect a stool sample as soon as possible and to send it to the Baden-Württemberg State Health Office in Stuttgart, where the samples were examined for the following parameters: *Salmonella*, *Shigella*, *Yersinia enterocolitica*, *Campylobacter*, EHEC, rotavirus, adenovirus, enteroviruses, norwalkvirus, *Cryptosporidium parvum* and *Giardia lamblia*. 32 participants made use of this opportunity.

Three weeks after the trials the participants received a last questionnaire by mail (Annex 45), which they had to fill in at home and send back to the organisers of the study. After receipt of this fourth questionnaire, each participant received a compensation of 50 DM (25 Euro) to cover personal expenses.

4.9 Data entry and verification

Questionnaire data were entered into an electronic data base using electronic questionnaires created in Epi Info Version 6.2 (CDC, USA and WHO, Switzerland). All

data were entered a second time by a different person and compared during entry by application of the data verification module supplied with the Epi Info software. If data were inconsistent a menu popped up showing both entries, providing an opportunity to decide which of the choices was the correct one. For further statistical analyses the complete data set was exported and subsequently imported into JMP version 5.0 Statistical Discovery Software ™ (SAS Institute Inc., USA). All statistical analyses were performed in JMP ™ with the exception of the calculation of confidence limits for relative risks, which were calculated in STATCALC (Epi Info) and some of the Pearson's Chi Square Tests, which were calculated in MS Excel 97 ™.

4.10 Calculation of individual exposure concentrations

For each minute of trial duration microbial concentrations in the water were calculated by arithmetic interpolation between the results obtained by analysing the water samples. This was done for all bathing areas and in both the swimmers' and the non-swimmers' zones (Annex 11). In addition, the arithmetic means between the concentrations in the swimmers' and in the non-swimmers' zones were calculated (Annex 12). First values were carried back and last values were carried forward for ten minutes. This procedure was also applied for missing sample results, which occasionally occurred due to rapid growth of contaminating bacterial or fungal flora on the membrane filters, especially on cetrimide agar or in one case due to oversight of a sample for E. coli and enterococci analysis. The microbial concentrations of each of the ten minutes of water contact were individually assigned to each of the bathers. The values from the non-swimmers' or the swimmers' zones were used, depending on where the participant was staying. If during any given minute a participant switched between the non-swimmers' and the the swimmers' areas, the arithmetic mean between both concentrations was assigned for that minute (Annex 13). Individuals for whom one or more of the ten minute-specific concentrations were not available, e.g. because they had entered the water too early or too late or due to the lack of analytical results, were excluded from further analysis (Annex 14). This procedure was performed separately for each of the six microbiological parameters.

4.11 Exposure definitions

Exposure was defined in two different ways. The first definition was "10 minutes bathing with at least three head immersions", which is equivalent to the instructions that the participants had received from the study organisers in the written participation agreement and from their supervisors before entering the water. For this definition the arithmetic mean of the ten concentrations which had been calculated and assigned as described above was used as the mean exposure concentration of every individual bather. The second definition takes into account the fact that the participants followed the instructions to immerse their heads at least three times to a very varying extent (Annex 16). As each immersion of the head might be looked upon as an equivalent for the uptake of a certain small amount of water via the eyes and the lacrimal duct, the nose and throat, and the mouth, the minute-specific concentrations of all minutes during which the head was immersed were multiplied with the number of head immersions during each of these minutes and added up. The result can be considered to be equivalent to a single head immersion at that concentration. This definition of exposure is therefore called "single head immersion" (Annex 15).

4.12 Disease definitions

To evaluate health effects the following diseases were defined by Boolean combination of symptom variables from the questionnaires (Boolean operators in capital letters; terms separated by commas belong to the same variable):

Abbreviation	Definition
AFRI	Acute Febrile Respiratory Infections:
	(fever AND sore throat, sphagitis)
	OR (fever AND pain in the chest)
	OR (fever AND dry cough)

OR (fever AND productive cough)
OR (fever AND breathing difficulties)
OR (fever AND runny nose, coryza)

CC Common Cold:

sore throat, sphagitis
OR dry cough
OR productive cough
OR runny nose, coryza

EAR Ear inflammation:

inflammation, suppuration, pain in the ears

EYE Eye inflammation:

inflammation, suppuration of the eyes, red painful eyes

GE_UK-wf Gastroenteritis, UK definition according to Kay et al. 1994, without consideration of stool frequency:

diarrhoea
OR vomiting
OR (nausea AND fever)
OR (indigestion AND fever)

GE_UK Gastroenteritis, UK definition according to Kay et al., 1994:

(diarrhoea AND 3 or more bowel movements per day)
OR vomiting

OR (nausea AND fever)
OR (indigestion AND fever)

GE_NL-2 Gastroenteritis, Netherlands-2 definition according to van Asperen et al., 1998:

diarrhoea
OR vomiting
OR nausea
OR stomach pains

SKIN Skin infections, cutireactions:

exanthema, skin eruption
OR skin irritation, itching

UTI Urinary Tract Infections:

inflammation of the urinary bladder, urinary tract

4.13 Evaluation interval

Similar research work performed at British sea water beaches (Kay et al., 1994) has been heavily criticised because it only evaluated data collected after a three-week interval (Mugglestone et al., 2000). There were two main points of criticism. First, the incubation periods for the diseases under evaluation are hardly longer than one week. Second, by extending the evaluation time to three weeks there is an increased risk for introduction of bias from other sources of infection and from compliance problems due to additional bathing. We therefore initially compared the crude incidence rates of disease among bathers and non-bathers after the one-week interval with the incidence rates after the three-week interval. After it became obvious from that comparison that

there was in fact no additional scientific benefit from using the three-week interval, all subsequent analyses concentrated on the results obtained after one week. Nevertheless, some evaluations of the data obtained after the three-week interval were performed for the purpose of direct comparison with the results from the UK studies.

4.14 Formation of disease-specific and parameter-specific cohorts

Of the 2196 volunteers who participated in the 1st interview, 139 (6.3%) were excluded because of incomplete follow-up, 39 (1.8%) were excluded because of medical reasons, and 37 (1.7%) were excluded because of unacceptable exposure data. Exposure data were considered unacceptable if participants did not comply with their randomisation status, if they entered the water too early or too late, or if the total exposure time was less or more than 10 minutes. From the final cohort consisting of 1981 participants, disease-specific cohorts were formed by additional exclusion of participants who reported in the exposure-day interview (2nd interview) any symptom that was part of the disease definition under evaluation or any symptom considered to be a possible precursor of the disease under evaluation. Such symptoms were: fever, sore throat, runny nose, dry cough, productive cough, pain in the chest or breathing difficulties for the AFRI-cohort and for the CC-cohort; ear inflammation (inflammation, suppuration, pain in the ears) for the EAR-cohort; eye inflammation (inflammation, suppuration of the eyes, red painful eyes) for the EYE-cohort; fever, loss of appetite, nausea, vomiting, stomach pains or cramps, indigestion, loose bowel motions or diarrhoea for the GE-cohort; exanthema, skin eruption, skin irritation, itching, skin ulcer for the SKIN-cohort and fever or inflammation of the urinary bladder or urinary tract for the UTI-cohort. Participants were also excluded if no clear decision could be made on whether the disease definition was fulfilled or not fulfilled. This could happen if participants were not sure whether one or more of the essential symptoms used in the disease definition had occurred, or if the information in the questionnaires was incomplete.

Additional reductions of the disease-specific cohort sizes were necessary for the group of bathers when statistical calculations were based on concentrations of specific microorganisms, and information on these concentrations was partially missing because laboratory results were not available for certain parameters in certain water samples. These cohorts were called parameter-specific cohorts.

4.15 Calculation of crude relative risks

Crude relative risks were calculated for each of the nine disease definitions in the disease-specific cohorts. If a crude relative risk was < 1, no further analyses were performed for this disease definition. If a crude relative risk was > 1, further analyses were performed to determine threshold concentrations as explained below, irrespective of whether the crude relative risk was significantly > 1 or not. This was done because an existing significant effect within the group of bathers can be masked by a subgroup with no elevated risk due to low microbial exposure (exposure below a threshold).

4.16 Estimation of threshold concentrations and bathers' excess risks by application of a simplified dose-response model ("step-model") using a "minimal p procedure".

The model assumptions which were the basis of the statistical procedures used to estimate microbial threshold concentrations for increased risk within the group of bathers and to estimate bathers' excess risks compared to non-bathers were derived from the dose-response model explained in WHO's "Guidelines for safe recreational water environments" (WHO, 1998; 2003). These assumptions were:

(1) that a baseline risk for the bathers exists which is not significantly lower than the non-bathers' risk (bathing is not protective for any of the specified diseases).
(2) that there is a maximum risk level (ceiling) which is less than 100%, as a certain portion of the exposed population can be expected either to be immune or to become infected without developing any symptoms ("silent infections").

(3) that there is a concentration range within which the risk increases from baseline level towards the ceiling.

(4) that above and below this concentration range there is no trend or correlation between concentration and risk.

Based on these model assumptions (especially point 4) it was concluded that the application of statistical procedures to analyse the data for overall trends or overall correlations over the whole range of concentrations may be inappropriate and misleading. It was also recognised that if only few data (few cases) are available in the concentration range where the risk increases (point 3), it may be impossible to derive a perfect sigmoid-shaped function or even the exact concentration range and the slope of the risk increase between baseline and ceiling. To avoid over-interpreting the data as well as losing information due to overly complex model assumptions, the dose-response model used for data analysis was therefore further reduced to a simple step function, which can be described by only three parameters: the baseline risk, a threshold concentration and the maximum risk. In this "step model" the disease incidence in the group of non-bathers represents an estimate for the baseline, the threshold is an estimate of the microbial exposure concentration above which there is increased risk from bathing, and the disease incidence in the bathers exposed above the threshold concentration is an estimate for the ceiling of the dose response curve (maximum risk). To find the most probable value for the threshold concentration in the group of bathers, the participants were sorted by their individual exposure concentration in ascending order. For every actually occurring concentration a Pearson's Chi-square Test was performed, comparing the incidence rate of a disease in the group of bathers exposed at or below that concentration with the incidence rate in the group of bathers exposed above that concentration. For each of these Chi-square tests the p value (probability of a Chi-square value at least as large as the value observed under the null-hypothesis of no threshold effect) was calculated. The concentration with the minimal p value below 0.05 and a test result which was not suspect due to expected cell values of less than five, was considered to be the most reasonable estimate of the potential threshold. If all Pearson's p values below 0.05 were suspect, Fisher's exact test was performed for the

concentration with the minimal Pearson's p, and the result was documented but not analysed any further. A threshold concentration was considered to be potentially valid if three additional conditions were fulfilled: 1st, the incidence rate in the group of bathers exposed at or below this concentration was lower than the incidence rate above that concentration (exclusion of paradox effects); 2nd, the incidence rate in the group of bathers exposed at or below that concentration was not significantly lower ($p<0.05$) than the incidence rate in the group of non-bathers (exclusion of pseudo-effects due to accidental imbalance of cases within the group of bathers), and 3rd, the incidence rate in the group of bathers exposed above that concentration was significantly higher than in the group of non-bathers ($p<0.05$). If the incidence rate in the group of bathers exposed at or below the threshold concentration was significantly higher than in the group of non-bathers, it was concluded that the microbial indicator concentration could explain (or indicate) the effect only partially. In this case the proposed dose-response model was modified from a one-step model to a two-step model with the non-bathers' incidence rate as the baseline risk, the incidence rate below the threshold as bathing-associated baseline risk attributable to other independent risk factors, and the incidence rate above the threshold as indicator-associated risk.

Thresholds which were potentially valid were further analysed as described below to control for possible confounding effects from other risk factors or from protective factors. Thresholds were finally accepted if there was no statistical evidence that they were confounded by any other variable (Annex 19).

4.17 Control for bias

To control for bias a variety of potential confounding factors (variables) were analysed. Initially all variables were univariately screened for possible effects in the total final cohort of bathers and non-bathers (Pearson's Chi square tests). Variables showing significant univariate effects ($p<0.05$) were considered to be potential confounders of the threshold concentrations (Annex 22), and were further analysed using a multivariate nominal logistic regression procedure (effect likelihood ratio tests).

If microbiological threshold concentrations remained significant effects ($p<0.05$) in a model consisting of the disease as response variable and the threshold and the potential confounder as model effects, thresholds were considered to be unbiased, and the potential confounder was considered to be an independent predictor of disease. In addition all models were analysed for possible interaction effects between threshold values and potential confounding variables by crossing both effects in separate effect likelihood ratio tests. Significant interaction effects ($p<0.05$) were recorded but the thresholds were not rejected (Annex 23).

Factors considered to be potential confounders for all disease definitions were:

Ambient conditions:

study location (Kirchentellinsfurt, Uhldingen-Mühlhofen, Berlin, Lübeck, München), weather conditions (cool or warm)

Interviewer effect:

number of the interviewer in the 3rd interview

Age: age grouped by 10-year intervals, age grouped by 10-year intervals and 60+

Gender: male or female

Socio-economic status:

degree (none, primary school, non-classical secondary school, secondary school, university), occupation (unemployed, child or pupil, student, homemaker, practicing a profession)

Household:

number of household members (1, 2, 3, 4, 5, >5), number of children < 5 years of age living in household (none, 1, >1), children < 5 years living in household (yes, no), other household members participating in the study (yes, no)

Chronic symptoms or diseases:

any severe illness in 6 months before 1st interview (yes, no), stay in hospital in the 6 months before 1st interview (yes, no)

Medicines:

consumption of prescription drugs in the 4 weeks before 1st interview (yes, no), antibiotics (yes, no), steroids (yes, no)

Consumption of alcohol or tobacco:

consumption of alcohol (yes, no), amount of alcohol consumed in the week before 1st interview (0, 1-5, 6-10, >10 units (1 unit = 0.5 l beer, 0.25 l wine, 0.02 l spirits)), was this the normal amount of alcohol (yes, no), smoking (yes, no), number of cigarettes per day (0, 1-5, 6-20, >20)

Risk factors related to additional water contact:

additional swimming or bathing in the week after the trial day (yes, no), normal frequency of bathing in natural recreational waters (fresh or sea water) per month during summer (never; sometimes: 1-3; often: >3), frequency of bathing in 4 weeks before 1st interview (0, 1-5, >5), maximal swimming distance (-50, -500, -5000 m), maximal swimming duration (10 min, 20 min, 30 min, 1 h, 2 h), normal frequency of going to a beach without entering the water per month (never, sometimes: 1-3, often: >3), number of visits to a beach without entering the water in 4 weeks before 1st interview (0, 1-5, >5), normal frequency of using a public pool per month (never, sometimes: 1-3, often: >3), utilisation of private pools (yes, no), visit to a leisure park with water activities in 4 weeks before 1st interview (yes, no), normal frequency of each of the following water sports activities per month during summer: canoeing, motor-boating, paddling, surfing, diving, fishing (never, sometimes: 1-3, often: >3)

Leisure activities:

normal frequency of pub visits per month (never, sometimes: 1-3, often: >3), normal frequency of party or disco visits per month (never, sometimes: 1-3, often: >3), normal frequency of sports activities per month (never, sometimes: 1-3, often: >3)

Travel history:

overnight stays outside household in the 4 weeks before 1st interview (yes, no), travel abroad in the 4 weeks before 1st interview (yes, no)

Information about the study and about environmental subjects:

initial source of information about this study (partner, recruitment team, television, newspaper, other), having seen or heard news about this study (yes, no), regular reading of a newspaper (yes, no), member of an environmental organisation (yes, no), informed about the monitoring of beaches in Germany (yes, no), quality of that information (positive or negative), worried about that information (no, a bit, very much), information on the cleanliness of recreational waters in Germany (yes, no), quality of that information (positive or negative)

Risk perception:

are water-related activities considered to be dangerous (yes, no), ever gone to a beach while feeling ill (yes, no), did feeling ill prevent from entering the water (yes, no), ever refused to go bathing because beach was too dirty (yes, no), ever refused to go bathing because water was too dirty (yes, no), ever refused to go bathing because waves were too rough (yes, no), ever refused to go bathing because of fear of becoming ill (yes, no)

Factors considered to be potential confounders only for specific disease definitions were:

Disease definition CC:

Household:

household member with one of the following symptoms or diseases in the 2 weeks before 1st interview: common cold, sore throat, lung infection, fever (yes, no)

Acute symptoms or ailments:

one or more of the following symptoms or ailments in the three weeks before 1st interview: fever, headache, runny nose, ear infection, dry cough, productive cough, sore throat, pain in the chest, breathing problems, pain in the joints, loss of appetite (yes, no)

Chronic symptoms or diseases:

ear ailments, chest ailments, hay fever, diabetes (yes, no)

Leisure activities:

normal frequency of cinema visits per month (never, sometimes: 1-3, often: >3), normal frequency of other activities involving contact to large groups of people (never, sometimes: 1-3, often: >3)

Risk factors related to additional water ingestion during the trial:

exposed and swallowed water during trial (no, teaspoon, tablespoon, cup), exposed and swallowed water during trial (yes, no), swallowed water during trial (unexposed or exposed and no, teaspoon, tablespoon, cup), swallowed water during trial (unexposed or exposed and no, exposed and yes)

Disease definition EAR:

Household:

household member with one of the following symptoms or diseases in the 2 weeks before 1st interview: ear infection (yes, no)

Acute symptoms or ailments:

one or more of the following symptoms or ailments in the three weeks before 1st interview, in the two or three days between the 1st and the 2nd interview and in the week after exposure: fever, headache, runny nose, dry cough, productive cough, sore throat, pain in the chest, breathing difficulties (yes, no)

Chronic symptoms or diseases:

Ear ailments (yes, no)

Disease definition EYE:

Acute symptoms or ailments:

one or more of the following symptoms or ailments in the three weeks before 1st interview: eye infection or inflammation (yes, no), vision disorders (yes, no)

Chronic symptoms or diseases:

chronic eye inflammation (yes, no), chronic hay fever (yes, no)

Disease definition GE:

Household:

household member with one of the following symptoms or diseases in the 2 weeks before 1st interview: common cold, fever, nausea, vomiting, diarrhoea (yes, no)

Acute symptoms or ailments:

one or more of the following symptoms or ailments in the three weeks before 1st interview: fever, loss of appetite, nausea, vomiting, loose bowel motions, diarrhoea, stomach pains, any of these symptoms (yes, no)

Chronic symptoms or diseases:

chronic stomach, gut, and liver ailments, diabetes (yes, no), normal frequency of diarrhoea (never, hardly ever: 1-2 per year, often: 1-2 per month), proneness to motion sickness (always, often, hardly ever, never)

Medicines:

consumption of the following prescription drugs in the 4 weeks before 1st interview: laxatives (yes, no), stomach remedies (yes, no)

Dietary factors:

consumption of the following foods in the two or three days before exposure (2nd interview) and in the week after exposure (3rd interview): ice cream, bought sandwiches, chicken, chicken meat, eggs, scrambled eggs, omelettes, tiramisu, home-made mayonnaise, hot dogs or grilled sausages, hamburgers, salads, raw milk, raw cheese, raw meat, sausages, fast food, sea food (yes, no), participation in a barbecue party in the week after exposure (yes, no)

Risk factors related to additional water ingestion during the trial:

exposed and swallowed water during trial (no, teaspoon, tablespoon, cup), exposed and swallowed water during trial (yes, no), swallowed water during trial (unexposed or exposed and no, teaspoon, tablespoon, cup), swallowed water during trial (unexposed or exposed and no, exposed and yes)

Disease definition SKIN:

Household:

household member with one of the following symptoms or diseases in the 2 weeks before 1st interview: fever, skin ailments (yes, no)

Acute symptoms or ailments:

one or more of the following symptoms or ailments in the three weeks before 1st interview: fever, exanthema or skin eruption, itching, tingling, skin ulcer (yes, no), fever in two or three days before 2nd interview (yes, no)

Chronic symptoms or diseases:

chronic skin, renal, neural and liver ailments, diabetes, hay fever (yes, no), normal frequency of diarrhoea (never, hardly ever: 1-2 per year, often: 1-2 per month), proneness to motion sickness (yes, no)

Risk perception:

frequency of sunburns after beach visits (always, often, hardly ever, never), are sunburns treated (always, often, hardly ever, never)

4.18 Incidence rates in quartile and quintile categories and in arithmetic concentration classes

To provide additional evidence for existing dose-response relationships between microbial concentrations and disease risk, the microbiological parameters for which unbiased threshold values could be established were analysed by classifying the data into quartiles and quintiles and into arithmetic concentration classes with equal concentration ranges. The disease incidences within the various classes were compared to the disease incidence in the unexposed group of non-bathers, and significant differences (Pearson's Chi-square test, $p<0.05$) were recorded.

For direct comparison between the results of previous studies performed in sea water with the results obtained in fresh water, gastroenteritis data were also classified using the quantile boundaries of the sea water studies published by Kay et al., 1994.

4.19 Combined effect of exposure concentrations and swallowing water

For the outcome variable "gastroenteritis" the term "dose response", which is often used to describe the relationship between concentrations of microorganisms in the water and disease risk, is misleading for at least two reasons. First of all, the microbiological parameters measured in the water samples are indicator organisms, not the causative agents themselves, and the pathogen-indicator ratio is variable. Second, a concentration of organisms is not really a dose if the outcome (ill or not ill) depends on the number of incorporated organisms. A dose could only be calculated by multiplication of a specific exposure concentration with the volume of water which was incorporated. With the applied study design this volume cannot be quantified exactly. However, one can assume that it depends on both the number of head immersions and the amount of water swallowed during exposure. The effect of the number of head immersions was assessed by including this number in the exposure definition as explained before. The effect of swallowing water was assessed by stratifying the exposure classes defined by the thresholds (below threshold or above threshold) into additional classes of participants who reported to have swallowed water and participants who reported not to have swallowed water (Annex 17). The resulting four classes (exposure below threshold and no water swallowed, exposure below threshold and water swallowed, exposure above threshold and no water swallowed, exposure above threshold and water swallowed) were considered to be the best possible estimate for the relative amount of incorporated microbial agents capable of causing gastroenteritis.

5 Results

5.1 Follow-up rate, randomisation result, and study population characteristics

Starting off with a total of 2196 participants in the 1st interview and ending up after exclusions and drop-out with 2018 participants who returned the 4th questionnaire, the follow-up rate was 91.9%. After exclusion of an additional 37 participants whose

exposure data were unacceptable or incomplete the final cohort size was 1981. The 2067 volunteers who participated in the 2nd interview were randomised into groups of 1033 bathers (50.0%) and 1034 non-bathers (50.0%), which demonstrates the success of the randomisation procedure. After disease specific exclusions the disease-specific cohort sizes one week and three weeks after exposure were (disease definition in brackets): 1569 and 1549 (AFRI), 1558 and 1534 (CC), 1953 and 1943 (EAR), 1943 and 1935 (EYE), 1757 and 1720 (GE_UK), 1768 and 1738 (GE_UK-wf), 1767 and 1731 (GE_NL-2), 1837 and 1816 (SKIN) 1946 and 1938 (UTI). For the parameters aeromonads (AE), *E. coli* (EC), intestinal enterococci (IE) and somatic coliphages (SOMCP) the parameter-specific cohorts were smaller than the disease-specific cohorts by approximately 10 participants; for the parameter *Pseudomonas aeruginosa* (PA) the cohort size was smaller by approximately 100 participants; no reduction was necessary for the parameter *Clostridium perfringens* (CP) because there were no missing laboratory data for this parameter. Tables with exact figures are in the annex (Annex 3, Annex 4, Annex 5).

The age distribution among bathers and non-bathers in the final cohort was almost identical. Minimum, 25th percentile, median, 75th percentile and maximum in the group of bathers were 4, 14, 23, 39 and 79 years vs. 4, 15, 25, 39 and 89 years in the group of non-bathers. The ratio of male to female participants in the group of bathers was 489/473 (50.8%/49.2%), and 474/545 (46.5%/53.5%) in the group of non-bathers. 33 participants (1.7%) were pre-school children, 661 (33.4%) were schoolchildren, 235 (11.9%) were students, 711 (35.9%) were practising a profession, 151 (7.6%) were homemakers or retired, 104 (5.2%) were unemployed, and 86 (4.3%) did not disclose their employment status. 140 (7.1%) of the 1981 participants were members of an environmental organisation, 1812 (91.5%) were not, and 29 (1.5%) did not answer the question.

5.2 Microbial concentrations in the water samples

The total number of available sample results per parameter from all five study locations was 421 for EC, 421 for IE, 423 for CP, 420 for SOMCP, 420 for AE and 385 for PA. The median concentrations and the concentration ranges were 136 EC /100ml [4.7;5344], 37 IE /100ml [3.0; 1504], 1.8 CP /10ml [0.9, 26], 20 SOMCP /100ml [10, 3780], 8200 AE /100ml [600; 31400] and 1 PA /10ml [1; 10]. With the exception of AE the lower range limit is equivalent to the lower detection limit. Median concentrations and concentration ranges at each of the five study sites are in the annex (Annex 7, Annex 10).

All faecal indicators correlated fairly well with each other, with correlation coefficients of the log10-transformed concentrations between 0.79 and 0.41 (orthogonal regression, univariate variances, after censoring values below detection limits), while all coefficients for correlations with aeromonads or *Pseudomonas aeruginosa* were between 0.31 and 0.07. The closest associations were between *E. coli* and intestinal enterococci, and between *E. coli* and somatic coliphages. Correlation coefficients for all 15 combinations of parameters in descending order were: 0.79 (EC-IE), 0.78 (EC-SOMCP), 0.57 (EC-CP), 0.51 (IE-CP), 0.50 (CP-SOMCP), 0.41 (IE-SOMCP), 0.31 (IE-PA), 0.29 (EC-PA), 0.28 (SOMCP-PA), 0.27 (EC-AE), 0.24 (SOMCP-AE), 0.23 (CP-AE), 0.17 (CP-PA), 0.11 (IE-AE) and 0.07 (AE-PA) (Annex 8).

The relationship between EC and IE which was found in this study is similar to the. relationship between faecal coliform and faecal streptococcal counts in United Kingdom bathing waters as published in Figure 4.7 of the WHO publication known as the "Farnham Protocol" (Bathing Water Quality and Human Health: Faecal Pollution. Outcome of an Expert Consultation, Farnham, UK, 2001). A reproduction of this figure overlaid with the EC-IE-relationship found in this study is in the annex. However, the formula of the linear regression function and the example calculations which are provided in the WHO publication together with Figure 4.7 are probably erroneous. They do not correspond to the graph and the example results are not plausible from an empirical point of view. It is therefore not surprising that the linear regression function revealed in this study differs from the function published by WHO:

UK (WHO, 2001): log faecal coliform count = 1.028 + 0.601 * log faecal strep. count
D (this study): log EC count = 0.728 + 0.940 * log IE count

In an orthogonal regression analysis revealing a variance ratio of 1.431 for EC/IE the regression function after censoring values below the detection limit was:

D (this study): log EC count = 0.323 + 1.196 * log IE count (Annex 9)

Enteroviruses in 10 litres could not be detected at any of the five study sites. *Bacteroides fragilis* phages in 10 ml could not be detected at four sites. At one site, the method did not work due to problems with a clumping agar batch. F-specific coliphages, *Cryptosporidium* oocyts and *Giardia* cysts could not be detected at four study sites. Only at one study site were F-specific coliphages detected at a concentration of 1 plaque forming particle/10ml in one of the four exposure areas. At this site *Cryptosporidium* oocyts could be detected in two of the four areas at a concentration of 1 oocyst/20 litres, and *Giardia* cysts could be detected in all four areas, three times at a concentration of 1 cyst/20 litres and once at a concentration of 3 cysts/20 litres. At this site the minimum concentration of EC was 146/100ml, the minimum concentration of IE was 110/100ml. EHEC (PCR detection of stx1 or stx2) could not be detected at any of the five sites.

The results of the retrospective analyses of frozen sample aliquots by quantitative real time PCR for *Escherichia coli* and intestinal enterococci were disapppointing. While nearly perfect standard curves could be achieved for given concentrations of target DNA, the quantitative results of the sample analyses did not correlate with the culture results. Moreover, the samples from certain locations seemed to contain inhibitory substances which could not be effectively removed by the applied DNA extraction procedure, and obviously caused large numbers of false negative results. Therefore, the PCR-based quantitative molecular detection methods which were evaluated in this project cannot be considered to be a promising alternative to culture

methods. The molecular analyses may have also been hampered by the storage conditions and by the lower sample volumes which were applied in the PCRs (effective volumes: 1.8 ml for *Escherichia coli*; 9 ml for intestinal enterococci). Details of the results are described in Annex 46.

5.3 External and internal quality control

The results obtained from the quantitative external quality control tests for EC and IE using 'lenticules K' were well within the range of results that could be calculated in a round robin trial performed by nine European expert labs in a EU research project under the supervision of the Institute Pasteur de Lille, France. The means and the standard deviations of the log10 transformed results obtained during the study trials were 2.76 ±0.08 EC/100ml and 2.47±0.09 IE/100ml (n=38) compared to 2.77±0.18 EC/100ml and 2.49±0.30 IE/100ml (n=43) obtained in the round robin trial (Annex 6). Internal quality control procedures for culture media and incubators did not reveal any results which would have justified doubts about the analytical quality of the sample results.

5.4 Compliance with current EU standards

According to the official report of the EU all five bathing sites complied with the standards of the European bathing water guideline of 1976 at the end of the season in which the studies were performed (Annex 1). This evaluation is based on the test results of the official monitoring programs, which were performed independently by the responsible local authorities throughout the bathing seasons, and excludes all test results obtained during the study trials.

In 95.2% (401/421) of the samples collected during the five trial days EC concentrations were below the imperative value of 2000/100ml. Thus, in the time periods during which the trials were performed the total water quality of all five study locations would have just passed the current European standard (95% of sample results below 2000/100ml). If the study sites are considered separately, only one site would

have failed to comply with the current standard during the trial with a compliance rate of only 80.0% (64/80) of the samples. The compliance rates of the other four sites in ascending order were 97.7% (85/87), 97.7% (86/88), 100% (78/78) and 100% (88/88).

5.5 Crude incidence rates, bathers' excess rates and relative risks

The crude incidence rates of bathers vs. non-bathers one week after exposure were AFRI: 0.9 % vs. 1.1 % (p=0.7098); CC: 15.4 % vs. 13.6 % (p=0.3051); EAR: 1.6 % vs. 0.7 % (p=0.0652); EYE: 1.7 % vs. 1.6 % (p=0.8717); GE_UK: 3.3 % vs. 1.4 % (p=0.0074); GE_UK-wf: 5.4 % vs. 2.8 % (p=0.0056); GE_NL-2: 7.4 % vs. 5.2 % (p=0.0531); SKIN: 9.3 % vs. 2.8 % (p=0.0000); UTI: 0.2 % vs. 0.4 % (p Pearson's Chi square test = suspect, p Fisher's exact test =0.8764). The crude relative risks with lower and upper 95% confidence limits were AFRI: 0.83 [0.31; 2.22]; CC: 1.13 [0.89; 1.45]; EAR: 2.26 [0.93; 5.52]; EYE: 1.06 [0.53; 2.10]; GE_UK: 2.37 [1.23; 4.54]; GE_UK-wf: 1.92 [1.20; 3.08]; GE_NL-2: 1.43 [0.99; 2.06]; SKIN: 3.30 [2.16; 5.05]; UTI: 0.53 [0.10; 2.89].

The comparison of crude incidence rates, bathers' excess risks and relative risks calculated after the one-week interval and after the three-week interval revealed that the evaluation of the data after the three-week interval yielded no advantage. All significant differences between the crude incidence rates of bathers vs. non-bathers after one week were less significant or no more significant after three weeks, and none of the differences which were insignificant after one week became significant or nearly significant after three weeks. The corresponding significant relative risks, especially those for gastroenteritis, declined and the excess risks stayed more or less the same, indicating that the majority of symptoms which were attributable to bathing occurred within incubation periods of less than one week. It was therefore concluded that data evaluation after an observation period of three weeks would only increase the risk of bias, e. g. due to additional exposure or underreporting, as symptoms which are not very severe are less likely to be remembered for a time period of three weeks than for a period of one week. Further data evaluation therefore focused on the results obtained

after one week. Detailed data and figures of the comparison between results obtained after one week and after three weeks may be found in the annex (Annex 18).

5.6 Threshold concentrations and specific relative risks

Disease definitions revealing crude relative risks >1 (bathers vs. non-bathers) were further analysed according to the procedures described in the materials and methods section to determine threshold concentrations for increased risk and upper limits of the disease incidence rates above threshold concentrations ("ceilings" of dose-response relationships). Threshold concentrations fulfilling the validity criteria (incidence rate above threshold significantly higher than incidence rate below threshold within the group of bathers; no suspect p in Pearson's Chi square test; incidence rate among bathers above threshold significantly higher than incidence rate among non-bathers; incidence rate among bathers below threshold not significantly lower than incidence rate among non-bathers; threshold unbiased by any of the potential confounding variables) could be established with exposure definition 1 ("10 min bathing; ≥ 3 head immersions") for all three definitions of gastroenteritis (GE_UK, GE_UK-wf and GE_NL-2) and all faecal indicators (EC, IE, CP and SOMCP), as well as for skin ailments (SKIN) and aeromonads. With exposure definition 2 ("1 head immersion") threshold concentrations fulfilling the validity criteria could be established for all three definitions of gastroenteritis (GE_UK, GE_UK-wf and GE_NL-2) and all faecal indicators (EC, IE, CP and SOMCP) with the exception of GE_UK-wf and EC. The differences between the incidence rates of gastroenteritis among bathers exposed up to threshold concentrations and the incidence rates of bathers above threshold concentrations were highly significant, with minimal p values (Pearson's Chi square test) between 0.0005 and 0.022. Due to the larger number of non-bathers than of bathers up to threshold concentrations, the differences between the incidence rates of non-bathers and the incidence rates of bathers above threshold concentrations were even more significant, with p values ranging from <0.0001 to 0.0055. The potential threshold concentration for exposure definition 2, GE_UK-wf and EC determined by the "minimal p method" was not considered to be valid because the incidence rate of

bathers below the threshold was significantly lower than the non-bathers' rate ($p<0.05$). Threshold concentrations for acute febrile respiratory infections (AFRI), common cold (CC), ear infections (EAR), eye infections (EYE) and urinary tract infections (UTI) were either non-existent or non-detectable with the available cohort size or within the range of microbial concentrations encountered during this study, or they were unacceptable because they did not fulfil the validity criteria.

The bathers' excess risk of gastroenteritis could entirely be attributed to the concentration of any of the four faecal indicator organisms as there was no significant difference between the incidence rates of bathers below threshold concentrations and that of non-bathers (p values ranging from 0.12 to 1.0). Hence, the data derived from this study do not provide any evidence for the existence of a "water-related risk of gastroenteritis" which is independent of the extent of faecal contamination of the water. A one-step model consisting of the incidence rate of non-bathers, the threshold concentration and the incidence rate of bathers above the threshold concentration can therefore be considered to be an acceptable simplification of the true dose-response relationship. In contrast, the incidence rate for skin ailments among bathers below the threshold concentration of aeromonads was significantly higher than the incidence rate of non-bathers ($p=0.028$). It can therefore be concluded that the concentration of aeromonads can only predict part of the water-related skin ailments, and the dose-response relationship has to be described by a two-step model consisting of the incidence rate of non-bathers, the incidence rate of bathers exposed up to threshold concentration, and the incidence rate of bathers exposed above threshold concentration.

The incidence rates of gastroenteritis in the non-bathers group ("base-line risks") corresponded well with the three different definitions of gastroenteritis, the most stringent definition (GE_UK) revealing the lowest rate of 1.4% (95% CI: 0.8; 2.4), the less stringent definitions (GE_UK-wf and GE_NL-2) revealing higher rates of 2.8% (95% CI: 1.9; 4.1) and 5.2% (95% CI: 3.9; 6.9). Threshold concentrations depended mainly on the microbial parameters and on the definition of exposure, and were astonishingly constant for all three definitions of gastroenteritis, especially for the

parameters IE and CP. For exposure definition 1 ("10 min bathing; ≥ 3 head immersions") the determined threshold concentrations were 180, 78 and 167 EC/100ml (for GE_UK, GE_UK-wf, and GE-NL-2 respectively); 24, 21 and 24 IE/100ml; 13, 13 and 13 CP/100ml; and 150, 10 and 10 SOMCP/100ml. For exposure definition 2 ("1 head immersion") they were 1453 and 2163 EC/100ml (only GE_UK and GE_NL-2); 123, 123 and 145 IE/100ml; 38, 38 and 36 CP/100ml; and 330, 50 and 119 SOMCP/100ml.

The threshold concentrations depended mainly on the definition of exposure ("10 min. bathing; ≥ 3 head immersions" or "1 head immersion"), with, as expected, significantly higher concentrations for exposure definition 2 ($p=0.0088$; ANOVA; log10 transformed data). They also depended on the microbiological parameter, with a significant difference between the log10 transformed threshold concentrations of EC, IE, CP and SOMCP ($p=0.0061$; ANOVA). They did not, however, depend on the definition of gastroenteritis ($p=0.32$; ANOVA).

Like the incidence rates of non-bathers and the incidence rates of bathers ≤ threshold concentrations, the incidence rates of bathers above threshold concentrations mainly depended on the definition of gastroenteritis, with a highly significant difference between the incidence rates of GE_UK, GE_UK-wf and GE_NL-2 ($p<0.0001$; ANOVA), and no significant difference between the two exposure definitions ($p=0.8997$) and between the four faecal indicator parameters ($p=0.8772$). The mean incidence rate of gastroenteritis among bathers above threshold concentrations was 5.2% for GE_UK (range: 4.4;6.5), 7.2% for GE_UK-wf (range: 6.9; 7.8), and 9.9% for GE_NL-2 (range: 8.9;12.1).

Mean relative risks of bathers above threshold vs. non-bathers were 3.7 (range: 3.12; 4.61) for GE_UK, 2.6 (range: 2.43; 2.78) for GE_UK-wf and 1.9 (range: 1.71; 2.33) for GE_NL-2. Bathers' excess risks were 3.8% (range: 3.1; 5.2), 4.4% (range: 4.2; 5.7) and 4.7% (range: 4.0; 8.6), respectively. This demonstrates that the majority of gastroenteritis cases attributable to bathing were cases meeting the most stringent

definition of gastroenteritis (vomiting or diarrhoea with three or more bowel movements), i.e. they were not simply mild forms of gastroenteritis.

Detailed raw numbers, p values and 95% confidence intervals for incidence rates and relative risks for all combinations of the two exposure definitions, the three definitions of gastroenteritis and the four faecal indicator organisms, and for skin ailments and aeromonads are listed in the annex (Annex 20, Annex 21, Annex 25).

5.7 Incidence rates in quartile and quintile categories and in arithmetic concentration classes

The results from classifying bathers into quartiles and quintiles of the exposure concentrations corresponded well with the predicted step models. The incidence rates of gastroenteritis in the first quartile and in the first quintile were below the upper limit of the 95% confidence interval of the incidence rate of non-bathers for all 24 combinations of exposure definition, definition of gastroenteritis and faecal indicator organism. Pearson's Chi square tests did not reveal a significant difference between the incidence rates of non-bathers and the incidence rates in the first quartile or quintile in any of the 24 combinations. On the other hand, all incidence rates within the fourth quartile and, with only one exception, all incidence rates within the fifth quintile were within the 95% confidence limits of the incidence rate ceilings of the predicted step models, i. e. the incidence rates of bathers above threshold concentration. For the majority of quartiles and quintiles with concentration ranges above threshold Pearson's Chi square tests revealed significant differences between the incidence rates within the quantiles and the incidence rate among non-bathers (Annex 26, Annex 27, Annex 29, Annex 30).

The classification of bathers into exposure classes of up to 20, >20 to 40, >40 to 60, >60 to 80, 80 to 158 and >158 IE/100 ml, which was done for the sake of direct comparison with the results from sea water exposure, provided no evidence for a dose-response relationship which could not be characterised by the proposed step model. For

all three definitions of gastroenteritis the incidence rate in the lowest exposure group was below the upper 95% confidence limit of the incidence rate of non-bathers, and 14 of the 15 incidence rates of quantiles with geometric quantile means above threshold concentrations were within the 95% confidence limits of the incidence rate ceilings of the corresponding step models. 10 of them were significantly higher than the incidence rates of non-bathers. Similar results could be observed for E. coli classes of 100 units (Annex 28).

5.8 Combined effect of exposure concentrations and swallowing water

The most convincing evidence for the existence of a true-dose response relationship for gastroenteritis attributable to bathing in faecally contaminated water comes from the analysis of the combined effect of bathing below or above threshold concentrations and swallowing water. Among bathers exposed below threshold concentrations swallowing water caused an increase in the incidence rate of gastroenteritis in 21 of the 23 combinations of exposure definitions, definitions of gastroenteritis and faecal indicator organisms for which threshold concentrations could be established. In one case the incidence rates were equal. In one case the incidence rate of bathers who had swallowed water was lower. Among bathers exposed above threshold concentrations the incidence rates of gastroenteritis were always higher for those who reported to have swallowed water (Annex 31). The mean risk attributable to swallowing water above threshold concentrations was significantly higher ($p<0.0001$; ANOVA) than the attributable risk below threshold concentrations (3.6% (95% CI: 3.1; 4.2) vs. 1.3% (95% CI: 0.7;1.8)). There was no significant difference, however, between the two exposure definitions (2.4% for definition 1 (95% CI: 1.7; 3.1) vs. 2.5% for definition 2 (95% CI: 1.8; 3.2); $p=0.86$, ANOVA) or between the three definitions of gastroenteritis (2.7% for GE_UK (95% CI: 1.8; 3.7) vs. 2.2% for GE_UK-wf (95% CI: 1.2; 3.1) and 2.5% for GE_NL-2 (95% CI: 1.6; 3.3); $p=0.72$, ANOVA) or between the four faecal indicator parameters (2.0% for CP (95% CI: 1.0; 3.0), 3.0% for EC (95% CI: 1.9; 4.1), 2.5% for IE (95% CI: 1.4; 3.5), and 2.5% for SOMCP (95% CI: 1.4; 3.5); $p=0.62$, ANOVA) (Annex 32). The fact that swallowing water containing a high

concentration of microbes (high dose) causes relatively more cases of illness in a defined population than swallowing water containing a low concentration of microbes (low dose) is plausible, and indicative for the existence of a true dose-response relationship. However, it also suggests that the determined threshold concentrations are not absolute values, as a slight effect of swallowing water was also detectable below threshold concentrations. As already demonstrated by the effect of exposure definition ("10 min bathing; 3 head immersions" or "1 head immersion"), the concentrations above which a significant increase in the incidence rates of disease is measurable depend on the intensity of activities which are associated with the contact of waterborne microorganisms with the mucosa of the lacrimal duct, the nasopharynx, the oral cavity or the gastrointestinal tract, i. e. the number of head immersions and the amount of water swallowed. They probably also depend on the size of the available cohort.

5.9 Results of the analyses of stool specimens

Four cases of gastroenteritis were laboratory-confirmed by identification of *Salmonella* sp. in stool specimens. Two of these cases occurred among non-bathers, two among bathers. It was concluded that the latter two cases were probably not bathing-associated, but were rather due to risk factors which were independent of this study. Other pathogens like *Shigella*, *Yersinia enterocolitica*, *Campylobacter*, EHEC, rotavirus, adenovirus, enteroviruses, norwalkvirus, *Cryptosporidium parvum* and *Giardia lamblia* could not be detected in any of the analysed specimens.

5.10 Derivation of parameters necessary for the estimation of cost-benefit effects

The socio-economic costs of bathing-acquired gastroenteritis on the one hand and the benefit from prevention of bathing-associated gastroenteritis by setting or tightening standards for faecal indicator organisms on the other hand depend to a great extent on the severity of symptoms. If subjective symptoms are so severe that the diseased person will seek professional medical advice or treatment or stay away from

work or cause other people (e. g. parents) to stay away from work, the disease will cause measurable costs.

In this study a total of five participants, four male and one female, reported having consulted a doctor in the week after exposure, and having been diagnosed with gastroenteritis. Two of them belonged to the group of non-bathers, three to the group of bathers. The two non-bathers were 8 and 21 years old, the bathers were 10, 28 and 53 years old. All five cases met the definition of GE_NL-2 and GE_UK-wf. One of the bathers did not meet the definition of GE_UK. The exposure concentrations of the three bathers were 4.1, 3.5 and 2.7 CP/10ml, 500, 392 and 371 EC/100ml, 49, 130 and 140 IE/100ml and 389, 172 and 112 SOMCP /100ml, i. e. all of them had been bathing clearly above threshold concentrations. Of course it is impossible to decide for individual cases whether they are attributable to bathing or not. The fact that all of them had been bathing above threshold, however, is not contradictory to the assumption that these cases might have been bathing-associated. Other risk factors for gastroenteritis were reported by all three bathers (e.g. history of travelling abroad and gastroenteritis of a household member in one case, consumption of raw milk and raw milk cheese in the other two cases) and might have been alternative sources of infection.

In summary, 2 of 26 non-bathers (7.7%; 95% CI: 0.9; 25.1) who developed gastroenteritis (Definition: GE_UK-wf; i. e. diarrhoea or vomiting) within one week after the trial day consulted a doctor vs. 3 of 40 bathers with gastroenteritis (7.6%; 95% CI: 1.6; 20.4) who were exposed above threshold concentrations for faecal indicators (p=0.66; Fisher's exact test).

Taking into consideration the fact that the majority of gastroenteritis cases attributable to bathing were cases meeting the most stringent definition of gastroenteritis (3.4% GE_UK vs. 4.4% GE_UK-wf and 4.7% GE_NL-2), these results do not justify the assumption that gastroenteritis acquired by bathing in faecally contaminated fresh water is less or more severe or causes less or more socio-economic costs than gastroenteritis acquired through other sources of infection. Standard estimates for the

socio-economic costs of gastroenteritis may therefore be used to analyse cost-benefit effects of setting or altering standards for faecal indicator organisms in fresh water bathing sites.

To estimate the costs of bathing-acquired gastroenteritis in Germany it must be determined how many people per year are bathing above threshold concentrations. It should also be evaluated whether a bathing duration of 10 minutes and four head immersions (the median number of head immersions in this study) are a reasonable estimate for typical bathing intensity. This, however, is beyond the scope of this study and should be determined in additional research projects.

6 Conclusions and Recommendations

The results of this study show that bathing in faecally contaminated German fresh water bathing sites which pass the current microbiological standards of the European bathing water directive of 1976 can increase the risk of acquiring gastroenteritis compared to the risk of non-bathers by a factor of two to four within one week after exposure. A significant increase in the risk of bathing vs. non-bathing became detectable when the extent of faecal contamination of the water exceeded certain threshold concentrations. Such threshold concentrations could be established for each of the four faecal indicator organisms, *Clostridium perfringens*, intestinal enterococci, *Escherichia coli* and somatic coliphages, which were monitored during exposure of the study participants.

Threshold concentrations for increased risk are not absolute. They depend on the intensity of bathing activities which allow the microorganisms present in the water to come into contact with the mucous membranes of the lacrimal duct, the nasopharynx, the oral cavity or the gastrointestinal tract. A bather who immerses his head only once encounters the same risk at a higher exposure concentration as a bather who immerses his head three or more times at a lower exposure concentration; and swallowing water with a high concentration of faecal indicators will result in a greater increase of risk

than swallowing water with a low concentration. Any threshold and any dose-response relationship which is derived from exposure studies with standardised bathing activities is therefore only valid for the type of exposure which has been applied in the corresponding trials, i. e. for bathing activities which are similar to those of the study participants.

Ultimately, the type of exposure which was chosen in this study (10 minutes of bathing and at least three head immersions) is probably an underestimation of the actual mean exposure intensity in "real life". Only 9% of the participants immersed their heads less often than required, while 53% voluntarily did it more often (median = 4; mean = 5.5; range = 0-87 head immersions).

Considering both the results of fitting step models for dose-response relationships between the concentration of indicator organisms and gastroenteritis and the results of the calculation of incidence rates within classes of concentration ranges defined by quartiles and quintiles, and with respect to the threshold of 32 faecal streptococci / 100ml which could be derived from similar studies in sea water, the following threshold concentrations for an increased risk of gastroenteritis from 10 minutes of bathing and at least three head immersions are recommended: 10/100ml for *Clostridium perfringens*, 25/100ml for intestinal enterococci, 100/100ml for *Escherichia coli*, and 10/100ml for somatic coliphages.

Using step models as an approximation for dose-response relationships has three main advantages. First, they are easy to compute because they can be described by only three parameters: the baseline risk, the threshold concentration and the maximum risk. Second, they provide conservative estimates for the maximum risk, since extrapolation into higher concentration ranges do not produce results which are beyond what is verifiable by experimental data. Third, they are easy to communicate to the public. The models, however, overestimate the risk for concentrations slightly above the threshold, and for the same reasons they slightly underestimate the maximum risk. The assumption that there is no further risk increase at higher concentrations than those which were

encountered during this study is strongly supported by the results of the studies reported by Cabelli et al. 1982/83 on bathing-associated gastroenteritis at Egyptian beaches. According to an analysis performed for the WHO (Prüss, 1998) these are the only studies which provide experimental data for higher exposure concentrations. In these studies no increase in the incidence rates of gastroenteritis could be detected for bathers exposed to 9160 enterococci /100ml (geometric mean concentration) vs. bathers exposed to 954 enterococci /100ml. The incidence rates among bathers exposed to the roughly tenfold concentrations were even slightly lower: 4.5% vs. 4.8% for visitors from Cairo, and 1.9% vs. 2.1% for local residents. Van Asperen et al., 1998, report similar results for triathletes exposed in Dutch fresh waters. The incidence rate for exposure concentrations of ca. 1200 E. coli / 100 ml was 6.7% vs. 10.9% for exposure concentrations of 500/100ml.

The incidence rate of gastroenteritis clearly depends on the definition of the disease. More stringent definitions result in lower incidence rates and in higher relative risks for bathers above threshold concentrations than less stringent definitions. Excess risks for bathers above threshold concentrations are higher for less stringent definitions. Threshold concentrations, however, were independent of the definition of gastroenteritis. They are probably also independent of the portion of susceptible individuals in different cohorts, e. g. cohorts of tourists. Standards for faecal indicator organisms should therefore be based on a threshold concept rather than on estimates of how many bathers will become ill ("disease burden"). The percentage of bathers who become ill may differ dramatically depending on the cohort that is used as the basis for the estimate. According to Prüss, 1998, maximum excess risks range between 0.4% (Dufur et al., 1984) and 27.7% (Fewtrell et al., 1992) in studies involving fresh water exposure and between 0.53% (Cheung et al., 1989) and 19.5% (Kay et al., 1994) in studies involving sea water exposure. To base regulatory standards on maximal acceptable excess risks, e. g. 5%, as it is has been done in the current proposal for a revised European bathing water directive (Commission, 2002) and in WHO publications (Havelaar et al. 2001; WHO 1998 and 2003), is therefore inappropriate. If this concept were applied to the results of this study or the results of the Cabelli studies in Egypt, no

imperative or guide values would be necessary at all for German fresh waters or Egyptian beaches or bathing areas in any other part of the world where the maximal excess risks were below 5% (Annex 33).

In contrast, a threshold concept would consist of calculating the percentage of microbial concentrations exceeding the threshold during the bathing season. This percentage is identical with the percentage of bathers exposed above threshold, i. e. the percentage of bathers who are at increased risk, under the assumption that bathing activities are randomly distributed over the season. A threshold concept would therefore provide a classification of beaches according to their overall health risk from faecal contamination and should consist of the following components:

(1) The threshold concentrations; e. g. 25 IE/100ml and 100 EC/100ml.
(2) Estimates for the mean water quality on the days of sampling; e. g. the concentrations determined by area-integrated sampling (one pooled sample) from four representative sampling points on a beach.
(3) A standardised (constant) estimate for the standard deviation of lognormal distributed microbiological concentrations on the day of sampling; e.g. 0.4 for log10 transformed concentrations of IE or EC.
(4) The calculation of the most probable percentage of microbial concentrations above threshold on the day of sampling. This percentage can be easily computed with any standard spread sheet calculation program like Excel(TM) or can be read from a prepared table (s. Annex).
(5) A time weight for the monitoring results to ensure that more frequent sampling apart from the regular sampling intervals, e. g. after incidences of high contamination, is not discouraged (or "punished") by a disproportionate influence of these sample results on the final result for the complete season. The sample results should therefore be weighted by the number of days for which they are assumed to be valid, i.e. the number of days until the next sample is collected.

(6) The calculation of the mean percentage of indicator concentrations exceeding the thresholds during the bathing season, as an estimate for the percentage of bathers at increased risk.

(7) A political decision on which percentages are acceptable in different quality categories.

Examples and illustrations to explain this concept may be found in the annex (Annex 24, Annex 34, Annex 35, Annex 36).

The current European standard for *Escherichia coli* in bathing water requires a compliance rate of 95% of the samples with an imperative value of 2000 faecal coliforms /100ml. In the proposal for a revision of this standard a 95th percentile approach is favoured, and the imperative value is reduced to 500 *E. coli* /100ml. This value was proposed by the German Federal Environmental Agency using preliminary data (four of five data sets) from this study and a tentative dose-response model (López-Pila, 2002). It was argued that the proposed reduction of the imperative value would result in a 50% reduction of the disease burden of bathing sites which just comply with the standard, assuming lognormal distributed concentrations with a SD of 0.75 during the bathing season. A repetition of this analysis using the complete data set and the step model approach revealed almost the same results (ca. 55% reduction) for all three definitions of gastroenteritis. The 95th percentiles for IE concentrations which would correspond to the risk levels associated with 95th percentile concentrations of 500 and 250 EC/100ml (the proposed imperative and guide value in the current EU proposal) would be ca. 120 and 60 IE/100ml. These values are considerably lower than those being proposed (200 and 100 IE/100ml), indicating that the proposed standards for *E. coli* would provide a higher degree of health protection than the standards for intestinal enterococci. The relation between the proposed standards for these two indicator parameters should therefore be reconsidered.

The risk for bathing-associated skin ailments (disease definition "SKIN") in this study was not correlated to the concentration of any of the four faecal indicators. It

could, however, be partially predicted by the concentration of aeromonads. The incidence rates among non-bathers, bathers below threshold concentration (ca. 5600 aeromonads /100ml) and bathers above the threshold concentration were 2.8%, 5.3% and 12.2% respectively, i.e. ca. 75% of the maximum excess risk occurred above the threshold concentration, while ca. 25% were obviously due to other independent bathing-associated factors, for example the presence of parasites of the Trichobilharzia genus. As the possible association between aeromonads and skin ailments has not yet been widely studied, it is recommended that this parameter be included in future studies to find out whether these results are reproducible.

No other significant and unbiased association between any of the six microbiological parameters and any of the other disease definitions (AFRI, CC, EAR, EYE, UTI) could be observed in this study.

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Umweltforschungsplan
des Bundesministeriums für Umwelt,
Naturschutz und Reaktorsicherheit

Umwelt und Gesundheit

Förderkennzeichen (UFOPLAN) 298 61 503

Epidemiological Determination of Disease Risks from Bathing
Epidemiologische Erfassung des Erkrankungsrisikos beim Baden
- Annex -

von

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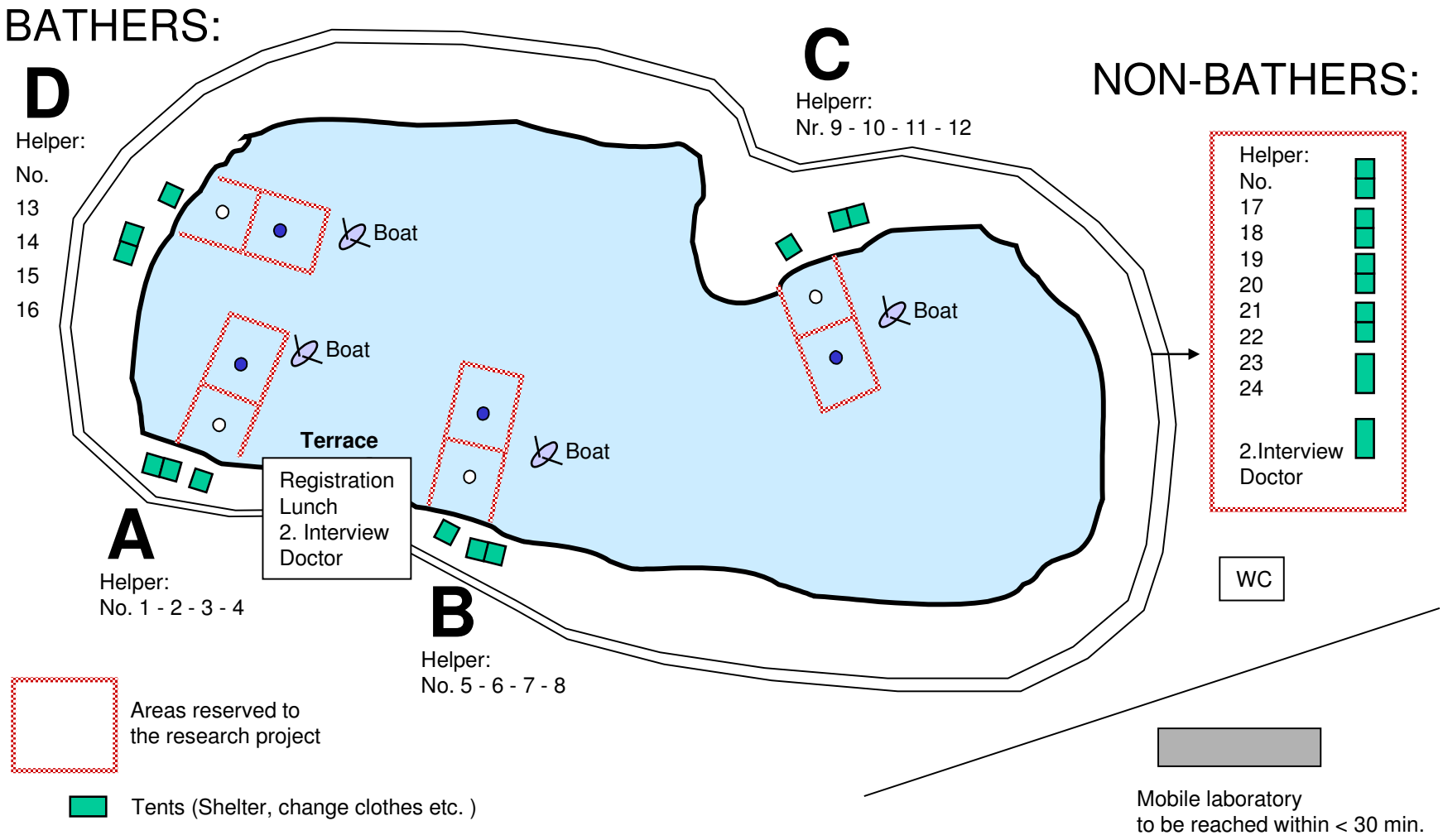
IM AUFTRAG
DES UMWELTBUNDESAMTES
Dezember 2004

Annex 1. Classification of the five study sites according to the European Bathing water directive 76/160/EWG in the year of the study and in the years before

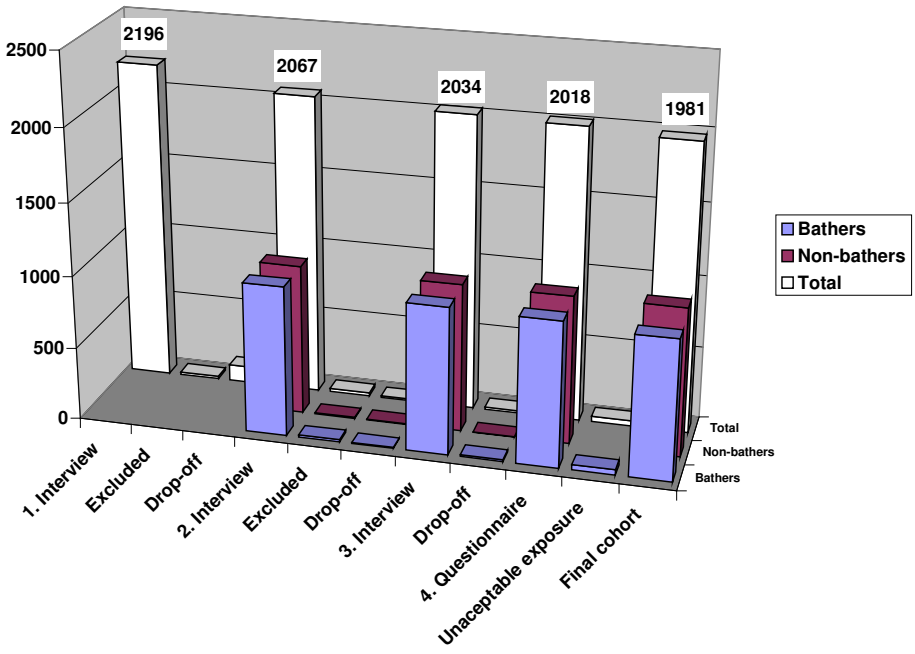
All study sites complied with the European bathing water directive 76/160/EWG (Commission of the European Communities, 1976) in the year the study was performed and for at least the three preceding years (blue or green marker coulour). The figures are copied from the official web site of the European Commission.

Annex 2. Schematic site map with study arrangements at one of the five study sites ("Lerchenauer See" in Munich)

The graphic representation on the following page is an example for the study arrangements on the beach. At all sites participants received maps like this at the registration desk to assist them in finding their way to their individual bathing area or to the non-bathers area and to one of the supervisors (project helpers). Supervisors were numbered and uniformly dressed with caps and t-shirts: blue ones for bathers, and red ones for non-bathers.


BATHERS:
D
Helper:
No.
13
14
15
16
C
Helperr:
Nr. 9 - 10 - 11 - 12
NON-BATHERS:
Helper:
No.
17
18
19
20
21
22
23
24
2.Interview
Doctor
Boat
Boat
Boat
Boat
Terrace
Registration
Lunch
2. Interview
Doctor
A
Helper:
No. 1 - 2 - 3 - 4
B
Helper:
No. 5 - 6 - 7 - 8
WC
Areas reserved to
the research project
Tents (Shelter, change clothes etc.)
Mobile laboratory
to be reached within < 30 min.

Annex 3. Follow-up rate, medical exclusions, randomisation result, exclusions due to unacceptable exposure status, and final cohort size



	Total	Follow-up rate	Bathers		Non-bathers	
	n	(%)	n	(%)	n	(%)
1. Interview	2196	(100.0)				
Medical Exclusions	17					
Drop-off	112					
2. Interview	2067	(94.1)	1033	(50.0)	1034	(50.0)
Medical Exclusions	22		15		7	
Drop-off	11		6		5	
3. Interview	2034	(92.6)	1012	(49.8)	1022	(50.2)
Drop-off	16		13		3	
4. Postal Questionnaire	2018	(91.9)	999	(49.5)	1019	(50.5)
Unaceptable exposure data	37		37			
Final cohort	1981		962	(48.6)	1019	(51.4)

Annex 4. Disease-specific cohorts

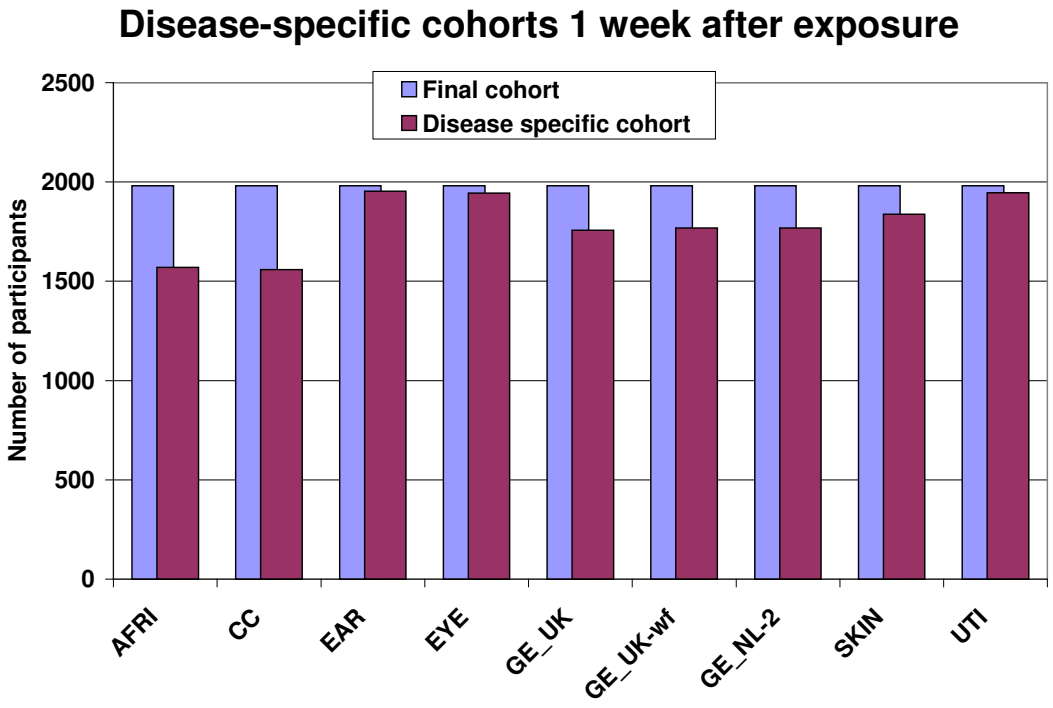
Definition (1)	Final Cohort	Excluded (2)	Excluded (3)	Disease-specific Cohort	Bathers		Non-bathers	
	n	n	n	n	n	(%)	n	(%)
1 week after exposure								
AFRI	1981	405	7	1569	759	(48.4)	810	(51.6)
CC	1981	405	18	1558	754	(48.4)	804	(51.6)
EAR	1981	26	2	1953	950	(48.6)	1003	(51.4)
EYE	1981	34	4	1943	944	(48.6)	999	(51.4)
GE_UK	1981	188	36	1757	837	(47.6)	920	(52.4)
GE_UK-wf	1981	188	25	1768	847	(47.9)	921	(52.1)
GE_NL-2	1981	188	26	1767	846	(47.9)	921	(52.1)
SKIN	1981	138	6	1837	880	(47.9)	957	(52.1)
UTI	1981	32	3	1946	944	(48.5)	1002	(51.5)
3 weeks after exposure								
AFRI	1981	405	27	1549	747	(48.2)	802	(51.8)
CC	1981	405	42	1534	741	(48.3)	793	(51.7)
EAR	1981	26	12	1943	942	(48.5)	1001	(51.5)
EYE	1981	34	12	1935	938	(48.5)	997	(51.5)
GE_UK	1981	188	73	1720	818	(47.6)	902	(52.4)
GE_UK-wf	1981	188	55	1738	831	(47.8)	907	(52.2)
GE_NL-2	1981	188	62	1731	823	(47.5)	908	(52.5)
SKIN	1981	138	27	1816	869	(47.9)	947	(52.1)
UTI	1981	32	11	1938	939	(48.5)	999	(51.5)

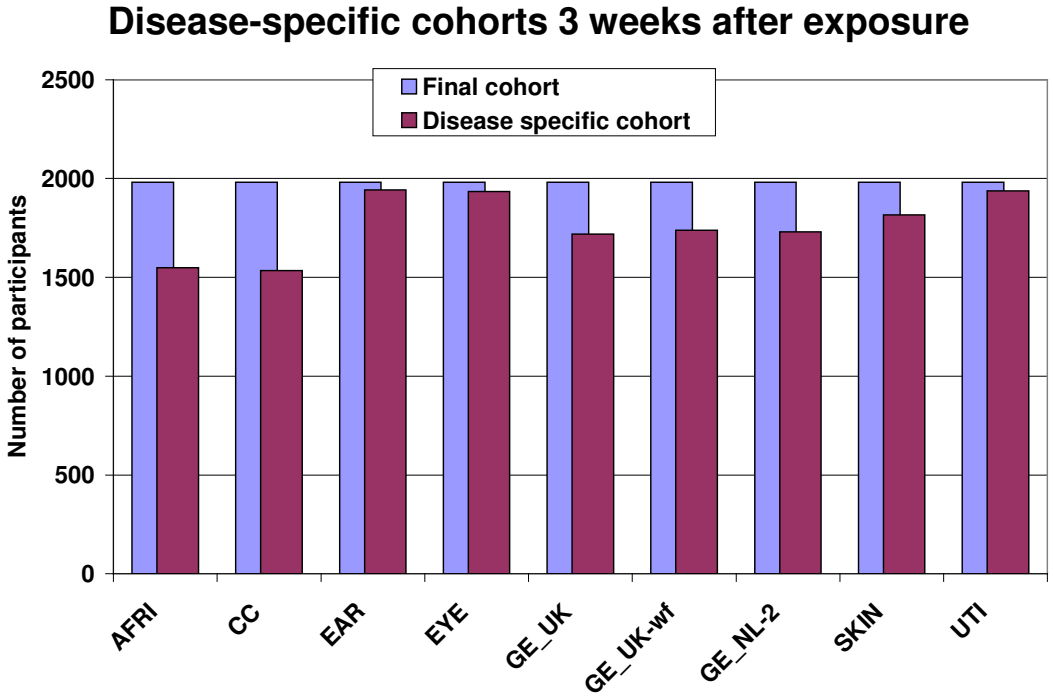
(1) Disease definitions

AFRI	Acute febrile respiratory infections
CC	Common cold
EAR	Ear inflammation
EYE	Eye inflammation
GE_UK	Gastroenteritis, UK definition
GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis, NL-2 definition
SKIN	Skin infections, cutireactions
UTI	Urinary tract infections

(2) Excluded from analysis because of disease-specific precursor symptoms at 2nd interview

(3) Excluded from analysis because of undefined disease status (missing answers or answers of "not sure" in any of the three interviews or in the postal questionnaire).


Disease-specific cohorts 1 week after exposure
Final cohort
Disease specific cohort
Number of participants
2500
2000
1500
1000
500
0
AFRI
CC
EAR
EYE
GE_UK
GE_UK-wf
GE_NL-2
SKIN
UTI


Disease-specific cohorts 3 weeks after exposure
Final cohort
Disease specific cohort
Number of participants
2500
2000
1500
1000
500
0
AFRI
CC
EAR
EYE
GE_UK
GE_UK-wf
GE_NL-2
SKIN
UTI

Annex 5. Parameter-specific cohorts

Disease definition (1)	Disease-specific cohort	AE result missing (2)	AE cohort	CP result missing	CP cohort	EC result missing	EC cohort	IE result missing	IE cohort	PA result missing	PA cohort	SOMCP result missing	SOMCP cohort
1 week after exposure													
AFRI	1569	9	1560	0	1569	9	1560	9	1560	107	1462	7	1562
CC	1558	9	1549	0	1558	8	1550	8	1550	107	1451	7	1551
EAR	1953	17	1936	0	1953	10	1943	10	1943	121	1832	13	1940
EYE	1943	17	1926	0	1943	10	1933	10	1933	117	1826	13	1930
GE_UK	1757	12	1745	0	1757	9	1748	9	1748	108	1649	12	1745
GE_UK-wf	1768	12	1756	0	1768	9	1759	9	1759	110	1658	12	1756
GE_NL-2	1767	12	1755	0	1767	9	1758	9	1758	110	1657	12	1755
SKIN	1837	15	1822	0	1837	10	1827	10	1827	107	1730	11	1826
UTI	1946	15	1931	0	1946	10	1936	10	1936	120	1826	12	1934
3 weeks after exposure													
GE_UK	1720	12	1708	0	1720	8	1712	8	1712	108	1612	12	1708

(1) Disease definitions

AFRI	Acute febrile respiratory infections
CC	Common cold
EAR	Ear inflammation
EYE	Eye inflammation
GE_UK	Gastroenteritis, UK definition
GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis, NL-2 definition
SKIN	Skin infections, cutireactions
UTI	Urinary tract infections

(2) Microbiological parameters

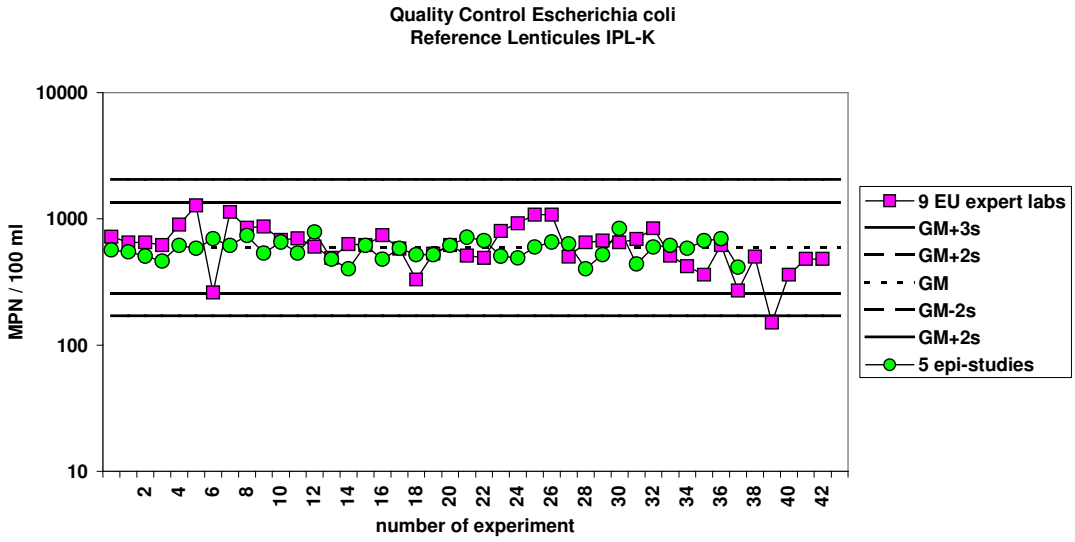
AE	Aeromonads
CP	*Clostridium perfringens*
EC	*Escherichia coli*
IE	Intestinal enterococci
PA	*Pseudomonas aeruginosa*
SOMCP	Somatic coliphages

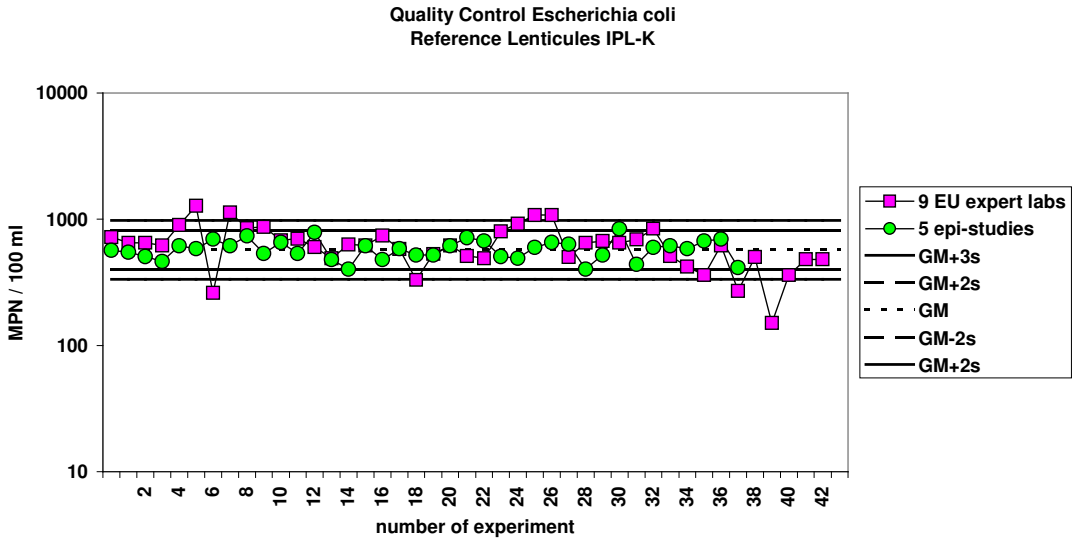
Annex 6. Quality control of the enumeration procedure for *Escherichia coli* and intestinal enterococci using quantitative external reference materials

Quality control procedures included positive and negative media controls for target organisms, or in the case of *E. coli* and intestinal enterococci the application of quantitative reference materials which had been evaluated in an earlier international round robin trial funded by a European Union research project ("reference lenticules K", donated by Institute Pasteur de Lille, European Community Contract SMT4-CT95-1603; DG12-RSMT).

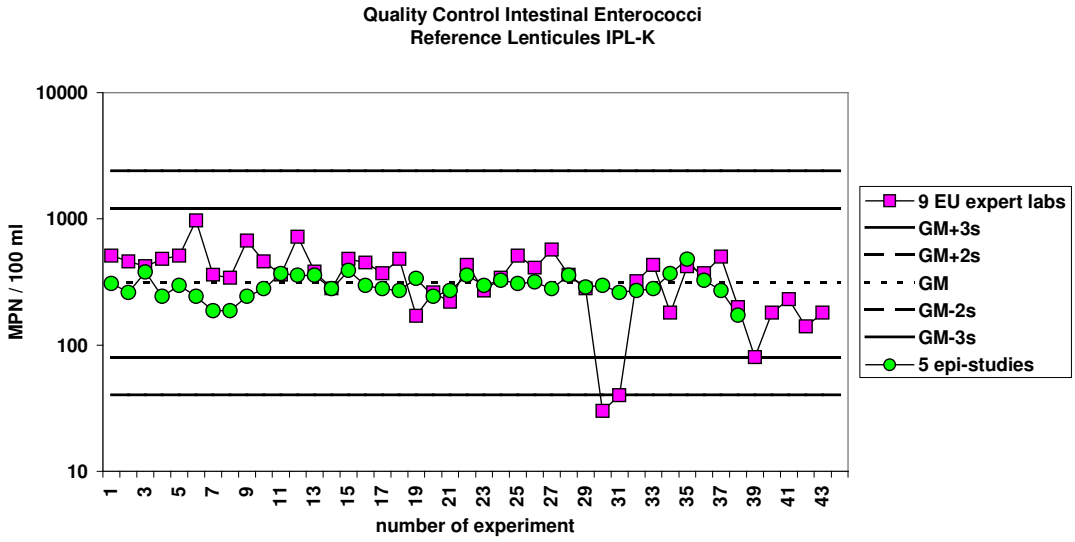
The figures in this annex show the results achieved with these reference materials during the five epidemiological studies in comparison with the results obtained by 9 EU expert labs in a round robin trial performed in the EU project mentioned above.

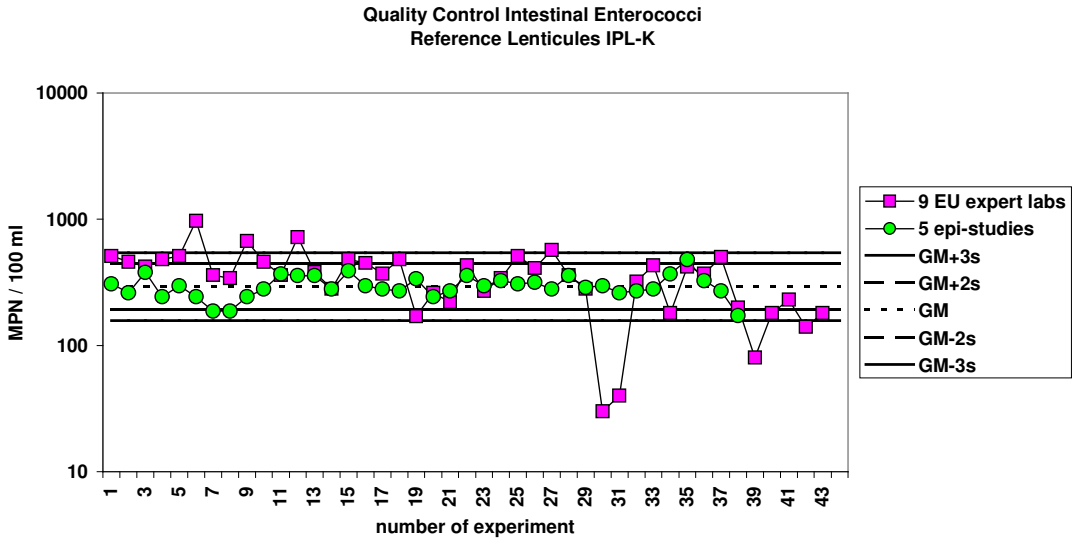
Geometric means ± 2 or 3 standard deviations are shown for the results of the European round robin trial and for the results achieved during the five epidemiological studies.





MPN /100 ml	Most Probable Number per 100 ml
GM + 3s	Geometric mean + 3 x standard deviation
GM + 2s	Geometric mean + 2 x standard deviation
GM	Geometric mean
GM - 2s	Geometric mean - 2 x standard deviation
GM - 3s	Geometric mean - 3 x standard deviation





MPN /100 ml	Most Probable Number per 100 ml
GM + 3s	Geometric mean + 3 x standard deviation
GM + 2s	Geometric mean + 2 x standard deviation
GM	Geometric mean
GM - 2s	Geometric mean - 2 x standard deviation
GM - 3s	Geometric mean - 3 x standard deviation

Annex 7. Concentration ranges and median concentrations of the microbiological parameters at the five study sites

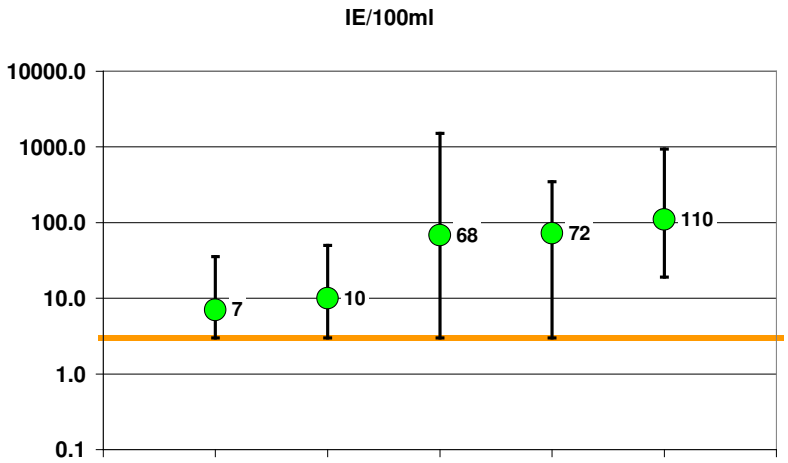
The figures in this annex show the concentration ranges and median concentrations of the six microbiological parameters at the five study sites. Lower detection limits are marked by a horizontal line in orange. The sites are sorted from left to right in ascending order according to the median concentration of intestinal enterococci.

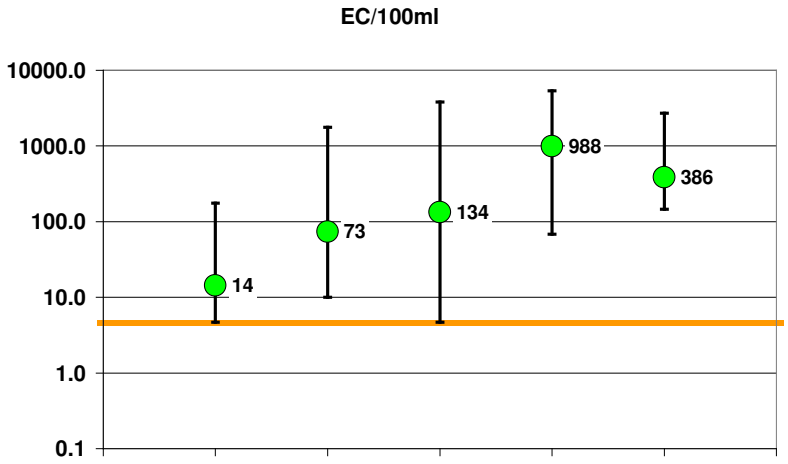
Legend

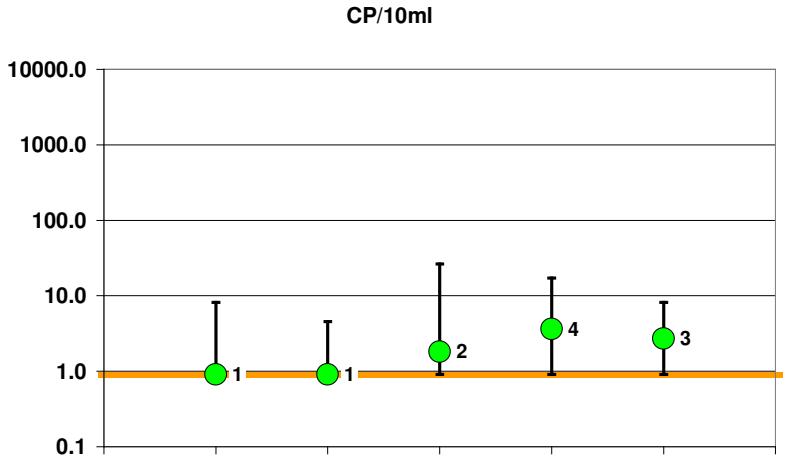
Parameters:

IE	Intestinal enterococci
EC	*Escherichia coli*
CP	*Clostridium perfringens*
SOMCP	Somatic coliphages
AE	Aeromonads
PA	*Pseudomonas aeruginosa*

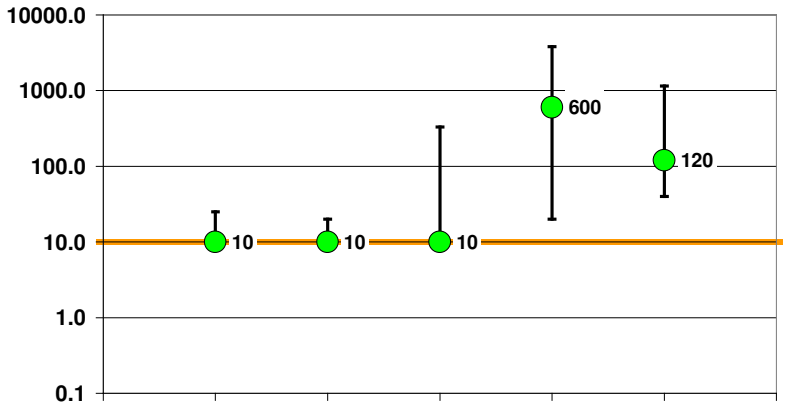
orange line	lower detection limit


IE/100ml
10000.0
1000.0
100.0
10.0
1.0
0.1
7
10
68
72
110

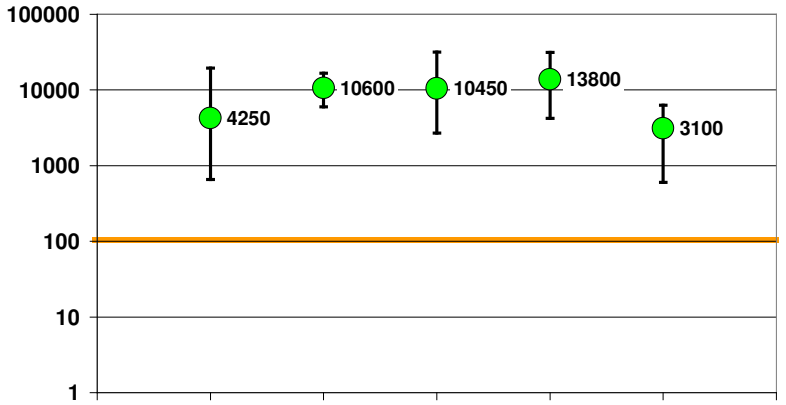

EC/100ml
10000.0
1000.0
100.0
10.0
1.0
0.1
14
73
134
988
386


CP/10ml
10000.0
1000.0
100.0
10.0
1.0
0.1
1
1
2
4
3

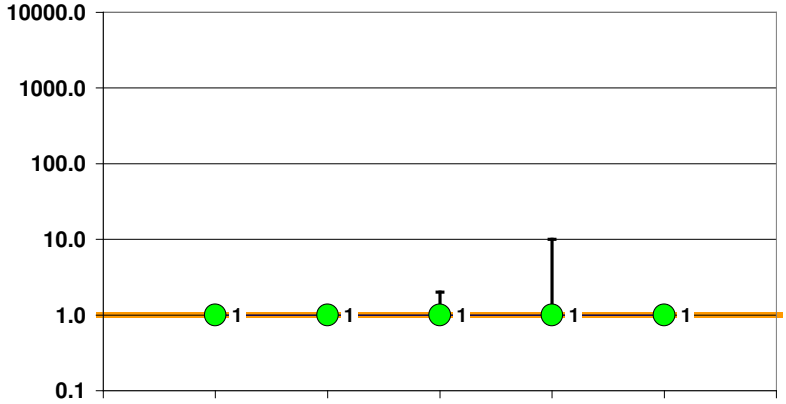
SOMCP/100ml



AE/100ml



PA/10ml

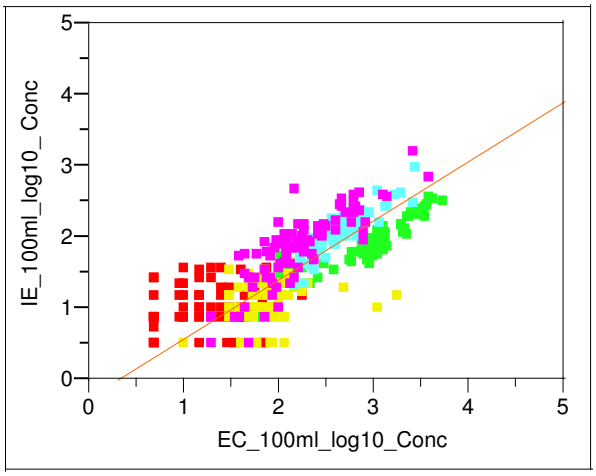


Annex 8. Correlation of microbiological parameters: Orthogonal fits of log10 transformed data after censoring values below detection limit

The correlation between each of the six microbiological parameters was analysed by orthogonal regression. ANOVA test results revealed significant differences between the parameter variances for all 15 possible combinations. Orthogonal regression was therefore based on the specific variance ratio of each of the 15 combinations. Intercepts, slopes and correlation coefficients of the resulting orthogonal fit lines are listed in the table below. Results are sorted in descending order according to the correlation coefficient. The graphs on the following pages display the data points in different colours according to the study site. The tables next to the graphs list the number of samples which were censored ("1") or not censored ("0"). The variance ratios for the various combinations of parameters are displayed as well. Orthogonal fit lines can be calculated using the following formula:

log10(parameter Y) = intercept + slope * log10(parameter X)

Parameter X	Parameter Y	N Samples	Intercept	Slope	Correlation
EC	EN	391	-0.270	0.836	0.79
EC	SOMCP	243	-1.112	1.243	0.78
EC	CP	285	-0.907	0.537	0.57
EN	CP	278	-0.714	0.631	0.51
CP	SOMCP	209	1.137	2.115	0.50
EN	SOMCP	241	-0.802	1.525	0.41
EN	PA	50	-1.370	0.841	0.31
EC	PA	50	-1.685	0.640	0.29
SOMCP	PA	49	-1.099	0.483	0.28
EC	AE	405	2.779	0.492	0.27
SOMCP	AE	242	2.760	0.529	0.24
CP	AE	285	3.473	1.030	0.23
CP	PA	47	-0.252	0.795	0.17
EN	AE	399	2.871	0.617	0.11
AE	PA	48	-4.789	1.211	0.07



EC	IE	N Samples
-	-	3
0	0	391
0	1	18
1	0	12

Orthogonal Fit Ratio=0.699

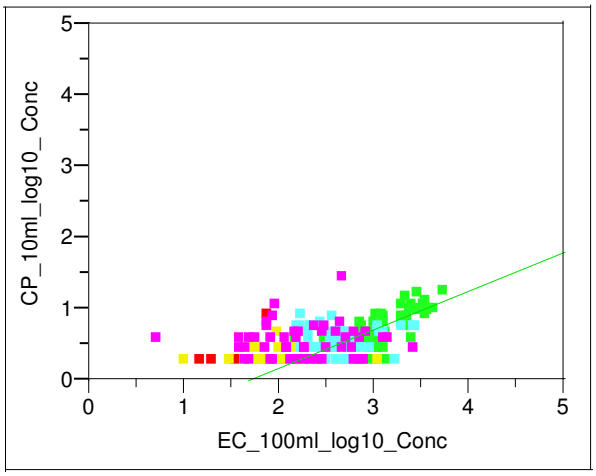
Legend

Parameters:

EC	*Escherichia coli*
IE	Intestinal enterococci
SOMCP	Somatic coliphages
CP	*Clostridium perfringens*
PA	*Pseudomonas aeruginosa*
AE	Aeromonads

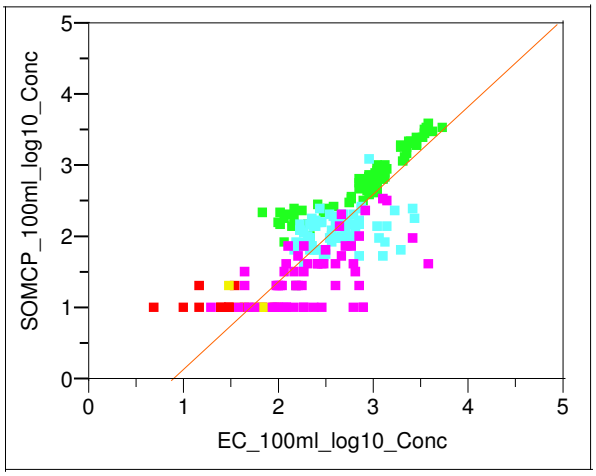
Censoring:

0	uncensored result (result within the detection limits)
1	censored (result below detection limit)
-	no result available (analytical failure)



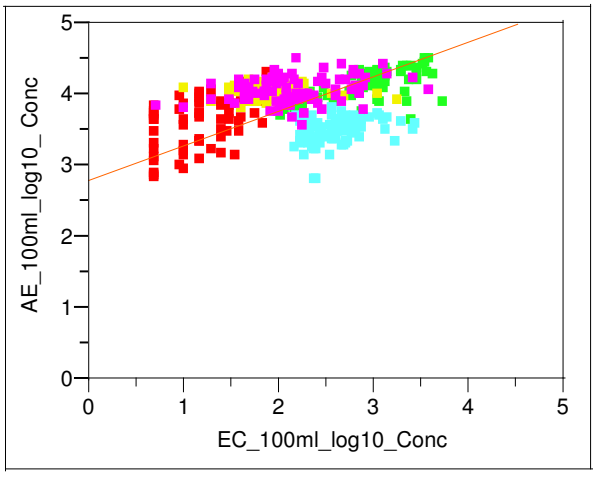
EC	CP	N Samples
-	0	1
-	1	2
0	-	1
0	0	285
0	1	123
1	0	2
1	1	10

Orthogonal Fit Ratio=0.289



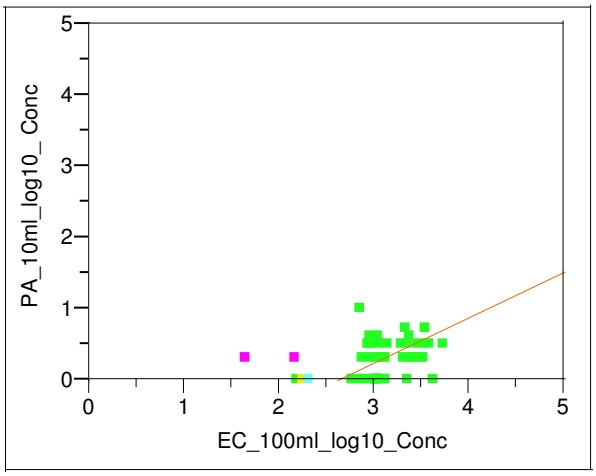
EC	SOMCP	N Samples
-	0	2
-	1	1
0	-	4
0	0	243
0	1	162
1	0	1
1	1	11

Orthogonal Fit Ratio=1.544



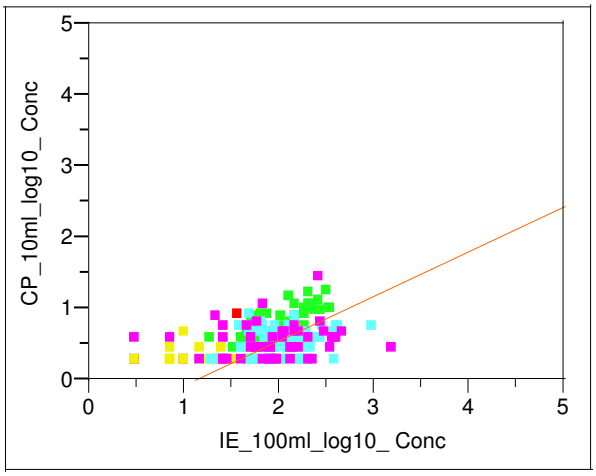
EC	AE	N Samples
-	0	3
0	-	4
0	0	405
1	0	12

Orthogonal Fit Ratio=0.242



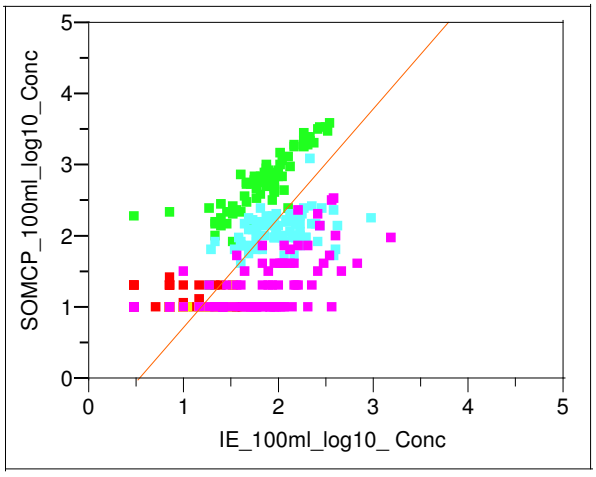
EC	PA	N Samples
-	1	3
0	-	39
0	0	50
0	1	320
1	1	12

Orthogonal Fit Ratio=0.409



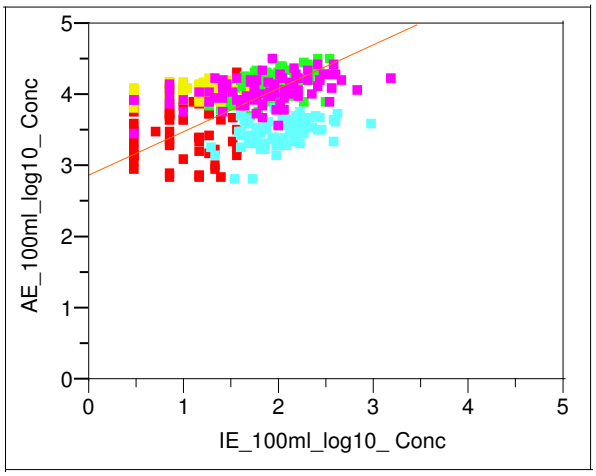
IE	CP	N Samples
-	0	1
-	1	2
0	-	1
0	0	278
0	1	124
1	0	9
1	1	9

Orthogonal Fit Ratio=0.399



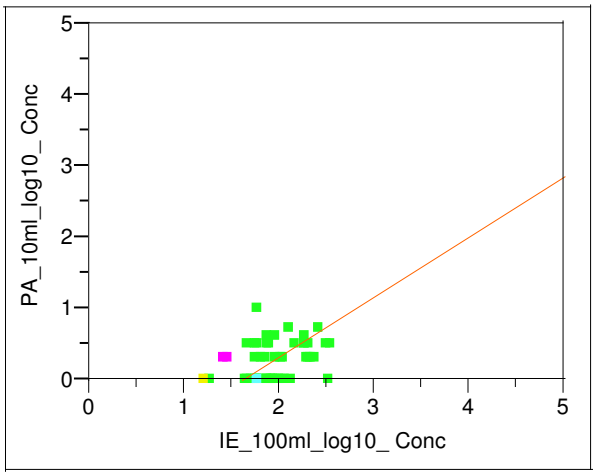
IE	SOMCP	N Samples
-	0	2
-	1	1
0	-	4
0	0	241
0	1	158
1	0	3
1	1	15

Orthogonal Fit Ratio=2.326



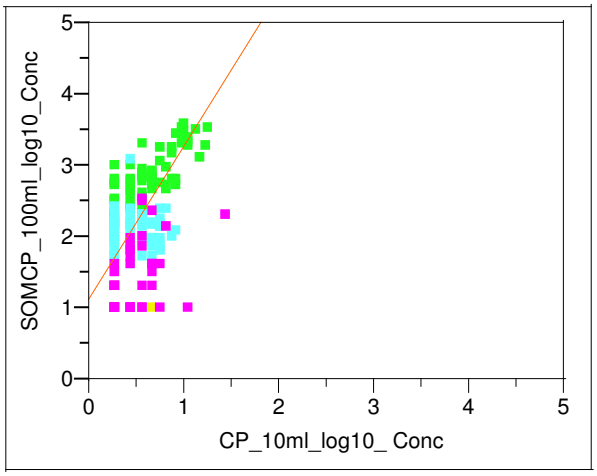
IE	AE	N Samples
-	0	3
0	-	4
0	0	399
1	0	18

Orthogonal Fit Ratio=0.381



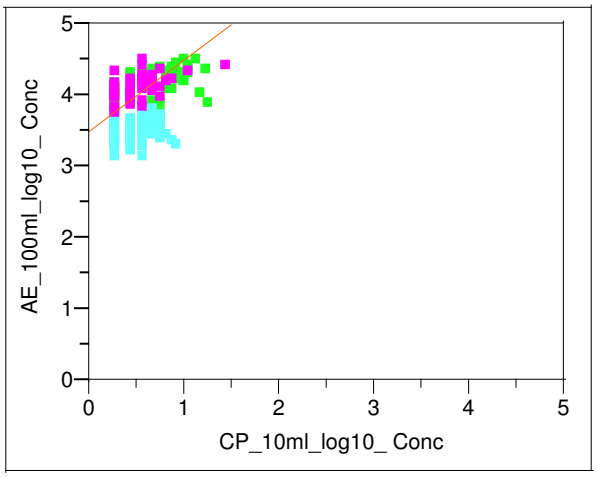
IE	PA	N Samples
-	1	3
0	-	39
0	0	50
0	1	314
1	1	18

Orthogonal Fit Ratio=0.707



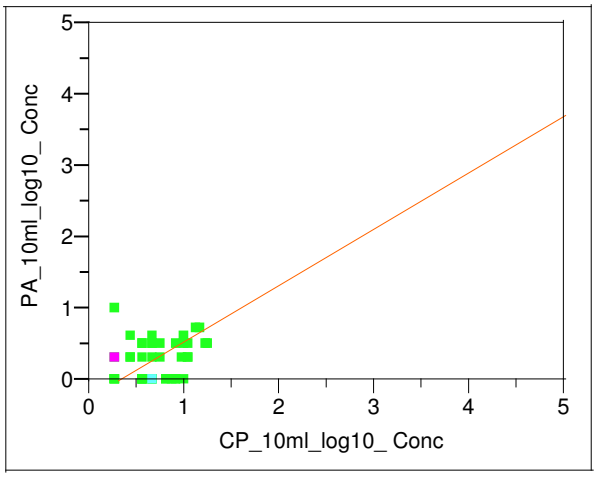
CP	SOMCP	N Samples
-	0	1
0	-	2
0	0	209
0	1	77
1	-	2
1	0	36
1	1	97

Orthogonal Fit Ratio=4.473



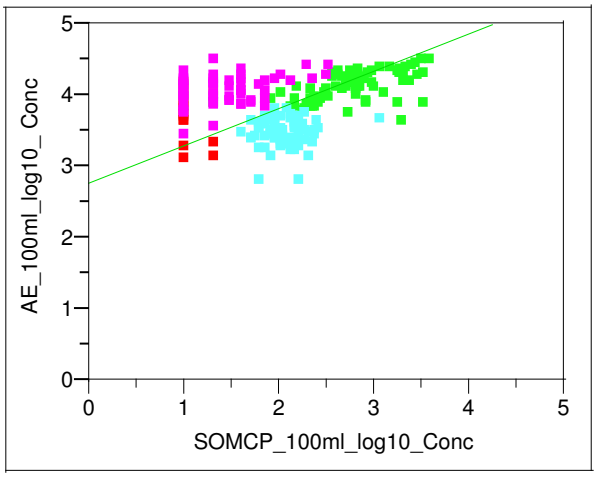
CP	AE	N Samples
-	-	1
0	-	3
0	0	285
1	0	135

Orthogonal Fit Ratio=1.061



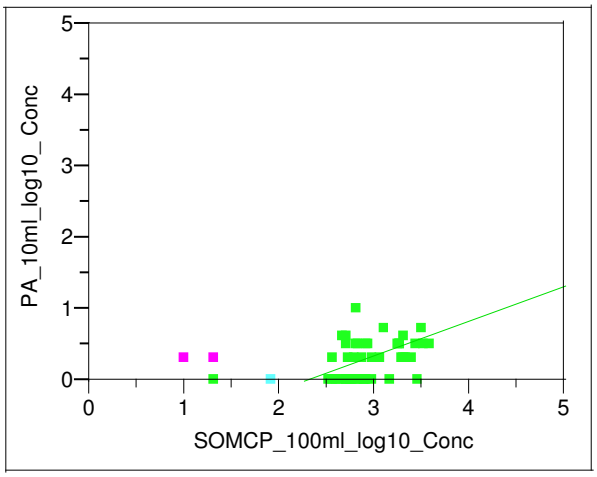
CP	PA	N Samples
-	-	1
0	-	27
0	0	47
0	1	214
1	-	11
1	0	3
1	1	121

Orthogonal Fit Ratio=0.631



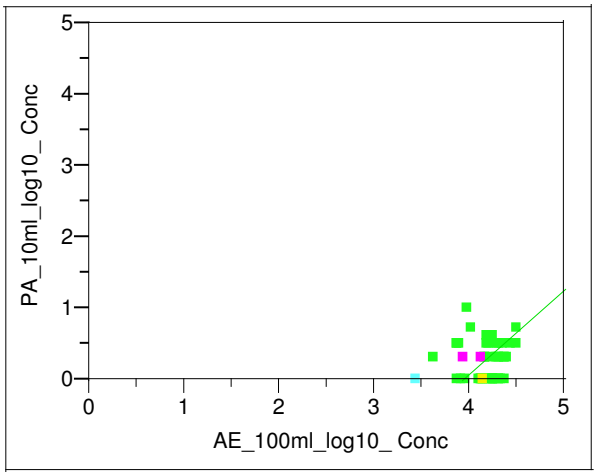
SOMCP	AE	N Samples
-	0	4
0	-	4
0	0	242
1	0	174

Orthogonal Fit Ratio=0.279



SOMCP	PA	N Samples
-	1	4
0	-	29
0	0	49
0	1	168
1	-	10
1	0	1
1	1	163

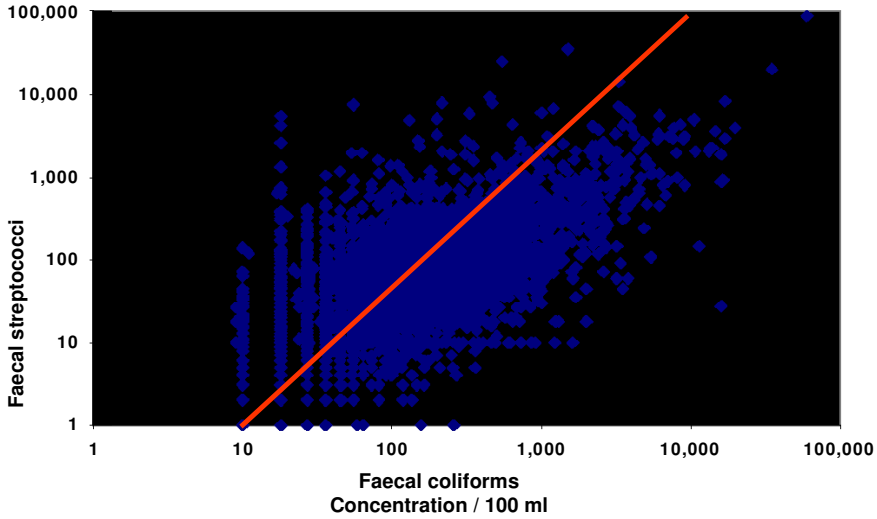
Orthogonal Fit Ratio=0.233



AE	PA	N Samples
-	-	1
-	0	2
-	1	1
0	-	38
0	0	48
0	1	334

Orthogonal Fit Ratio=1.467

Annex 9. Relationship of E. coli and intestinal enterococci counts in comparison with data from the UK

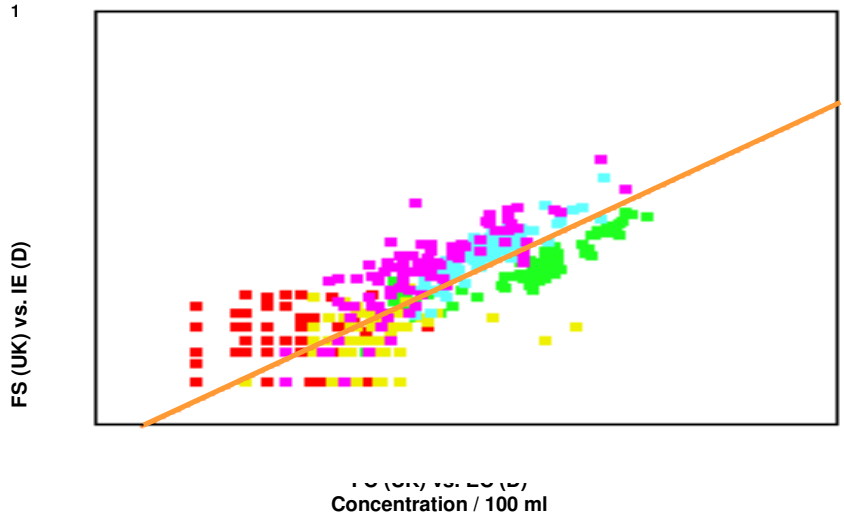


Relationship between faecal coliform and faecal streptococcal counts in United Kingdom bathing waters after censoring zero values (reprod. from WHO, 2001, Figure 4.7., p. 38), overlaid by the regression function given in the text quoted below (red line)

The regression function does not seem to correspond well with the dot plot, and the results of the sample calculations in the text quoted below do not correspond with the empirical observation that faecal coliform concentrations tend to be higher than faecal streptococci concentrations. The formula for the regression function is therefore probably erroneous. WHO, 2001, p. 38-39 ("Farnham protocol"): "There is no exact relationship between faecal streptococci and *E. coli* counts (Figure 4.7). Nevertheless, a relationship can be expressed by the equation below, which may help in interpreting historical data:

log faecal coliform count = 1.028 + 0.601 * log faecal streptococcal count.

Consequently, counts of =100 faecal coliforms/100 ml can be equated to =40 faecal streptococci/100 ml, =250 faecal coliforms/100 ml to =200 faecal streptococci/100 ml and =450 faecal coliforms/100 ml to =500 faecal streptococci/100 ml."



Relationship between faecal coliform and faecal streptococcal counts in United Kingdom bathing waters after censoring zero values (reprod. from WHO, 2001, Figure 4.7., p. 38) overlaid by the relationship between E. coli and intestinal enterococci counts at the five German fresh water bathing sites

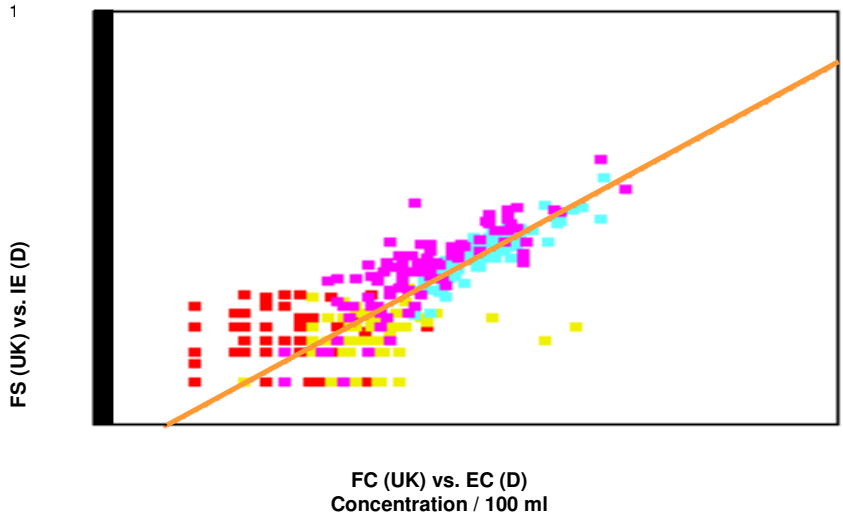
Legend

Country Codes:
UK United Kingdom
D Germany

Indicator organisms:
FC Faecal Coliforms
EC Escherichia coli
FS Faecal streptococci
IE Intestinal enterococci

Markers:
Dark blue: UK results
Red, yellow, pink, light blue, green: German results at five study sites

Orthogonal regression formula for log10 transformed German data:
Orange line
log10 EC count = 0.323 + 1.196 * log10 IE count
log10 IE count = -0.270 + 0.836 * log10 EC count



Relationship between faecal coliform and faecal streptococcal counts in United Kingdom bathing waters after censoring zero values (reprod. from WHO, 2001, Figure 4.7., p. 38) overlaid by the relationship between E. coli and intestinal enterococci counts at four of the five German fresh water bathing sites

Results from the site which was probably influenced by tertiary treated sewage effluent (green dots; see page before) are omitted.

Legend

Country Codes:
UK United Kingdom
D Germany

Indicator organisms:
FC Faecal Coliforms
EC Escherichia coli
FS Faecal streptococci
IE Intestinal enterococci

Markers:
Dark blue: UK results
Red, yellow, pink, light blue: German results at four study sites

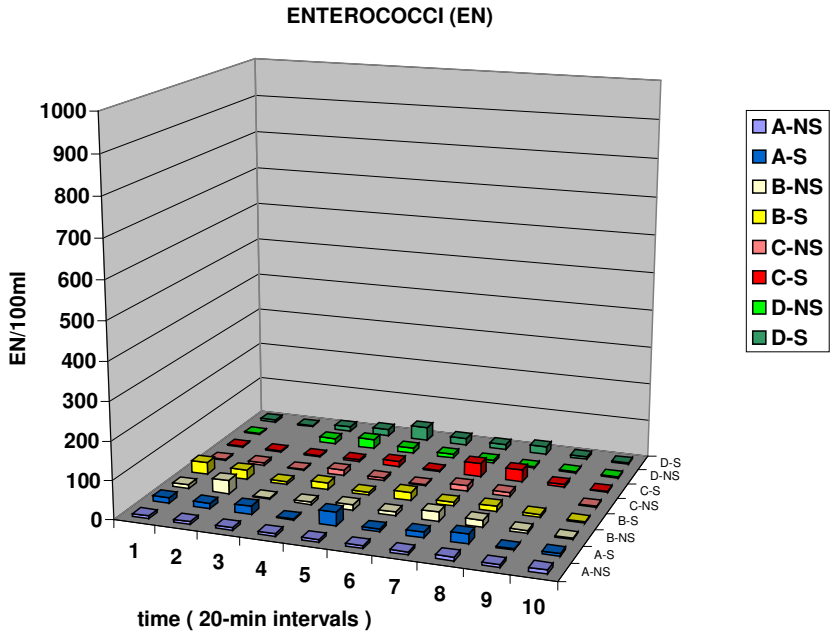
Orthogonal regression formula for log10 transformed German data (orange line)
log10 EC count = 0.485 + 1.028 * log10 IE count
log10 IE count = -0.472 + 0.973 * log10 EC count

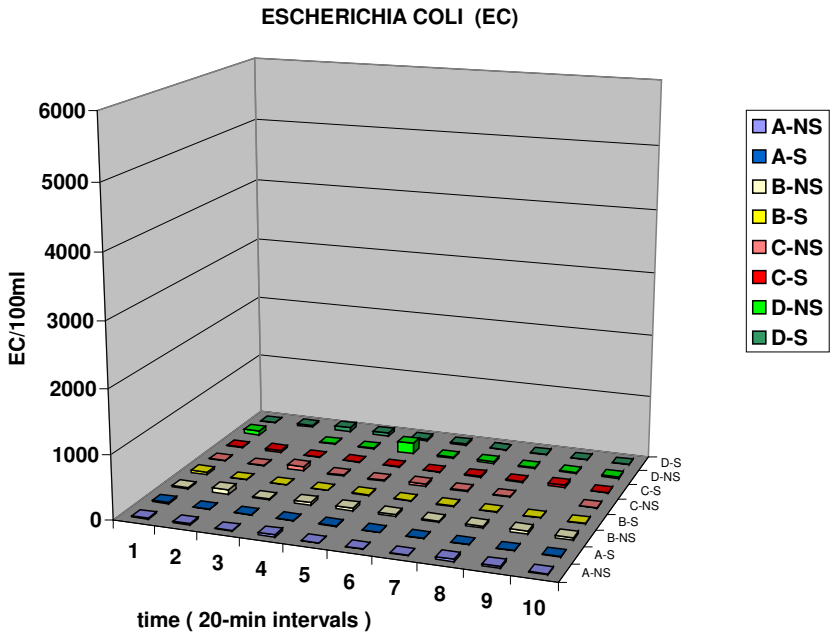
Annex 10. Temporal and spatial variability of the microbiological water quality at the five study sites: Concentrations of *Escherichia coli* and intestinal enterococci

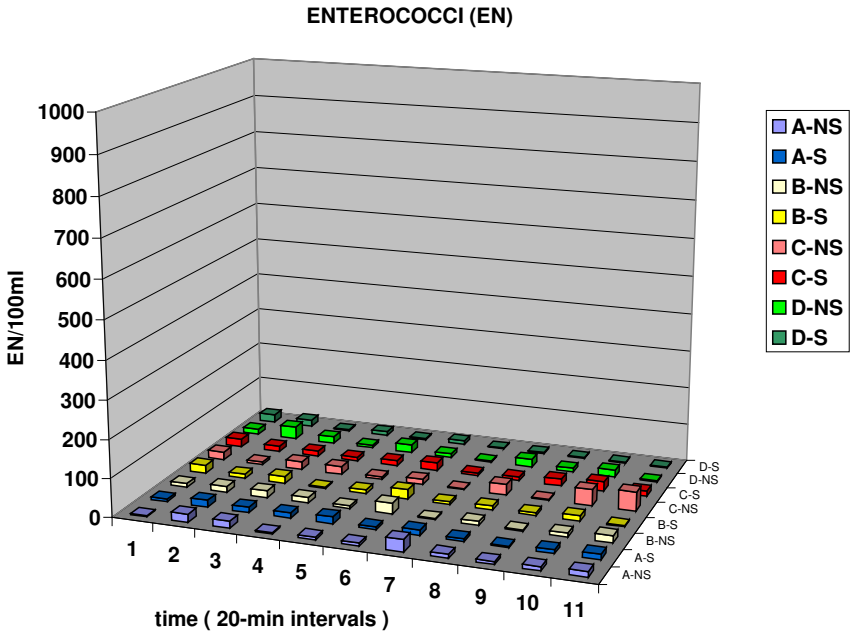
Water samples were collected every 20 minutes in the centres of the swimmers' and non-swimmers' zones of four roped-off areas at each of the five study sites. The figures in this annex demonstrate the temporal and spatial variability of the test parameters *Escherichia coli* and intestinal enterococci at the five sites. Sites were sorted according to the level of faecal contamination in ascending order.

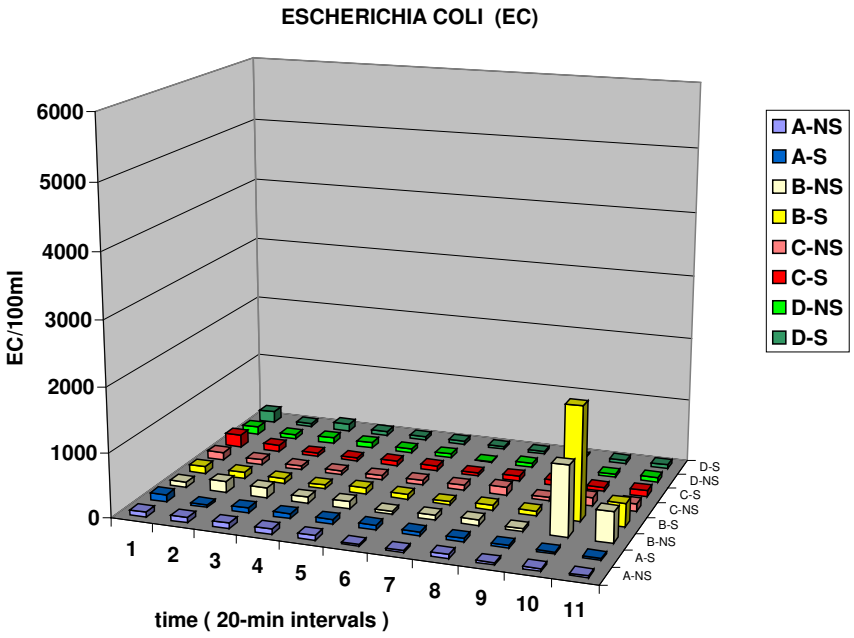
Legend:

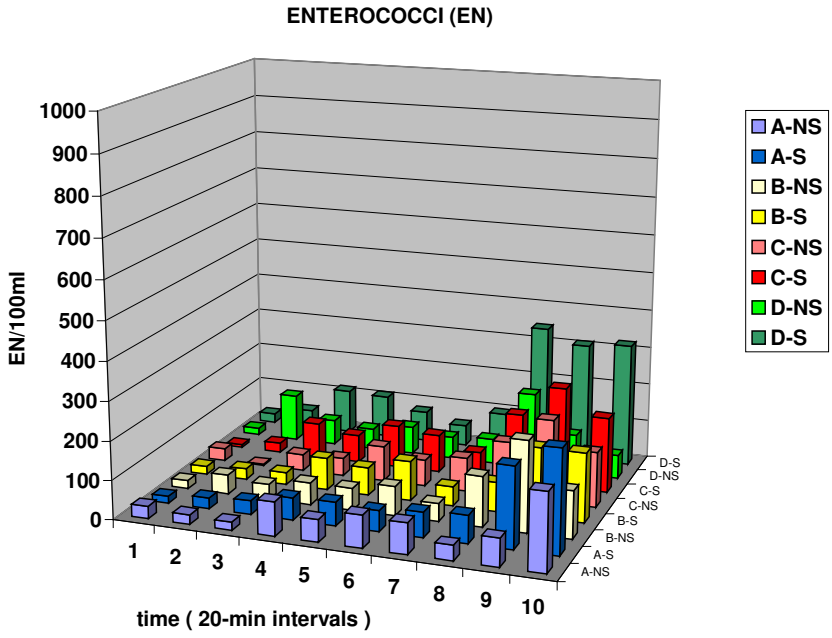
A, B, C, D	The four roped-off bathing areas on the beach
S	Swimmers' zone within bathing area (approx. 10 x 10 m)
NS	Non-swimmers' zone within bathing area (approx. 10 x 10 m)

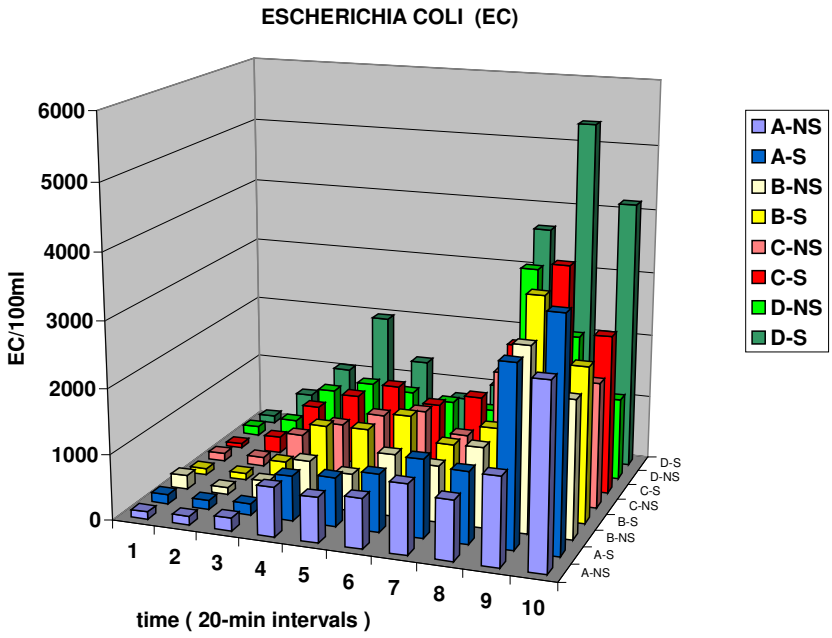

ENTEROCOCCI (EN)
EN/100ml
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
time (20-min intervals)
A-NS
A-S
B-NS
B-S
C-NS
C-S
D-NS
D-S

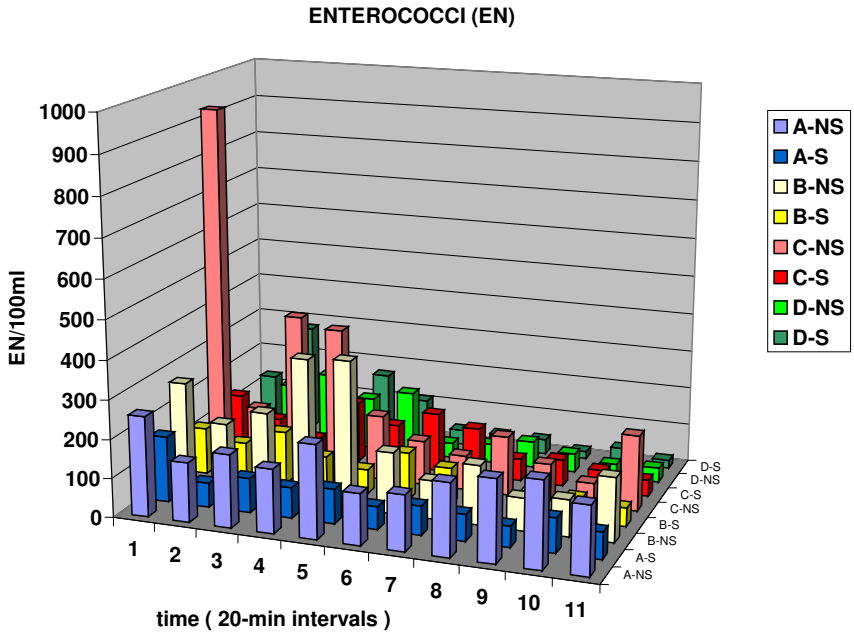

ESCHERICHIA COLI (EC)
EC/100ml
6000
5000
4000
3000
2000
1000
0
1
2
3
4
5
6
7
8
9
10
time (20-min intervals)
A-NS
A-S
B-NS
B-S
C-NS
C-S
D-NS
D-S

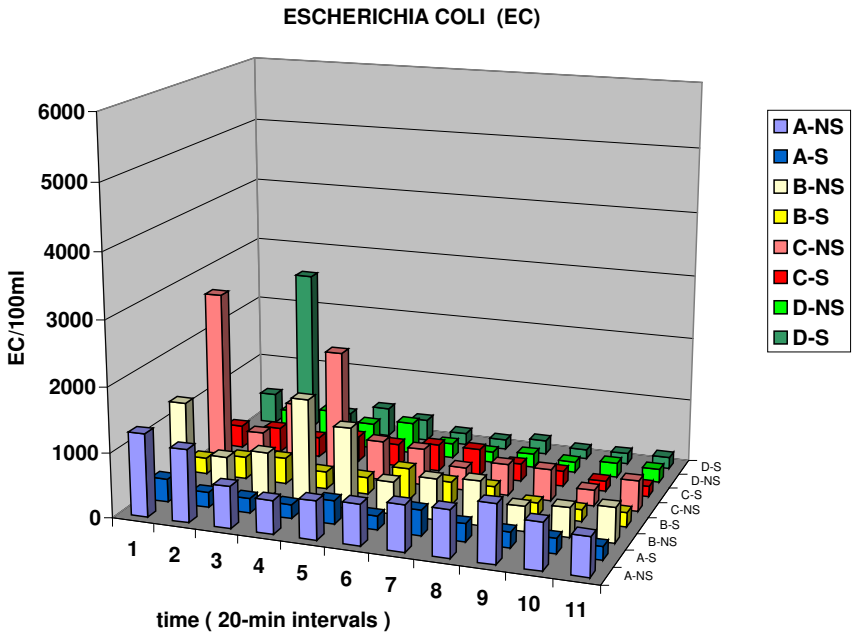

ENTEROCOCCI (EN)
EN/100ml
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
time (20-min intervals)
A-NS
A-S
B-NS
B-S
C-NS
C-S
D-NS
D-S

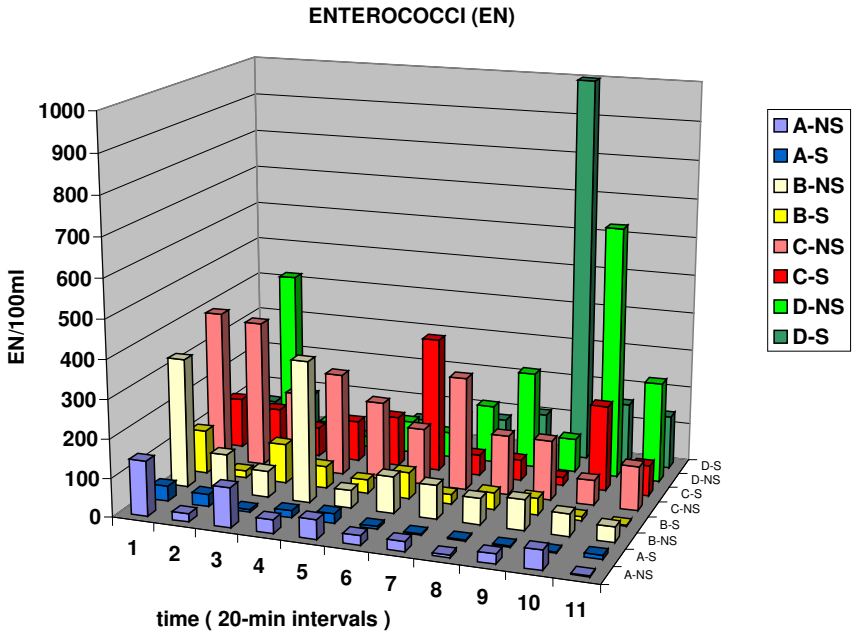

ESCHERICHIA COLI (EC)
EC/100ml
6000
5000
4000
3000
2000
1000
0
1
2
3
4
5
6
7
8
9
10
11
time (20-min intervals)
A-NS
A-S
B-NS
B-S
C-NS
C-S
D-NS
D-S

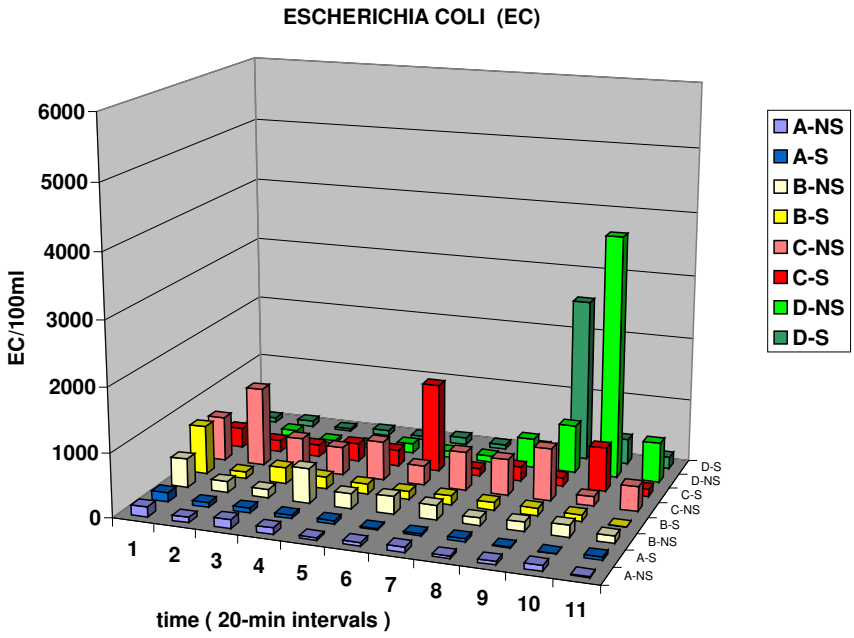

ENTEROCOCCI (EN)
EN/100ml
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
time (20-min intervals)
A-NS
A-S
B-NS
B-S
C-NS
C-S
D-NS
D-S


ESCHERICHIA COLI (EC)
EC/100ml
6000
5000
4000
3000
2000
1000
0
1
2
3
4
5
6
7
8
9
10
time (20-min intervals)
A-NS
A-S
B-NS
B-S
C-NS
C-S
D-NS
D-S

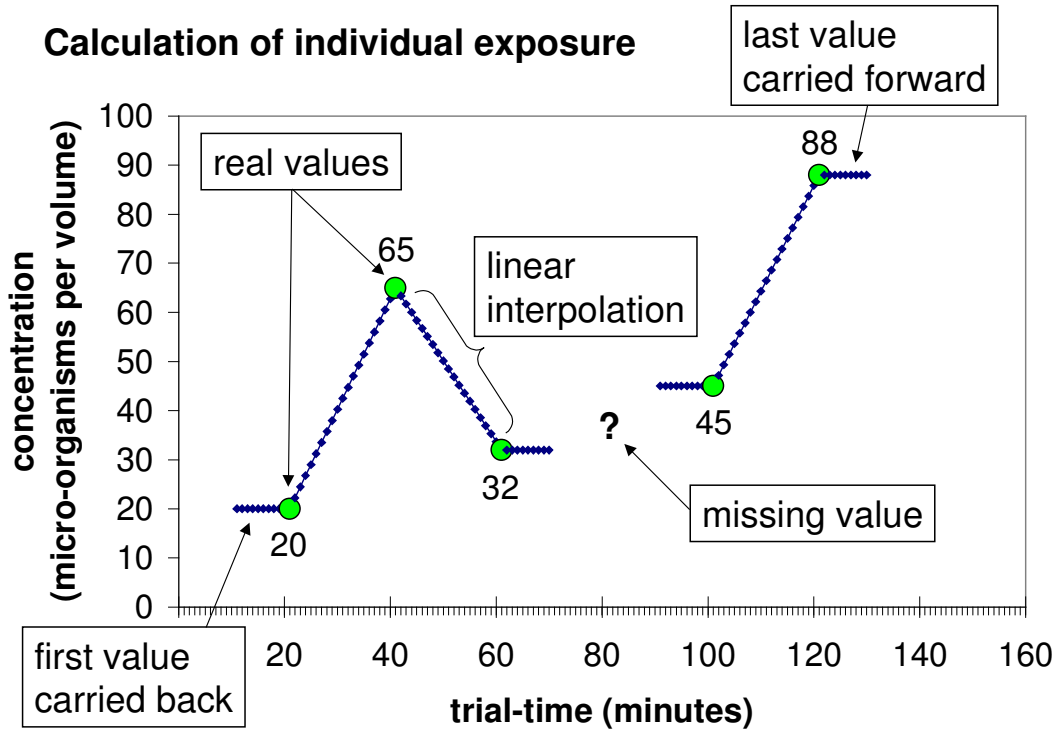

ENTEROCOCCI (EN)
EN/100ml
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
time (20-min intervals)
A-NS
A-S
B-NS
B-S
C-NS
C-S
D-NS
D-S


ESCHERICHIA COLI (EC)
EC/100ml
6000
5000
4000
3000
2000
1000
0
1
2
3
4
5
6
7
8
9
10
11
time (20-min intervals)
A-NS
A-S
B-NS
B-S
C-NS
C-S
D-NS
D-S


ENTEROCOCCI (EN)
EN/100ml
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
time (20-min intervals)
A-NS
A-S
B-NS
B-S
C-NS
C-S
D-NS
D-S


ESCHERICHIA COLI (EC)
EC/100ml
6000
5000
4000
3000
2000
1000
0
1
2
3
4
5
6
7
8
9
10
11
time (20-min intervals)
A-NS
A-S
B-NS
B-S
C-NS
C-S
D-NS
D-S

Annex 11. Principles of the calculation of individual exposure concentrations: interpolation between sample results and extrapolation of sample results

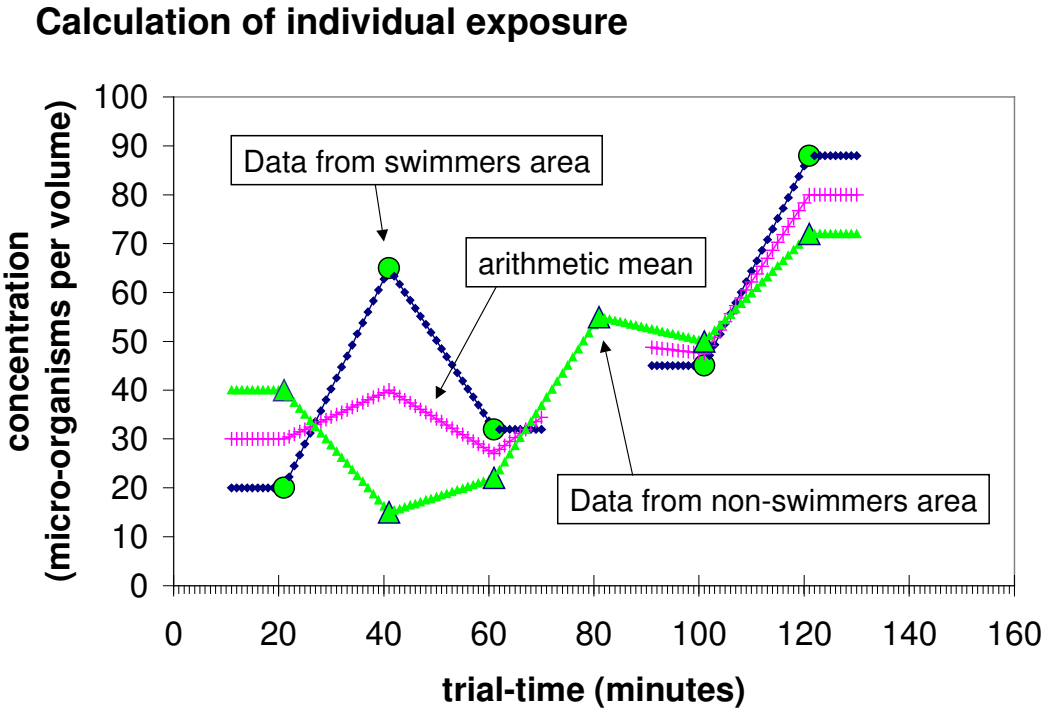


Legend

Green dots	Concentrations in the water samples collected at twenty-minute intervals
Blue dots	Concentrations calculated for the minutes before, after and between the sampling moments

First values were carried back, and last values were carried forward for a maximum of ten minutes. The same procedure was applied if sample results were missing due to analytical failures. Values between regular results were calculated by linear interpolation

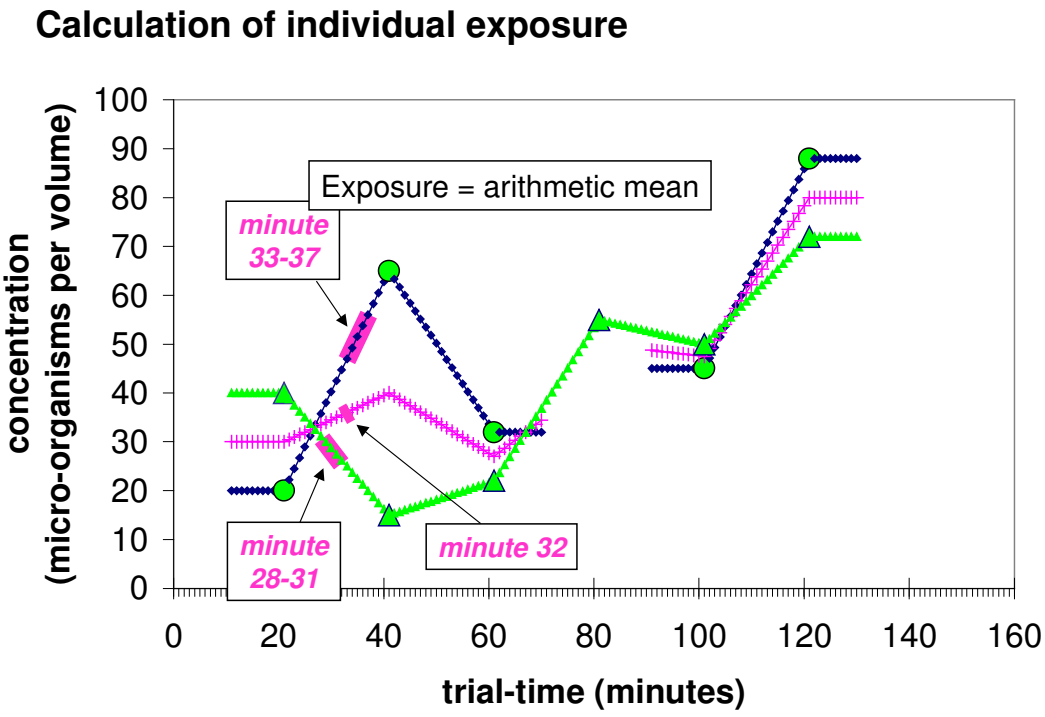
Annex 12. Principles of the calculation of individual exposure concentrations: interpolation between swimmers and non-swimmers areas



Legend

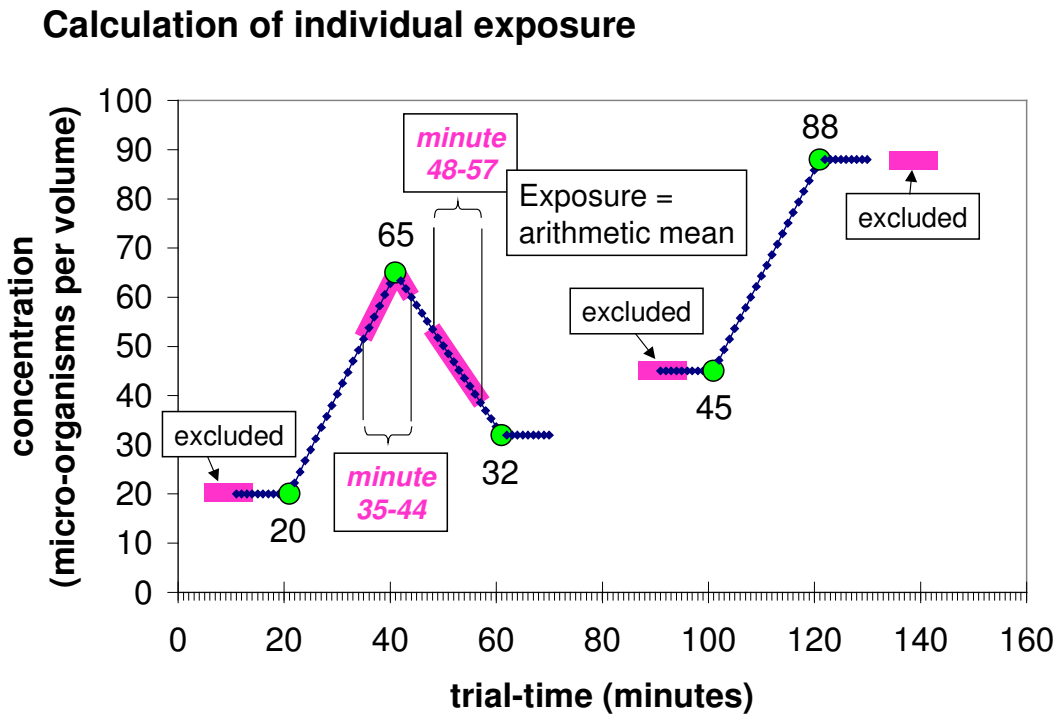
Green dots	Concentrations in the water samples collected at twenty-minute intervals in the swimmers' area
Green triangels	Concentrations in the water samples collected at twenty-minute intervals in the non-swimmers' area
Purple dashes	Arithmetic means between the concentrations in the swimmers' and in the non-swimmers' area after minute-wise interpolation between the sample results in both areas

Annex 13. Principles of the calculation of individual exposure concentrations: example for the assignment of calculated concentrations to an individual bather



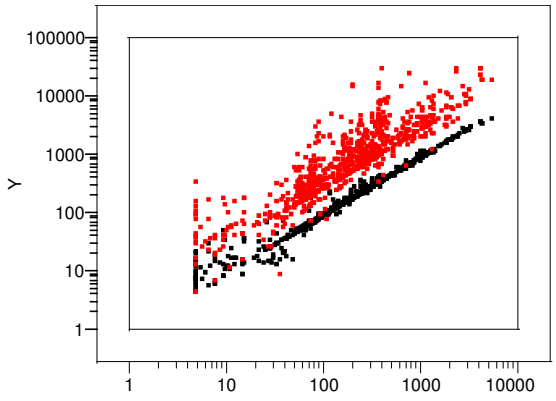
The bather stayed in the non-swimmers' area for four minutes. In the fifth minute the bather stayed in the non-swimmers' area as well as in the swimmers' area. In the following five minutes the bather stayed in the swimmers' area. The exposure concentration was calculated as the arithmetic mean of the concentrations in these ten minutes (concentrations marked in pink).

Annex 14. Principles of the calculation of individual exposure concentrations: examples for inclusions and exclusions

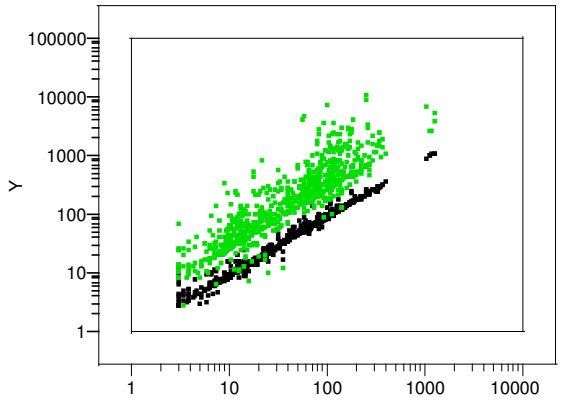


Only bathers for whom concentrations for each of the ten exposure minutes were available were included. If concentrations for any of the ten exposure minutes were missing either because the bathers entered the water too early or too late or because sample results were missing due to analytical failures, the bathers were excluded from the analysis.

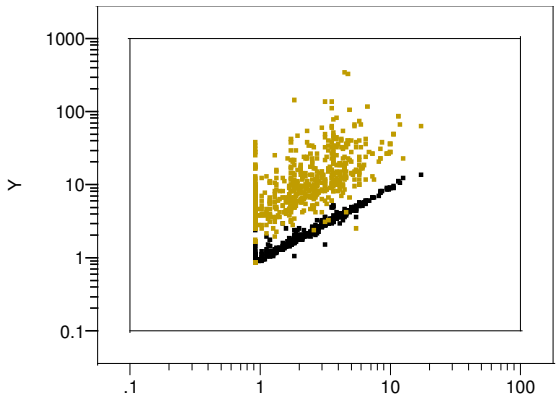
Annex 15. Influence of the exposure definition on the calculated exposure concentration for the four faecal indicator organisms



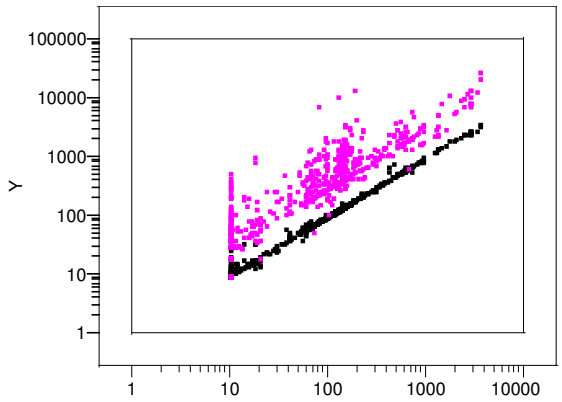
EC / 100 ml at 5th exposure minute



IE / 100 ml at 5th exposure minute



CP / 10 ml at 5th exposure minute

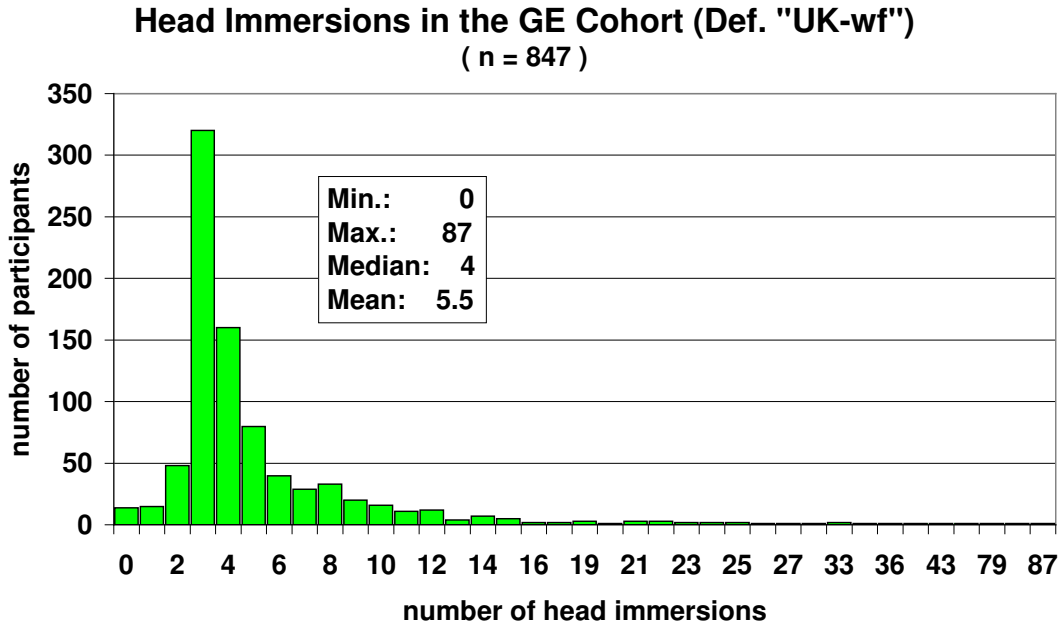


SOMCP / 100 ml at 5th exp. minute

Legend:

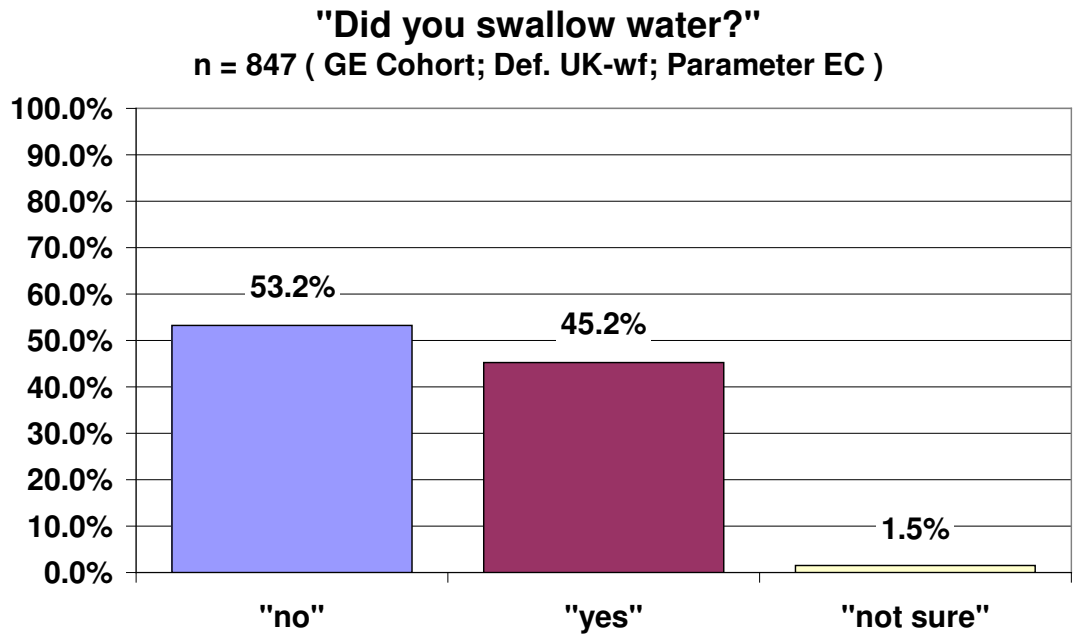
X	Concentration of the microbiological parameters at the 5th exposure minute of each bather
Y, black dots	Arithmetic mean of the 10 concentrations at the 10 individual exposure minutes of each bather (Exposure definition: "10 min bathing, 3 head immersions").
Y, colour dots	Sum of all concentrations at which the head was immersed, per bather (Exposure definition: "1 head immersion").
EC	*Escherichia coli*
IE	Intestinal Enterococci
CP	*Clostridium perfringens*
SOMCP	Somatic coliphages

Annex 16. Intensity of exposure: Number of head immersions



Participants were asked to immerse their head at least three times during the 10 minutes of bathing. Only 9% of the participants immersed their heads less often than required, while 53% voluntarily did it more often (median = 4; mean = 5.5; range = 0-87 head immersions). The type of exposure which was chosen in this study (10 minutes of bathing and at least three head immersions) is therefore probably rather an underestimation of the mean exposure intensity in "real life".

Annex 17. Intensity of exposure: Percentage of participants reporting to have swallowed water



For the outcome "gastroenteritis" in particular, the exposure dose, i. e. the number of microorganisms which were incorporated, depends not only on the concentration of the microorganisms in the water, but also on the incorporated volume of water. Besides the number of head immersions, swallowing water can therefore be considered a main influence factor. The role of swallowing water as a risk factor was studied by using it as an additional classification criterion for participants who were exposed at high or at low concentrations.

Annex 18. Crude incidence rates, excess risks (attributable risks) and relative risks of bathers vs. non-bathers 1 week and 3 weeks after exposure:

Disease definition	Bathers						Non-bathers					
	Cases				Participants		Cases				Participants	
	1 week		3 weeks		1 w	3 w	1 week		3 weeks		1 w	3 w
	n	(%)	n	(%)	n	n	n	(%)	n	(%)	n	n
AFRI	7	(0.9)	13	(1.7)	759	747	9	(1.1)	18	(2.2)	810	802
CC	116	(15.4)	171	(23.1)	754	741	109	(13.6)	162	(20.4)	804	793
EAR	15	(1.6)	21	(2.2)	950	942	7	(0.7)	13	(1.3)	1003	1001
EYE	16	(1.7)	25	(2.7)	944	938	16	(1.6)	25	(2.5)	999	997
GE_UK	28	(3.3)	54	(6.6)	837	818	13	(1.4)	43	(4.8)	920	902
GE_UK-wf	46	(5.4)	75	(9)	847	831	26	(2.8)	64	(7.1)	921	907
GE_NL-2	63	(7.4)	107	(13)	846	823	48	(5.2)	101	(11.1)	921	908
SKIN	82	(9.3)	113	(13)	880	869	27	(2.8)	41	(4.3)	957	947
UTI	2	(0.2)	4	(0.4)	944	939	4	(0.4)	11	(1.1)	1002	999

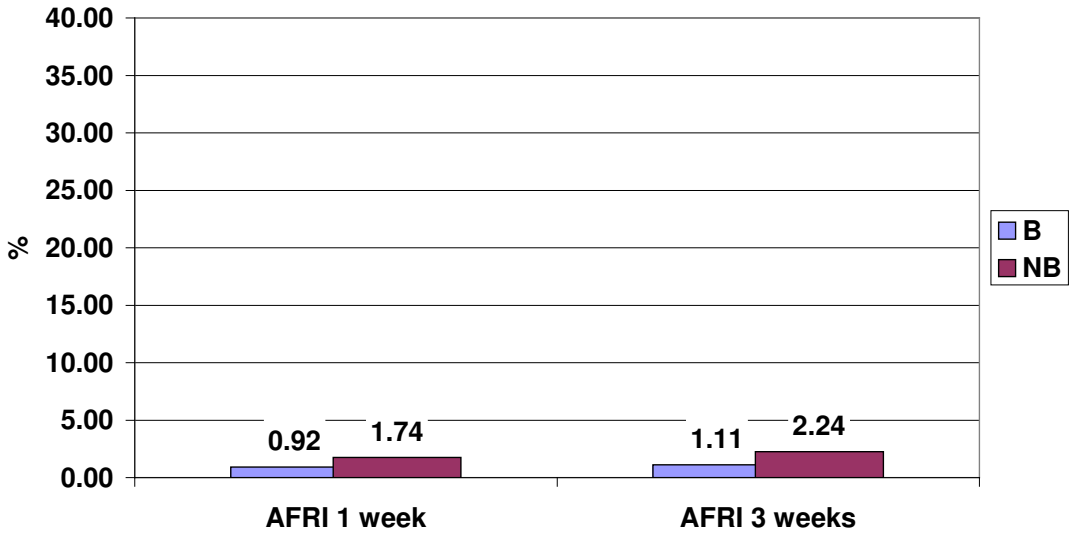
Disease definition	Excess cases		Excess risk		RR		p (Pearson's Chi sq. Test)				
	1 week	3 weeks	1 week	3 weeks	1 week	3 weeks	1 week		3 weeks		
	n	n	(%)	(%)							
AFRI	-2	-5	-0.2	-0.5	0.83	0.78	0.7098		0.4790		
CC	7	9	1.8	2.6	1.13	1.13	0.3051		0.2087		
EAR	8	8	0.9	0.9	2.26	1.72	0.0652		0.1179		
EYE	0	0	0.1	0.2	1.06	1.06	0.8717		0.8270		
GE_UK	15	11	1.9	1.8	2.37	1.38	0.0074	**	0.0996		
GE_UK-wf	20	11	2.6	2.0	1.92	1.28	0.0056	**	0.1306		
GE_NL-2	15	6	2.2	1.9	1.43	1.17	0.0531	(*)	0.2301		
SKIN	55	72	6.5	8.7	3.30	3.00	<.0001	***	<.0001	***	
UTI	-2	-7	-0.2	-0.7	0.53	0.39	0.4563		0.0901		

Significance: (*) border line
* < 0.05
** < 0.01
*** < 0.001

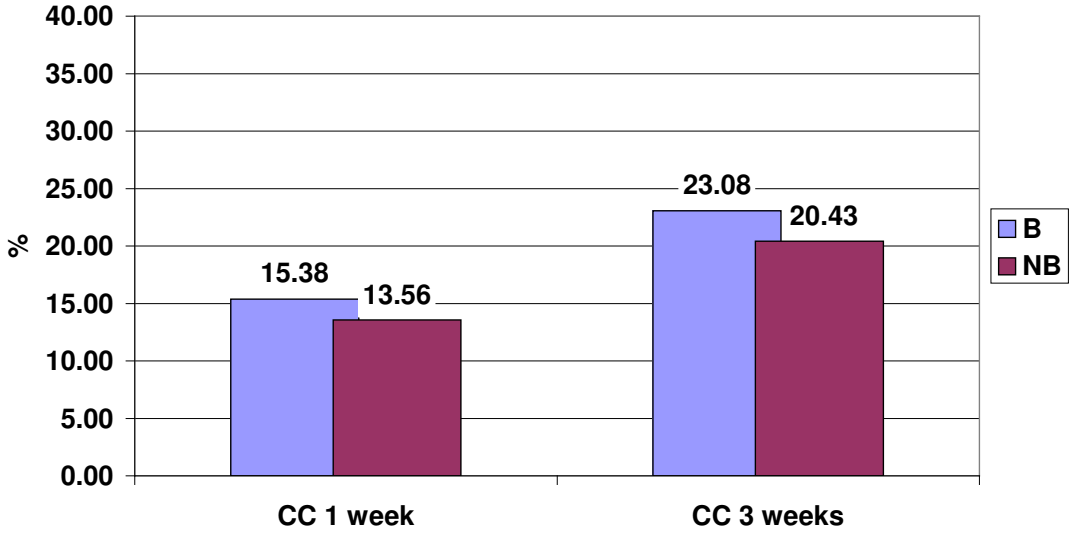
Disease definitions

AFRI	Acute febrile respiratory infections
CC	Common cold
EAR	Ear inflammation
EYE	Eye inflammation
GE_UK	Gastroenteritis, UK definition
GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis, NL-2 definition
SKIN	Skin infections, cutireactions
UTI	Urinary tract infections

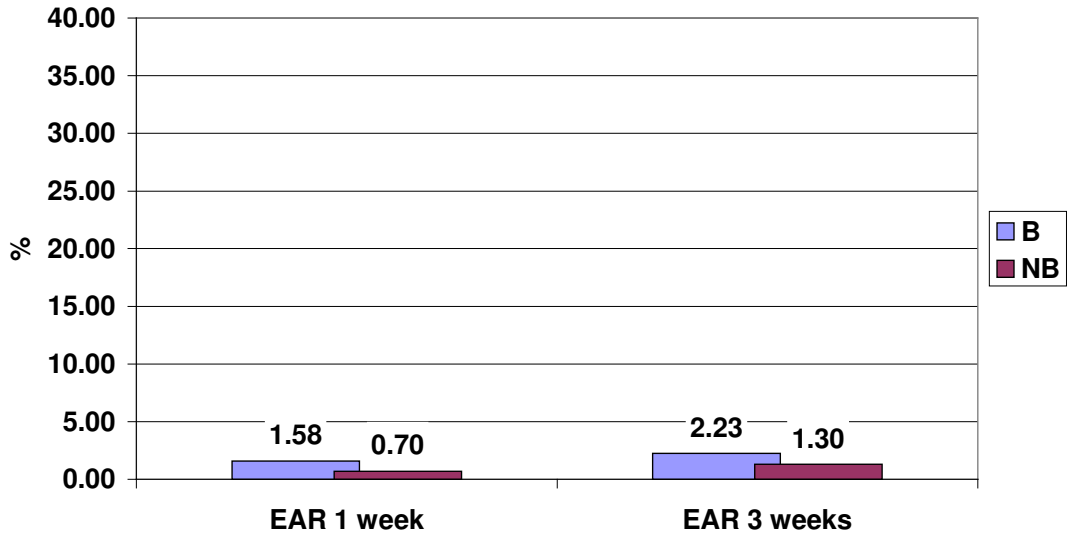
Crude incidence rates of AFRI (Acute febrile respiratory infections)
Bathers vs. non-bathers
1 week vs. 3 weeks after exposure



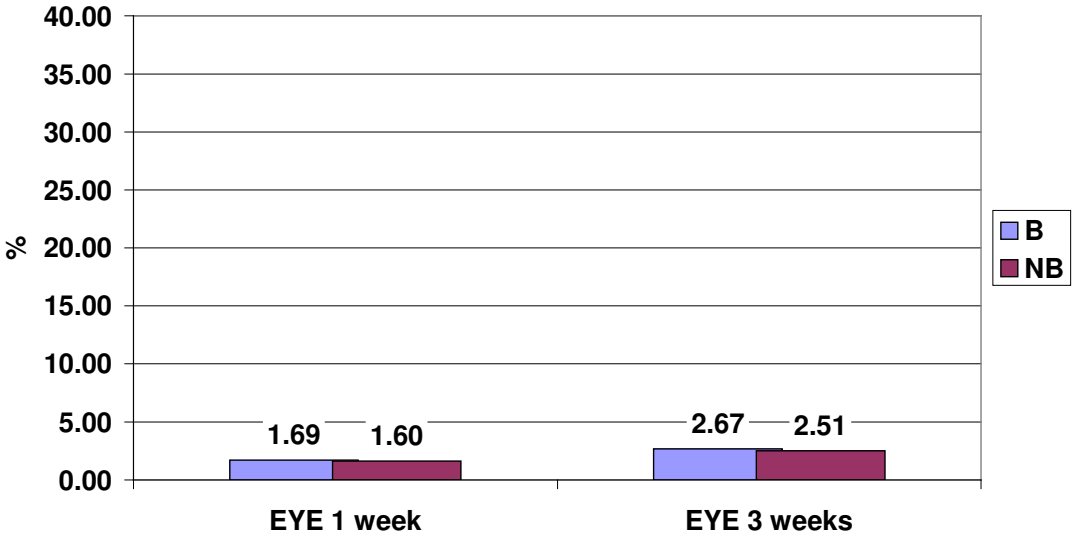
Crude incidence rates of CC (Common cold)
Bathers vs. non-bathers
1 week vs. 3 weeks after exposure



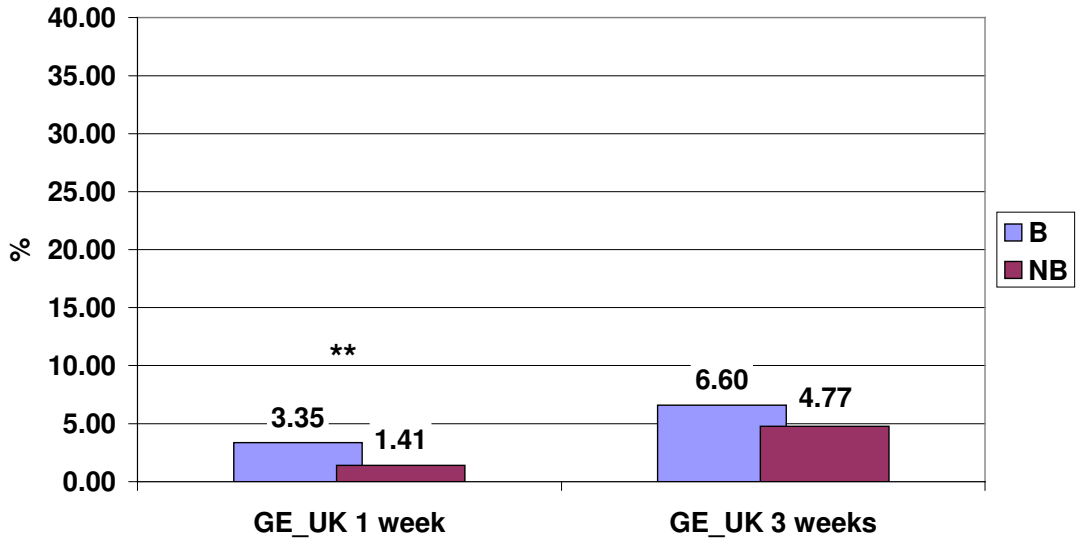
Crude incidence rates of EAR (Ear inflammation)
Bathers vs. non-bathers
1 week vs. 3 weeks after exposure



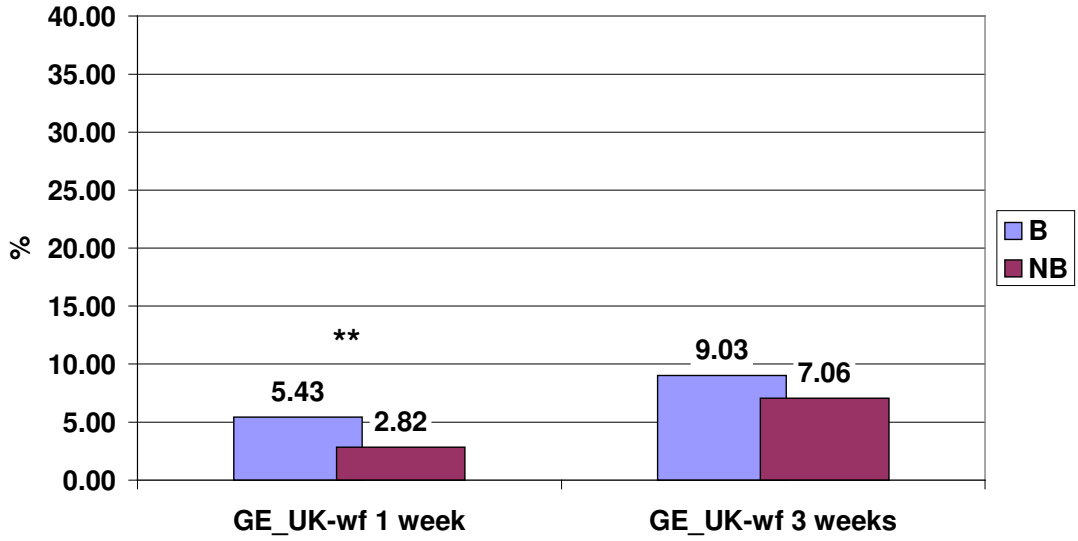
Crude incidence rates of EYE (Eye inflammation)
Bathers vs. non-bathers
1 week vs. 3 weeks after exposure



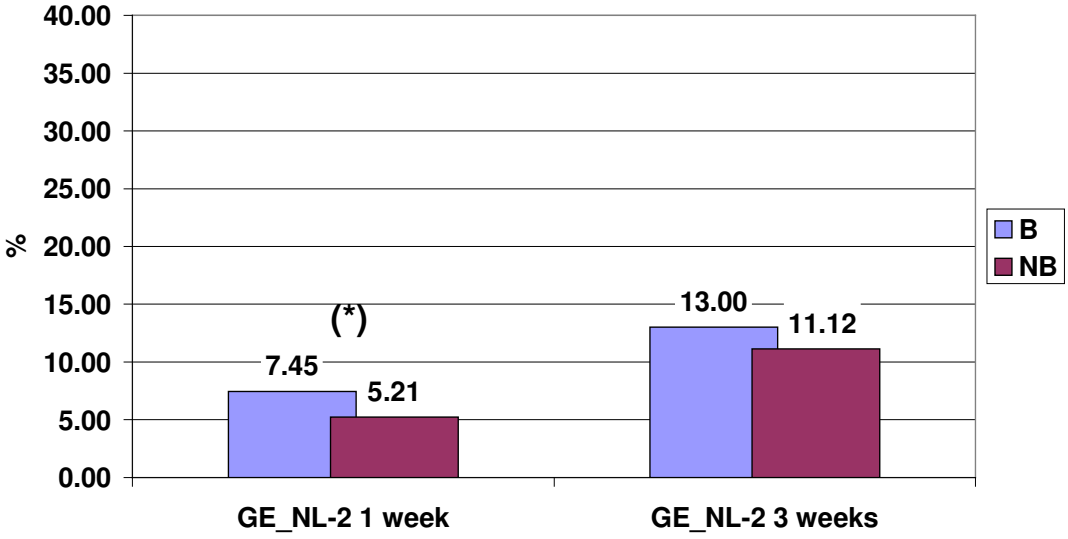
Crude incidence rates of GE_UK (Gastroenteritis, UK definition)
Bathers vs. non-bathers
1 week vs. 3 weeks after exposure



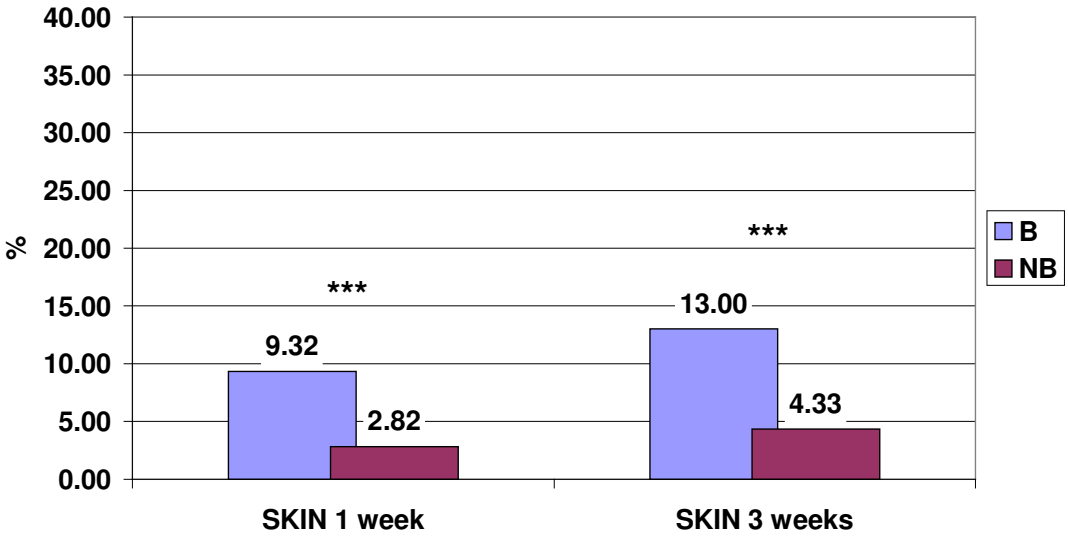
Crude incidence rates of GE_UK-wf
(Gastroenteritis, UK definition without consideration of stool frequency)
Bathers vs. non-bathers
1 week vs. 3 weeks after exposure



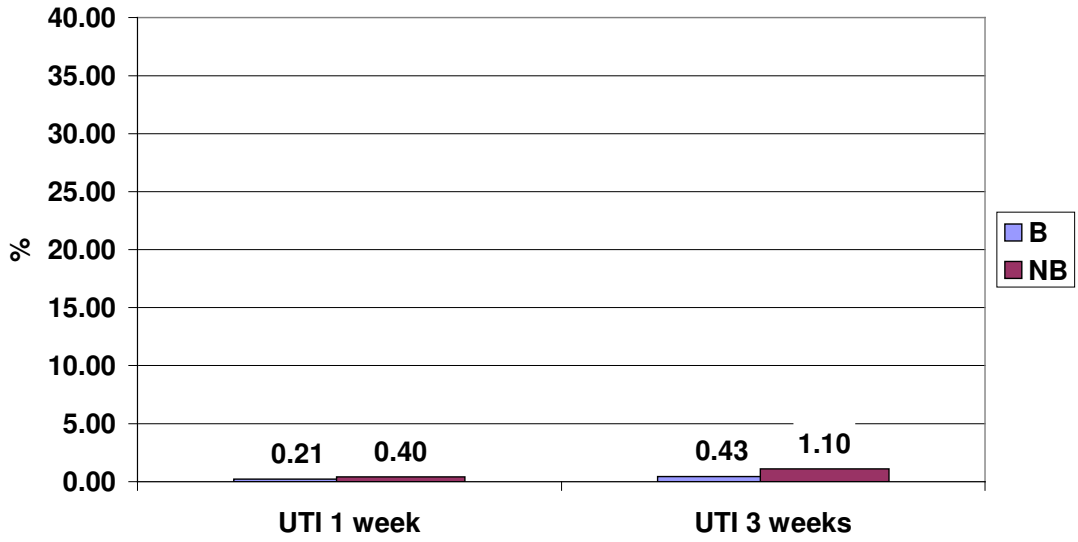
Crude incidence rates of GE_NL-2 (Gastroenteritis, NL-2 definition)
Bathers vs. non-bathers
1 week vs. 3 weeks after exposure



Crude incidence rates of SKIN (Skin infections, cutireactions)
Bathers vs. non-bathers
1 week vs. 3 weeks after exposure

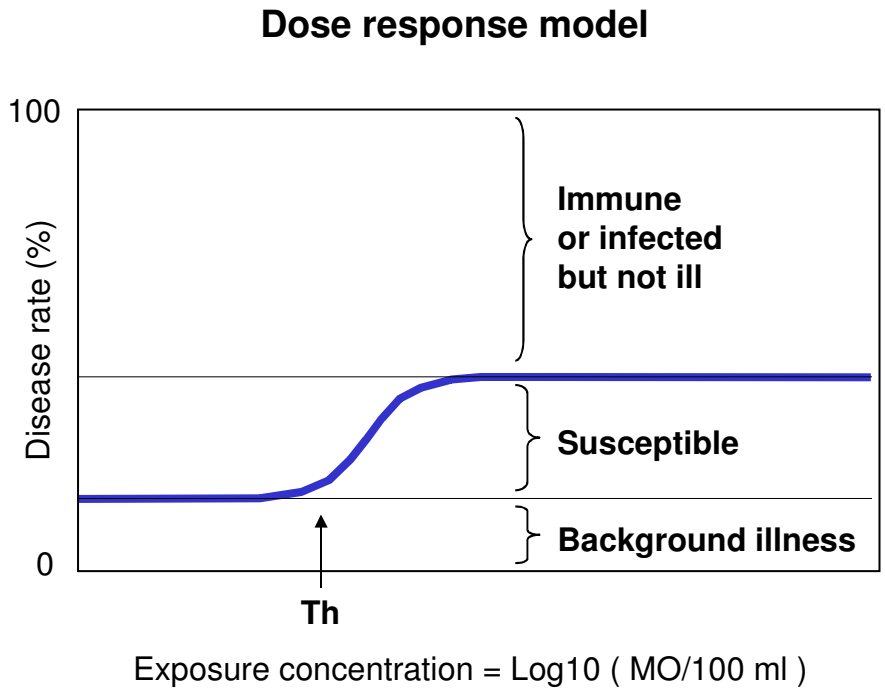


Crude incidence rates of UTI (Urinary tract infections)
Bathers vs. non-bathers
1 week vs. 3 weeks after exposure



Annex 19. Dose response model assumptions and control of bias

The figures in this annex illustrate the dose-response model assumptions on which the data analysis was based.



Legend:

Disease rate (%)	Incidence rate of disease (e. g. gastroenteritis) reported by study participants within one week or three weeks after exposure
Log10 (MO/100ml)	Concentration of microorganisms assigned to a bather
Th	Threshold of effect; NOAEL = no observed adverse effect level
Immune or infected but not ill	Part of exposed participants who had no symptoms either because they are immune or because the infection is asymptomatic
Susceptible	Part of exposed participants who are susceptible to symptomatic infections
Background illness	Part of exposed participants who have symptomatic infections due to independent other sources of infection, e. g. household contact, contaminated meals, etc.

The model assumptions were:

1. There is a baseline risk ("background rate of illness") for bathers which is due to other risk factors independent of bathing, and which is not significantly lower than the incidence rate of disease among non-bathers, i. e. it was assumed that a bathing duration of 10 minutes is not protective for any of the evaluated infectious diseases.

2. There is a maximum risk level (ceiling) which is less than 100%, as a certain part of the exposed population can be expected to be either immune or to become infected without developing any symptoms ("silent infections").

3. There is a certain concentration range within which the risk increases from baseline level towards the ceiling. When the exposure concentration of microorganisms is plotted on a log10 transformed scale on the x-axis this part of the curve is ideally sigmoid shaped.

4. The slope of the sigmoid part of the curve depends on the virulence of the pathogens which are present in the water.

5. Below and above that concentration range there is no trend or correlation between the concentration of monitored microorganisms and risk, because risk remains at a constant level.

6. For standardised exposure activities there is a threshold of effect (NOAEL = no observed adverse effect level), i. e. a concentration below which there is no detectable difference between the incidence rate among bathers and the incidence rate among non-bathers.

7. When a concentration of organisms (MO/100ml) is modelled as the x-variable instead of a dose (i.e. the number of organisms which are incorporated or which come into contact e.g. with the skin, the eyes etc.) the NOAEL depends on the intensity of exposure. A low exposure intensity (e.g. 1 head immersion) will result in a higher NOAEL than a high exposure intensity (e. g. 3 or more head

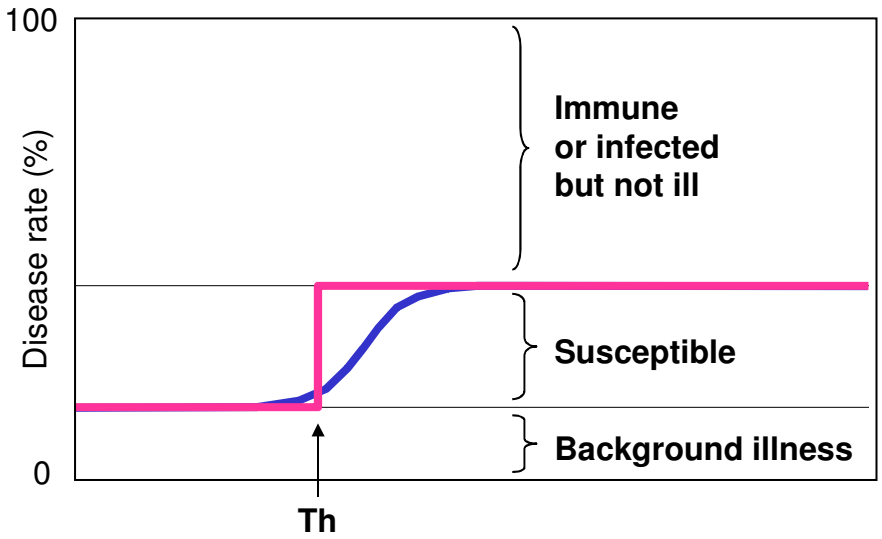
immersions) because the dose is the product of concentration and exposure intensity. E. g. the relevant dose with respect to gastroenteritis will be the product of the concentration and the volume of water which has been incorporated, and the relevant dose with respect to skin ailments will be the product of concentration and exposure duration.

8. When indicator organisms are used instead of pathogens the NOAEL will also depend on the pathogen/indicator ratio.

9. The maximum percentage of exposed persons who become ill depends on the susceptibility and vice versa on the immunity of the cohort. E. g. a cohort of tourists can be expected to have a much higher susceptibility than a cohort of local residents. This has already been demonstrated for bathers at Egyptian beaches by Cabelli et al. (Prüss, 1998).

To determine base line risk levels, threshold concentrations (NOAEL's) and maximum risk levels in this study, the sigmoid shaped dose response model was reduced to a simple step model consisting of only these three parameters: 1. Concentrations which revealed the most significant difference (minimum p value) in a series of Pearson's chi square tests comparing the incidence rates below and above the concentrations observed during the trials were considered to be the most probable estimates for the thresholds of effect (NOAEL's). 2. The incidence rate among non-bathers was used to estimate the base-line risk. 3. The incidence rate among bathers above threshold concentration was used to estimate the maximum risk level.

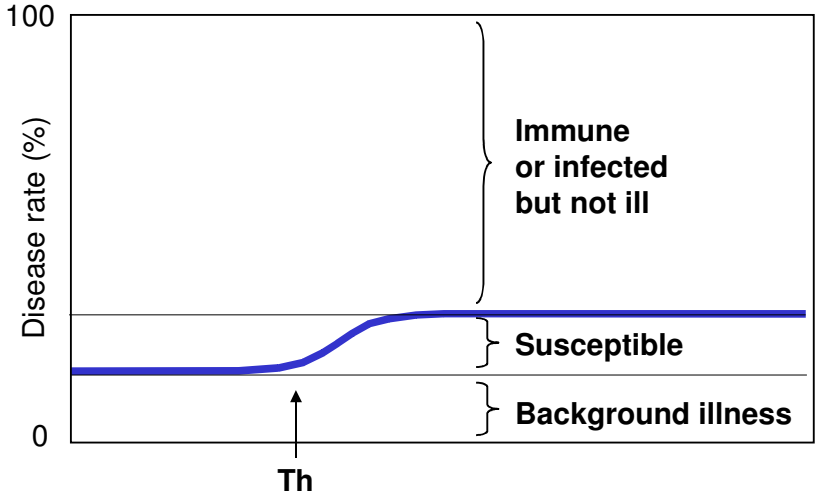
Simplified dose response model: Step model

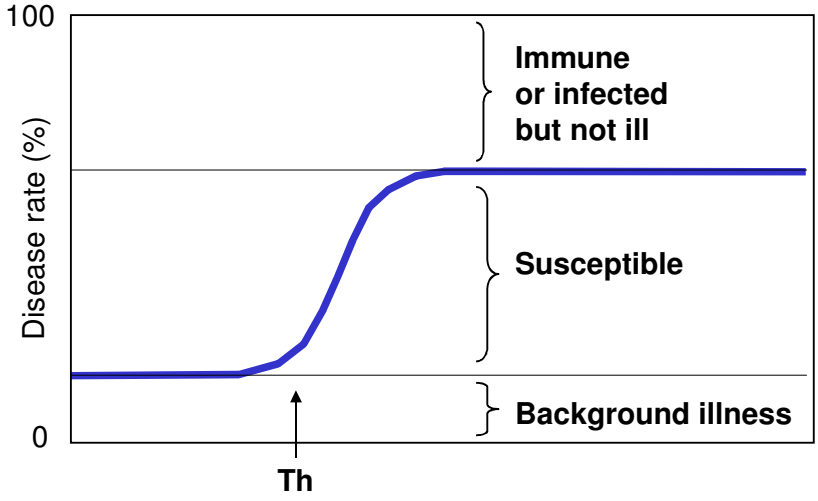


Legend:

Disease rate (%)	Incidence rate of disease (e. g. gastroenteritis) reported by study participants within one week or three weeks after exposure
Log10 (MO/100ml)	Concentration of microorganisms assigned to a bather
Th	Threshold of effect; NOAEL = no observed adverse effect level
Immune or infected but not ill	Part of exposed participants who show no symptoms either because they are immune or because the infection is asymptomatic
Susceptible	Part of exposed participants who are susceptible to symptomatic infections
Background illness	Part of exposed participants who have symptomatic infections due to independent other sources of infection, e. g. household contact, contaminated meals etc.

Different susceptibility of exposed cohort





Legend

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Log10 (MO/100ml)	Concentration of microorganisms assigned to a bather
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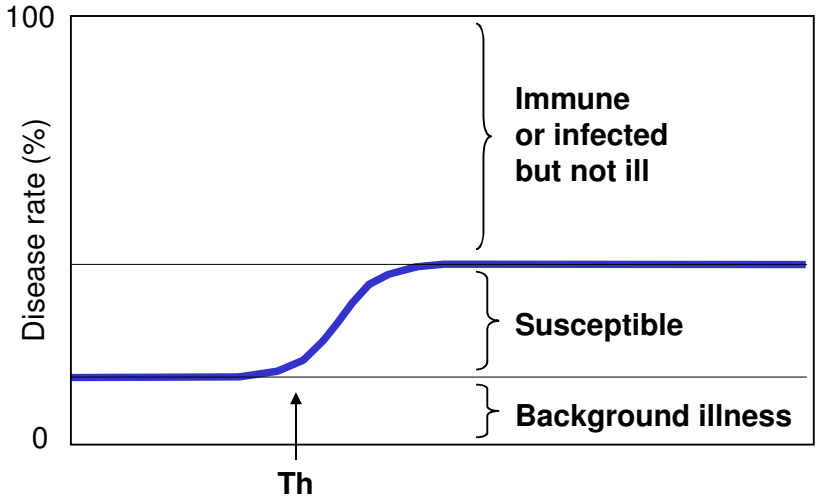
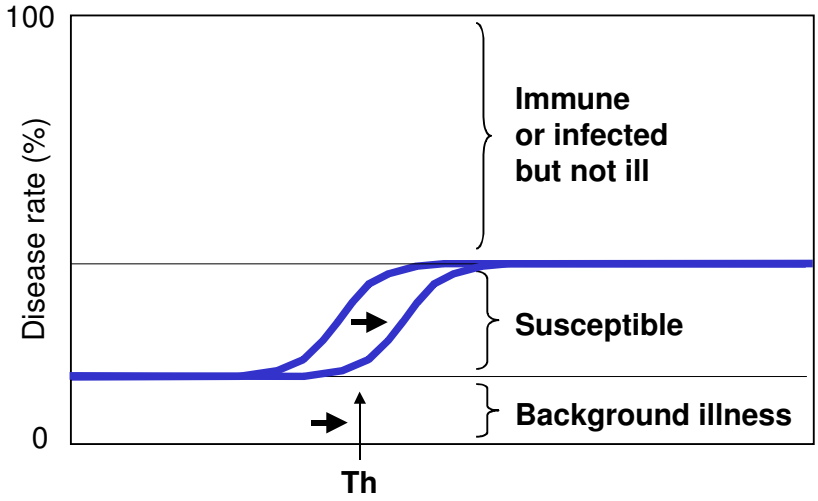
Susceptible	Part of exposed participants who are susceptible to symptomatic infections
Background illness	Part of exposed participants who have symptomatic infections due to independent other sources of infection, e. g. household contact, contaminated meals etc.

Different exposure intensity



Exposure concentration = Log10 (MO/100 ml)

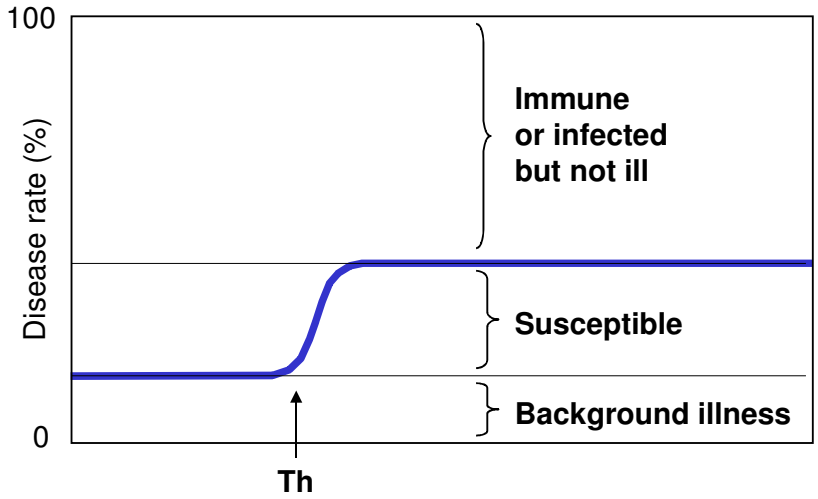


Exposure concentration = Log10 (MO/100 ml)

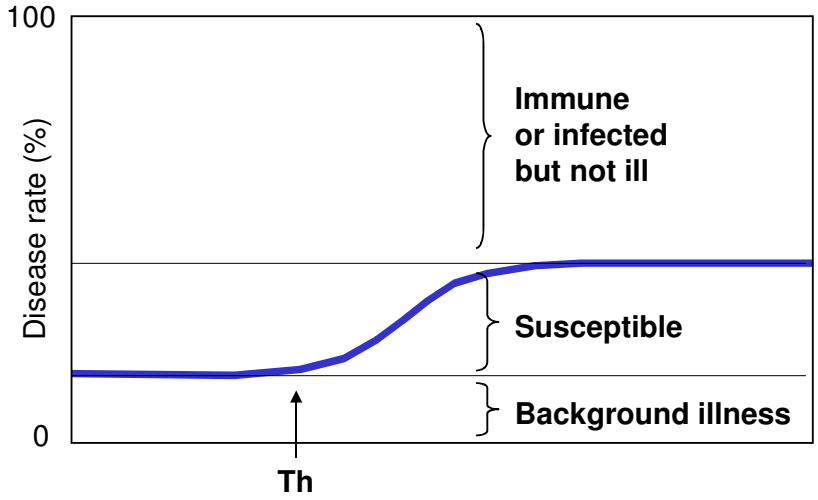
Legend

Disease rate (%)	Incidence rate of disease (e. g. gastroenteritis) reported by study participants within one week or three weeks after exposure
Log10 (MO/100ml)	Concentration of microorganisms assigned to a bather
Th	Threshold of effect; NOAEL = no observed adverse effect level
Immune or infected but not ill	Part of exposed participants who show no symptoms either because they are immune or because the infection is asymptomatic
Susceptible	Part of exposed participants who are susceptible to symptomatic infections
Background illness	Part of exposed participants who have symptomatic infections due to independent other sources of infection, e. g. household contact, contaminated meals etc.

Different virulence of pathogens



Exposure concentration = Log10 (MO/100 ml)



Exposure concentration = Log10 (MO/100 ml)

Legend

Disease rate (%)	Incidence rate of disease (e. g. gastroenteritis) reported by study participants within one week or three weeks after exposure
Log10 (MO/100ml)	Concentration of microorganisms assigned to a bather
Th	Threshold of effect; NOAEL = no observed adverse effect level
Immune or infected but not ill	Part of exposed participants who show no symptoms either because they are immune or because the infection is asymptomatic
Susceptible	Part of exposed participants who are susceptible to symptomatic infections
Background illness	Part of exposed participants who have symptomatic infections due to independent other sources of infection, e. g. household contact, contaminated meals etc.

The described dose-response relationships (better: concentration response relationships) may be influenced mainly by four different forms of bias: misclassification bias, recall bias, confounding and effect modification.

1. Misclassification bias would result from an imprecise assignment of exposure concentrations to individual bathers. It would produce false low NOAEL's and false shallow slopes of the dose-response curves. It would not, however, affect the base-line risk and the maximum risk level. Misclassification in this study was reduced to a minimum by monitoring the water quality at very short time intervals (20 minutes) at exactly the place where the bathers were exposed, and by applying very precise microbiological methods and strict quality control procedures, especially for the two parameters *Escherichia coli* and intestinal enterococci (three 96 well microtiter plates instead of one per sample; short transportation and storage times; application of external quality control materials).

2. Recall bias would result in higher incidence rates of disease among exposed individuals than among unexposed individuals because exposed individuals might tend to remember symptoms better than unexposed individuals. This effect could not be totally excluded as, of course, the participants knew whether they had been bathing or not. However, they were not aware of the water quality at the time of their exposure, nor were the organisers of the study. Recall bias would therefore most probably not affect the NOAEL. It might result in a higher base-line risk level among bathers and in a false high maximum risk level. For the outcome variable "gastroenteritis" such an effect could not be observed for the base-line risk level. It is therefore considered improbable that there was a recall bias effect solely influencing the maximum risk level.

3. Confounding would result from an imbalance of independent other risk factors among bathers and non-bathers, or within the group of bathers from an imbalance of such risk factors among those who were exposed at high or low concentrations. Confounding would mean that an effect is erroneously attributed to bathing or to

bathing at high concentrations of microorganisms. It could affect the base-line risk, the threshold concentration (NOAEL), the slope and the maximum risk level. In an extreme situation confounding could simulate a dose-response relationship which does not really exist at all. Confounding was therefore controlled by a multiple logistic regression procedure (effect likelihood ratio tests) which included non-bathers as well as bathers, and in which the threshold concentration and a variety of other identified risk factors were modelled as effect variables with the disease as outcome variable. Dose-response models were not considered to be valid when the determined threshold concentration (NOAEL) did not remain a significant effect ($p<0,05$) in these tests.

4. Effect modification would result from factors which are homogeneously distributed over the group of bathers and non-bathers, but affect (modify) the intensity of the outcome variable, i. e. the incidence rate of disease, when they occur in combination with other effect variables e. g. a high or low exposure concentration. Effect modification would also affect the NOAEL. however, as the cohort was recruited from the normal population, effect modification can be assumed to occur in "real life" to an extent similar to which it may have occurred in this study. It was therefore not considered to be a form of bias which has to be controlled for. If other risk factors revealed significant interaction effects in the effect likelihood ratio tests mentioned above, this was recorded but was not analysed further.

The model assumptions described in this annex in combination with the possible effects of bias are sufficient to explain the high degree of variability in the results from a variety of epidemiological studies investigating health effects from bathing in sea or fresh water (Prüss, 1998). They can also explain the differences and similarities between the results of this study and the results of studies with similar design performed at UK coastal bathing sites (Kay et al., 1994).

Annex 20. Step models for dose response relationships: Estimates for thresholds of effect (NOAEL's); incidence rates below and above threshold concentrations; relative risks and attributable risks

For all exposure concentrations which occurred in this study incidence rates of disease were calculated among bathers exposed up to this concentration and among bathers exposed above this concentration, and a Pearson's chi square test was performed at each concentration level. The concentration level which revealed a minimum p value was considered to be the most probable estimate for the threshold of effect (i. e. the NOAEL; no observed adverse effect level). Threshold concentrations were considered to be valid only when the following conditions were fulfilled: minimum $p < 0.05$; no suspect test result (no expected cell value < 5); incidence rate below threshold not significantly lower than incidence rate of non-bathers; incidence rate above threshold significantly higher than incidence rate of non-bathers; threshold concentration not confounded by any of the potential confounding variables which were evaluated in this study (evaluation by multiple logistic regression; effect likelihood ratio tests). The tables in this annex also list the corresponding relative risks and attributable risks.

Legend

Disease definitions:

GE_UK	Gastroenteritis, UK definition
GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis, NL-2 definition
SKIN	Skin infections, cutireaction

Microbiological parameters:

CP	*Clostridium perfringens*
EC	*Escherichia coli*
IE	Intestinal enterococci
SOMCP	Somatic coliphages
AE	Aeromonads

Parameter units:

MPN	Most probable number
CFP	Colony forming particles
PFP	Plaque forming particles

Miscellaneous:

Threshold	Threshold of effect (estimate for the NOAEL)
conc.	Concentration
p	Pearson's Chi square p value (bathers vs. non-bathers)
n	total number of participants per category
95% CI	95% confidence interval
RR	Relative risk (incidence rate of bathers / incidence rate of non-bathers)
aR	attributable risk (incidence rate of bathers - incidence rate of non-bathers); synonymous: "excess risk"
not applicable	Compared to bathing duration the number of head immersions cannot be assumed to relevantly effect the incidence rate of skin infections or cutireactions. It was therefore considered inappropriate to apply exposure definition 2 ("1 head immersion") to skin ailments.

Table 1: Incidence rates among non-bathers, bathers below threshold concentrations and bathers above threshold concentrations for exposure definition 1

Exposure definition 1:
("10 min bathing; 3 head immersions")

Disease definition	Parameter	Non-bathers				Bathers <= Threshold					Threshold	Bathers > Threshold				
		cases	n	(%)	[95% CI]	cases	n	(%)	[95% CI]	p	conc.	cases	n	(%)	[95% CI]	p
GE_UK	EC /100 ml	13	920	(1.4)	[0.8; 2.4]	7	409	(1.7)	[0.7; 3.5]	0.6800	180	21	419	(5.0)	[3.1; 7.6]	0.0001
GE_UK-wf	EC /100 ml	26	921	(2.8)	[1.9; 4.1]	5	259	(1.9)	[0.6; 4.4]	0.4275	78	41	579	(7.1)	[5.1; 9.5]	0.0001
GE_NL-2	EC /100 ml	48	921	(5.2)	[3.9; 6.9]	18	396	(4.5)	[2.7; 7.1]	0.6113	167	45	441	(10.2)	[7.5; 13.4]	0.0006
GE_UK	IE /100 ml	13	920	(1.4)	[0.8; 2.4]	5	320	(1.6)	[0.5; 3.6]	0.7905	24	23	508	(4.5)	[2.9; 6.7]	0.0003
GE_UK-wf	IE /100 ml	26	921	(2.8)	[1.9; 4.1]	6	307	(2.0)	[0.7; 4.2]	0.4080	21	40	531	(7.5)	[5.4; 10.1]	0.0000
GE_NL-2	IE /100 ml	48	921	(5.2)	[3.9; 6.9]	12	321	(3.7)	[1.9; 6.4]	0.2983	24	51	516	(9.9)	[7.4; 12.8]	0.0008
GE_UK	CP /10 ml	13	920	(1.4)	[0.8; 2.4]	4	329	(1.2)	[0.3; 3.1]	1.0000	1.3	24	508	(4.7)	[3.1; 6.9]	0.0002
GE_UK-wf	CP /10 ml	26	921	(2.8)	[1.9; 4.1]	8	331	(2.4)	[1.0; 4.7]	0.7079	1.3	38	516	(7.4)	[5.3; 10.0]	0.0001
GE_NL-2	CP /10 ml	48	921	(5.2)	[3.9; 6.9]	13	331	(3.9)	[2.1; 6.6]	0.3624	1.3	50	515	(9.7)	[7.3; 12.6]	0.0012
GE_UK	SOMCP /100 ml	13	920	(1.4)	[0.8; 2.4]	16	656	(2.4)	[1.4; 3.9]	0.1352	150 #	11	169	(6.5)	[3.3; 11.3]	0.0004
GE_UK-wf	SOMCP /100 ml	26	921	(2.8)	[1.9; 4.1]	7	305	(2.3)	[0.9; 4.7]	0.6326	10	37	530	(7.0)	[5.0; 9.5]	0.0002
GE_NL-2	SOMCP /100 ml	48	921	(5.2)	[3.9; 6.9]	11	304	(3.6)	[1.8; 6.4]	0.2694	10	49	530	(9.2)	[6.9; 12]	0.0031
SKIN *	AE /100 ml	27	957	(2.8)	[1.9; 4.1]	19	357	(5.3)	[3.2; 8.2]	0.0282	5622	62	508	(12.2)	[9.5; 15.4]	0.0000

* Incidence rate of bathers exposed <= threshold significantly higher than incidence rate of non-bathers (two-step dose response model)

\# threshold estimate probably too high, due to a second local minimum of Chi square p values

Table 2: Incidence rates among non-bathers, bathers below threshold concentrations and bathers above threshold concentrations for exposure definition 2

Exposure definition 2:
("1 head immersion")

Disease definition	Parameter	Non-bathers				Bathers <= Threshold					Threshold	Bathers > Threshold				
		cases	n	(%)	[95% CI]	cases	n	(%)	[95% CI]	p	conc.	cases	n	(%)	[95% CI]	p
GE_UK	EC /100 ml	13	920	(1.4)	[0.8; 2.4]	11	555	(2.0)	[1.0; 3.5]	0.4028	1453	17	273	(6.2)	[3.7; 9.8]	0.0000
GE_UK-wf	EC /100 ml	26	921	(2.8)	[1.9; 4.1]						---	---	---	---	---	---
GE_NL-2	EC /100 ml	48	921	(5.2)	[3.9; 6.9]	37	623	(5.9)	[4.2; 8.1]	0.5388	2163	26	214	(12.1)	[8.1; 17.3]	0.0002
GE_UK	IE /100 ml	13	920	(1.4)	[0.8; 2.4]	5	351	(1.4)	[0.5; 3.3]	1.0000	123	23	477	(4.8)	[3.1; 7.1]	0.0001
GE_UK-wf	IE /100 ml	26	921	(2.8)	[1.9; 4.1]	8	353	(2.3)	[1.0; 4.4]	0.5811	123	38	485	(7.8)	[5.6; 10.6]	0.0000
GE_NL-2	IE /100 ml	48	921	(5.2)	[3.9; 6.9]	16	370	(4.3)	[2.5; 6.9]	0.5066	145	47	467	(10.1)	[7.5; 13.2]	0.0007
GE_UK	CP /10 ml	13	920	(1.4)	[0.8; 2.4]	2	248	(0.8)	[0.1; 2.9]	1.0000	3.8	26	589	(4.4)	[2.9; 6.4]	0.0003
GE_UK-wf	CP /10 ml	26	921	(2.8)	[1.9; 4.1]	5	250	(2.0)	[0.7; 4.6]	0.4722	3.8	41	597	(6.9)	[5.0; 9.2]	0.0002
GE_NL-2	CP /10 ml	48	921	(5.2)	[3.9; 6.9]	5	194	(2.6)	[0.8; 5.9]	0.1170	3.6	58	652	(8.9)	[6.8; 11.3]	0.0041
GE_UK	SOMCP /100 ml	13	920	(1.4)	[0.8; 2.4]	9	487	(1.8)	[0.8; 3.5]	0.5315	330	18	338	(5.3)	[3.2; 8.3]	0.0001
GE_UK-wf	SOMCP /100 ml	26	921	(2.8)	[1.9; 4.1]	7	298	(2.3)	[0.9; 4.8]	0.6612	50	37	537	(6.9)	[4.9; 9.4]	0.0002
GE_NL-2	SOMCP /100 ml	48	921	(5.2)	[3.9; 6.9]	19	386	(4.9)	[3.0; 7.6]	0.8286	119	41	448	(9.2)	[6.6; 12.2]	0.0055
SKIN	AE /100 ml	not applicable														

--- potential threshold concentration not accepted because incidence rate below threshold is significantly lower than incidence rate of non-bathers

Table 3: Relative risks and attributable risks of bathers exposed above threshold concentrations vs. non-bathers

Disease definition	Parameter	Non-bathers		Exposure definition 1: ("10 min bathing; 3 head immersions") Bathers > Threshold					Exposure definition 2: ("1 head immersion") Bathers > Threshold				
		cases	n	cases	n	RR	[95% CI]	aR	cases	n	RR	[95% CI]	aR
GE_UK	EC	13	920	21	419	3.55	[1.79; 7.02]	3.6%	17	273	4.41	[2.17; 8.96]	5.2%
GE_UK-wf	EC	26	921	41	579	2.51	[1.55; 4.05]	4.3%	---	---	---	---	---
GE_NL-2	EC	48	921	45	441	1.96	[1.32; 2.89]	5.0%	26	214	2.33	[1.48; 3.67]	8.6%
GE_UK	IE	13	920	23	508	3.2	[1.64; 6.27]	3.1%	23	477	3.41	[1.74; 6.68]	3.7%
GE_UK-wf	IE	26	921	40	531	2.67	[1.65; 4.32]	4.7%	38	485	2.78	[1.71; 4.51]	5.7%
GE_NL-2	IE	48	921	51	516	1.9	[1.3; 2.77]	4.7%	47	467	1.93	[1.31; 2.84]	6.0%
GE_UK	CP	13	920	24	508	3.34	[1.72; 6.51]	3.3%	26	589	3.12	[1.62; 6.03]	3.2%
GE_UK-wf	CP	26	921	38	516	2.61	[1.6; 4.25]	4.5%	41	597	2.43	[1.5; 3.93]	4.6%
GE_NL-2	CP	48	921	50	515	1.86	[1.27; 2.73]	4.5%	58	652	1.71	[1.18; 2.47]	4.6%
GE_UK	SOMCP	13	920	11	169	4.61	[2.1; 10.11]	5.1%	18	338	3.77	[1.87; 7.61]	4.2%
GE_UK-wf	SOMCP	26	921	37	530	2.47	[1.51; 4.04]	4.2%	37	537	2.44	[1.49; 3.98]	4.6%
GE_NL-2	SOMCP	48	921	49	530	1.77	[1.21; 2.6]	4.0%	41	448	1.76	[1.18; 2.62]	4.9%
SKIN	AE	27	957	62	508	4.33	[2.79; 6.71]	9.4%	not applicable				
SKIN *	AE	19 *	357 *	62	508	2.29	[1.40; 3.77]	6.9%	not applicable				

* bathers exposed <= threshold (two-step dose response model)

--- potential threshold concentration not accepted because incidence rate below threshold is significantly lower than incidence rate of non-bathers

Annex 21. Plots of exposure concentrations (X) by Chi square p values (Y) ("minimal p procedure")

For all exposure concentrations "K" which were calculated using exposure definition 1 ("10 minutes bathing; ≥ 3 head immersions") or exposure definition 2 ("1 head immersion") Pearson's Chi-square Tests were performed to compare the incidence rates of disease in the group of bathers exposed at or below a certain concentration "K" with the incidence rate in the group of bathers exposed above that concentration. The concentration with the minimum p value below 0.05 and a test result which was not suspect due to expected cell values of less than five was considered to be the most probable estimate for a potential threshold of effect. The figures on the following pages show the "K" by p plots for all combinations of faecal indicator organisms (IE, EC, CP, SOMCP) with the three definitions of gastroenteritis (GE_UK, GE_UK-wf and GE_NL-2). Combinations with AE or PA which did not reveal any valid results are shown for comparison. The last figure shows the plot for AE and SKIN (skin infections, cutireactions). All other combinations of microorganisms and disease definitions either did not reveal any potentially valid threshold concentrations according to the criteria defined in the materials and methods section or they were confounded by other predictors of disease.

Legend

Indicator organisms:

IE	Intestinal enterococci
EC	*Escherichia coli*
CP	*Clostridium perfringens*
SOMCP	Somatic coliphages
AE	Aeromonads
PA	*Pseudomonas aeruginosa*

Disease definitions:

GE_UK	Gastroenteritis, UK definition
GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis, NL-2 definition (van Asperen et al., 1998)
SKIN	Skin infections, cutireactions

X- and Y-axis:

K	Exposure concentration. Concentrations of indicator organisms assigned to the participants using exposure definition 1 ("10 minutes bathing; ≥ 3 head immersions") or exposure definition 2 ("1 head immersion").
p	Probability of error in Chi square tests. Null-hypothesis: No difference between the incidence rate of disease (GE, SKIN) in participants exposed at concentrations ≤ K and incidence rate of disease in participants exposed at concentrations > K.

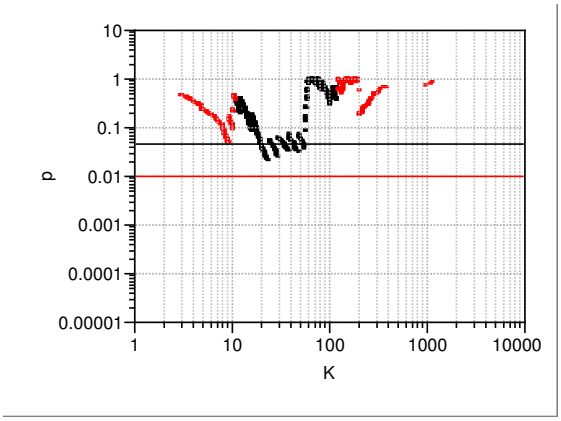
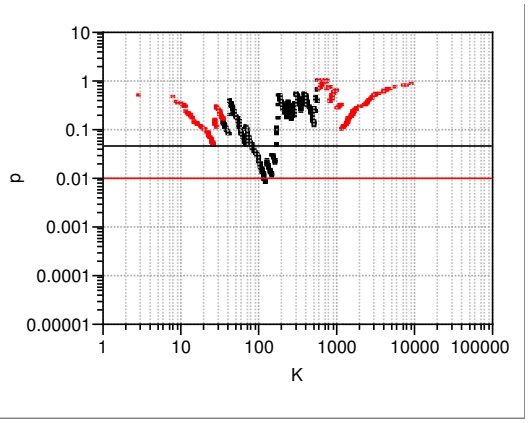
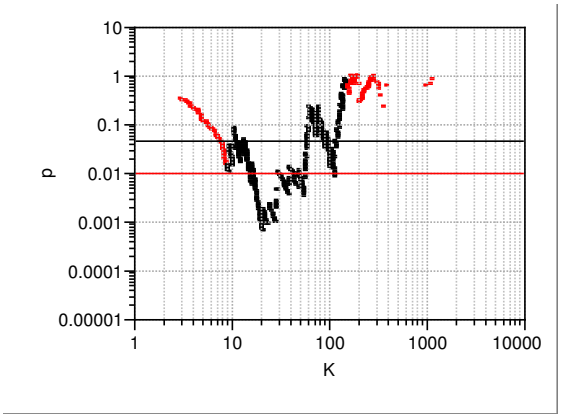
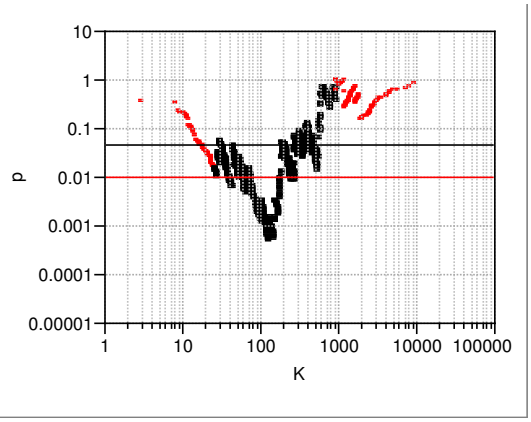
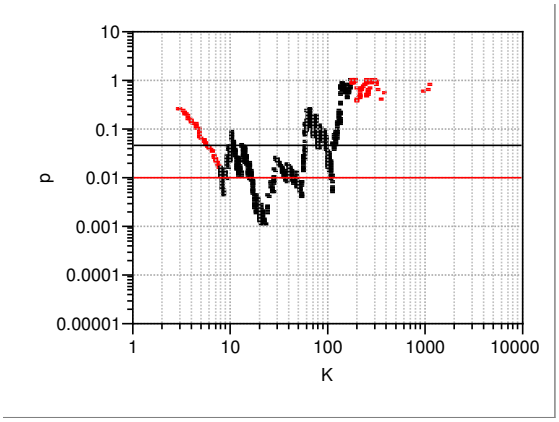
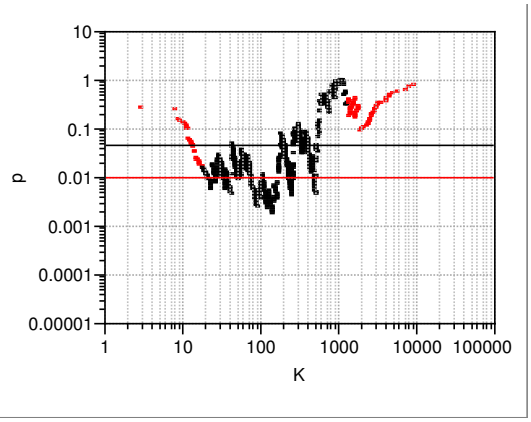
Markers:

Red dots	suspect p values (expected cell value < 5)
Black dots	p value not suspect

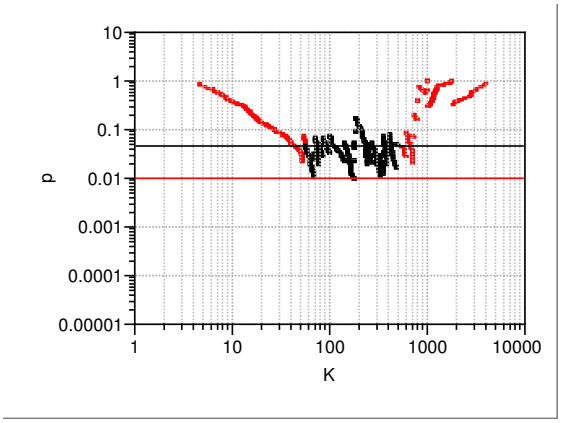
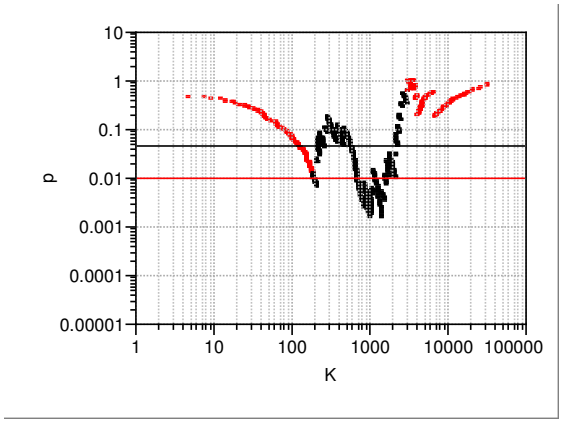
Horizontal lines:

Black line	alpha level (level of significance) = 0.05 (5%)
Red line	alpha level (level of significance) = 0.01 (1%)

K = IE / 100 ml

Exposure definition 1: "10 minutes bathing; ≥ 3 head immersions"	Exposure definition 2: "1 head immersion"
	
GE_UK	**GE_UK**
	
GE_UK-wf	**GE_UK-wf**
	
GE_NL-2	**GE_NL-2**

K = EC / 100 ml

Exposure definition 1: "10 minutes bathing; ≥ 3 head immersions"	Exposure definition 2: "1 head immersion"
	
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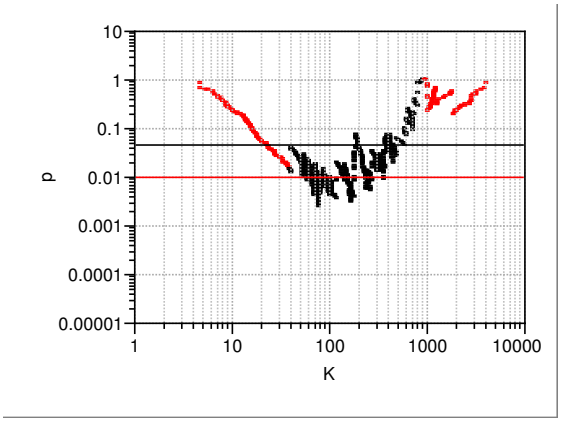
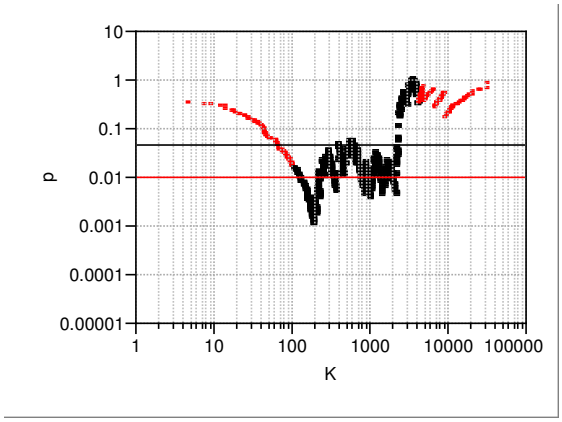
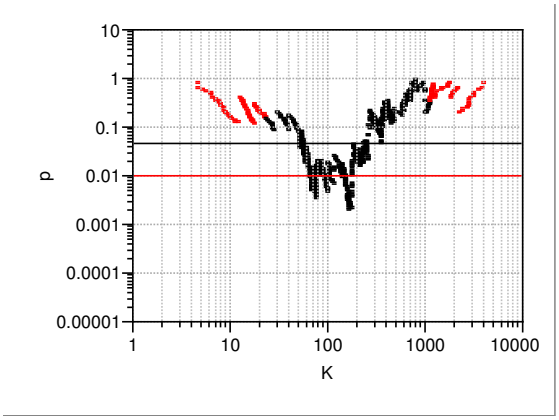
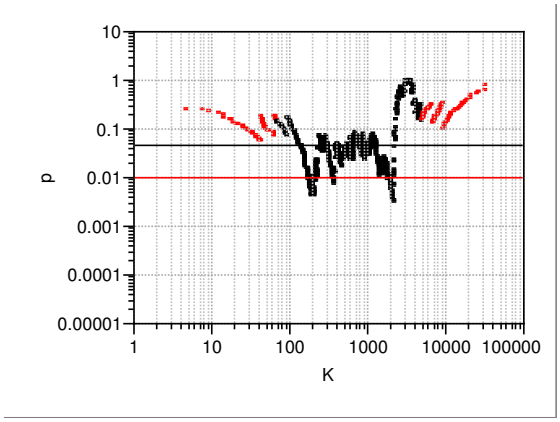
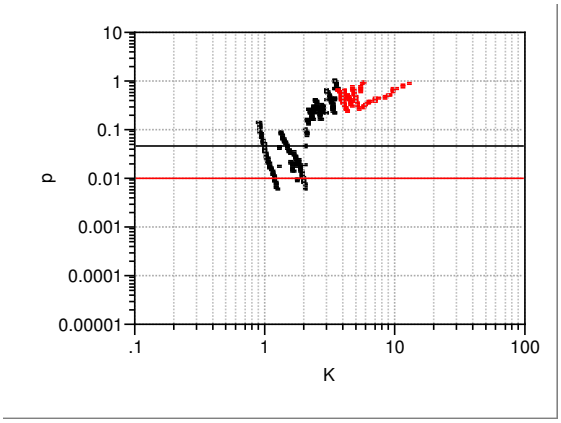
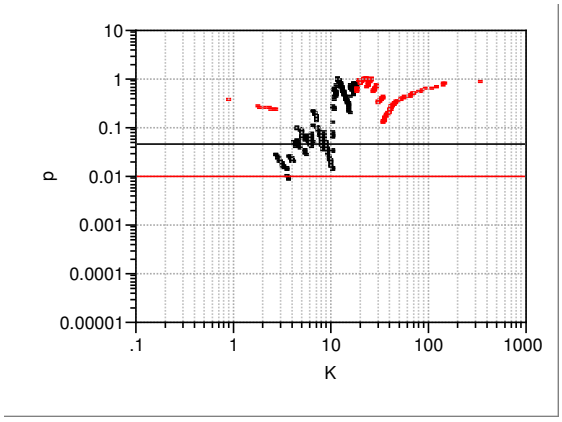
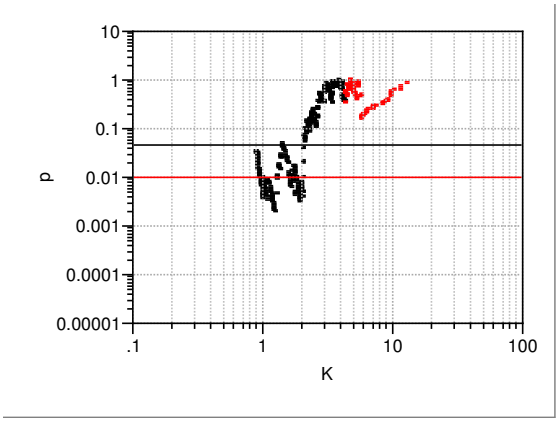
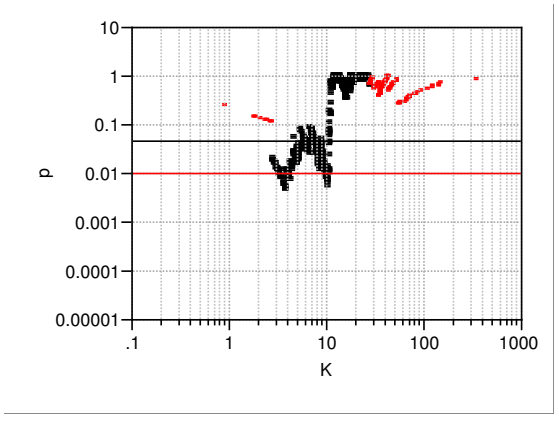
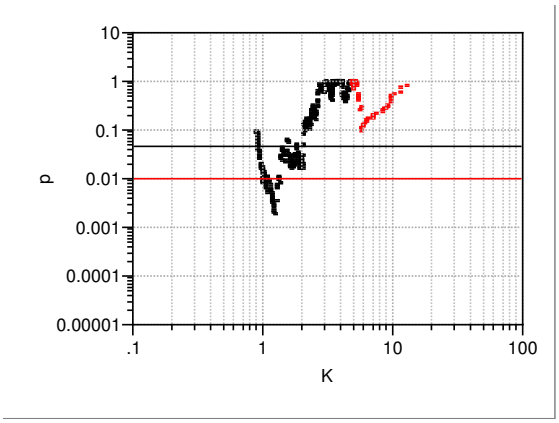
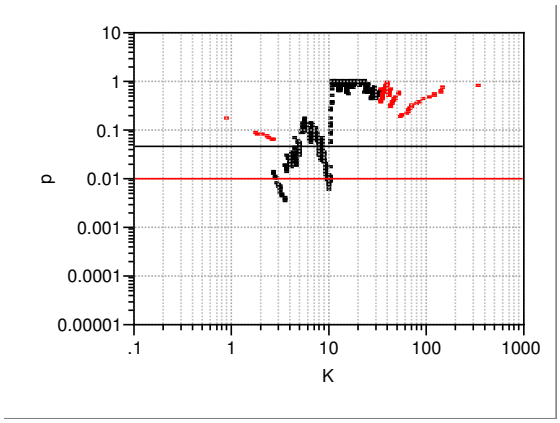
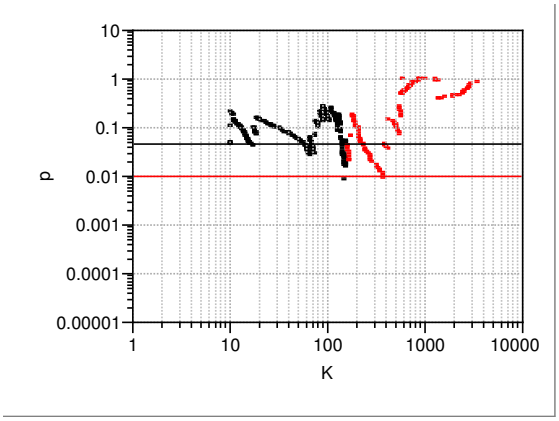
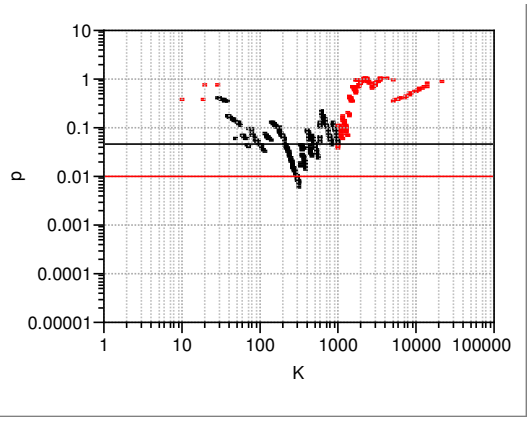
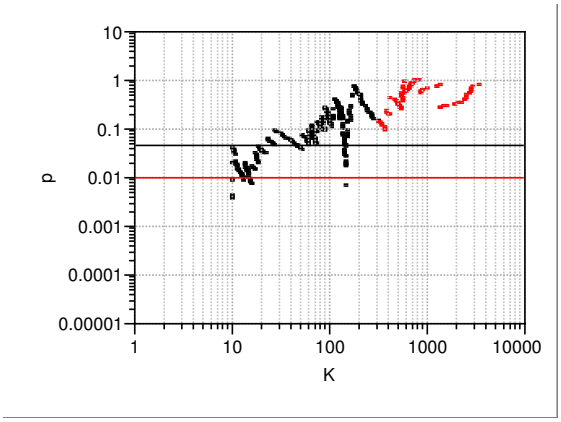
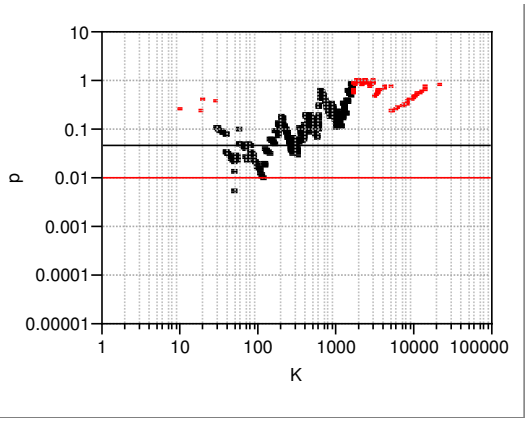
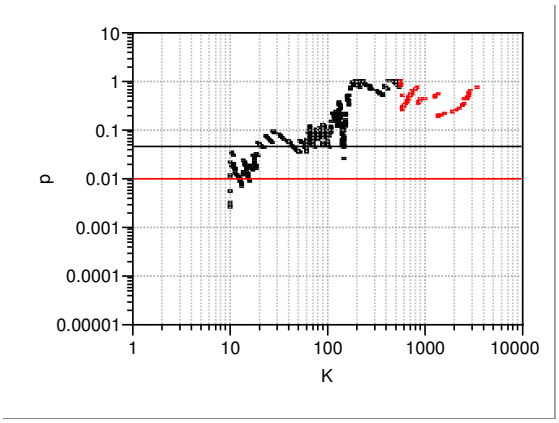
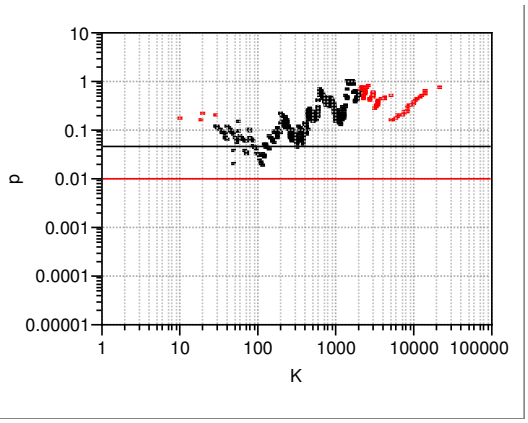
	
GE_UK-wf	**GE_UK-wf**
	
GE_NL-2	**GE_NL-2**

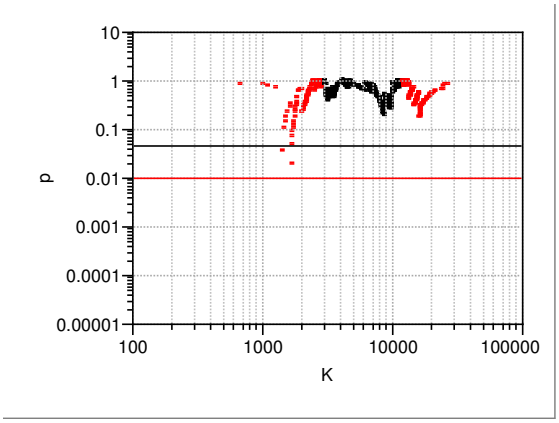
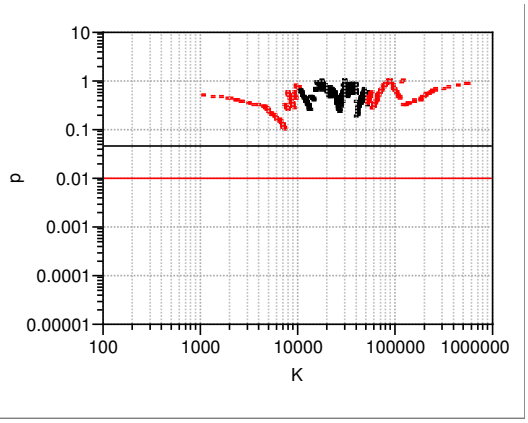
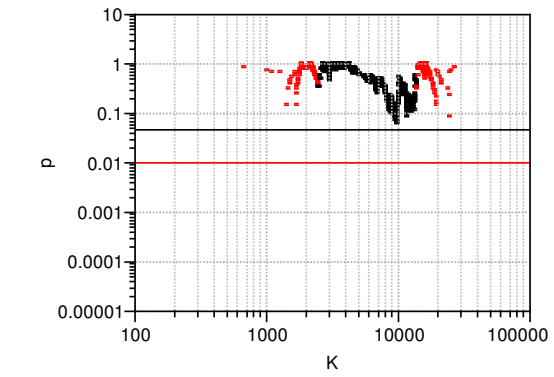
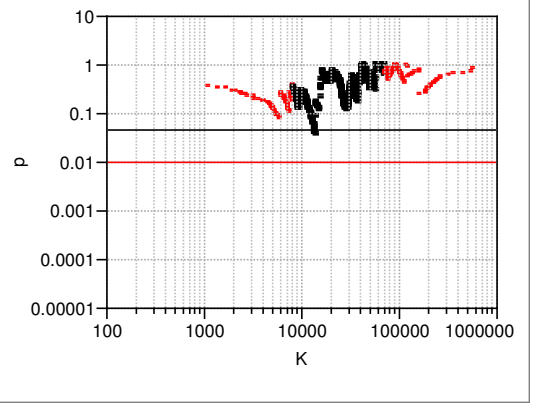
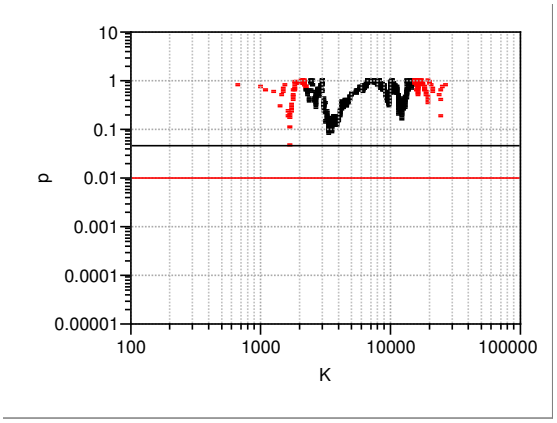
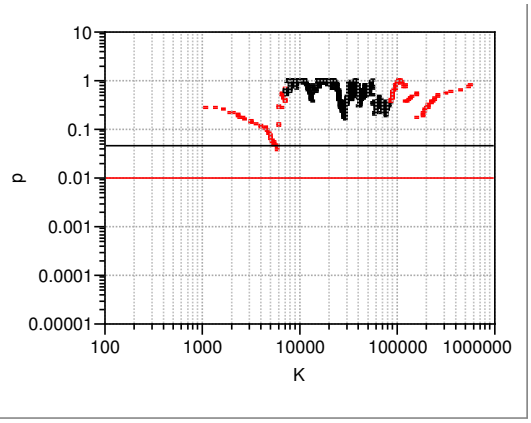
K = CP / 10 ml

Exposure definition 1: "10 minutes bathing; ≥ 3 head immersions"	Exposure definition 2: "1 head immersion"
	
GE_UK	**GE_UK**
	
GE_UK-wf	**GE_UK-wf**
	
GE_NL-2	**GE_NL-2**

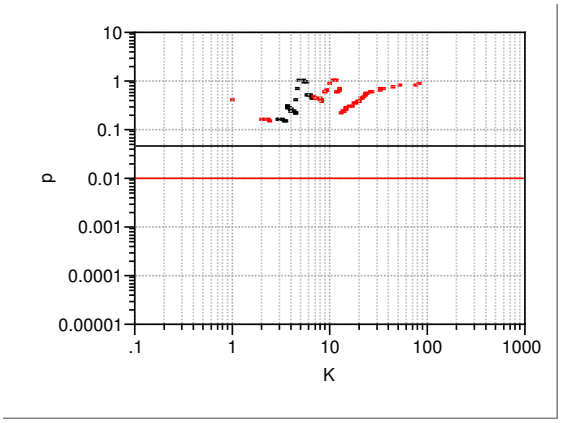
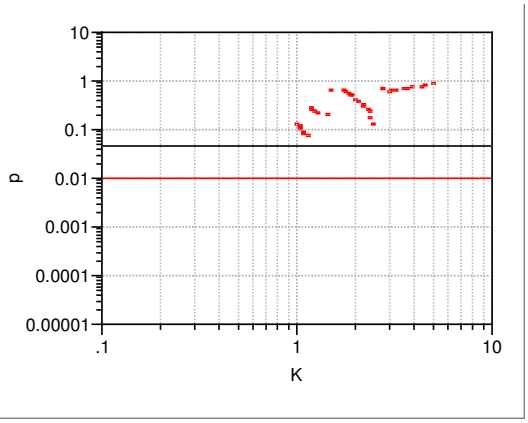
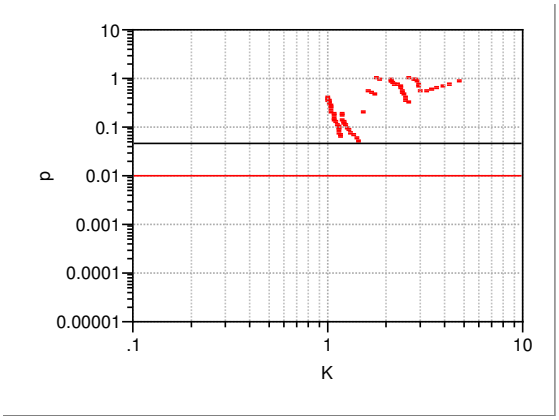
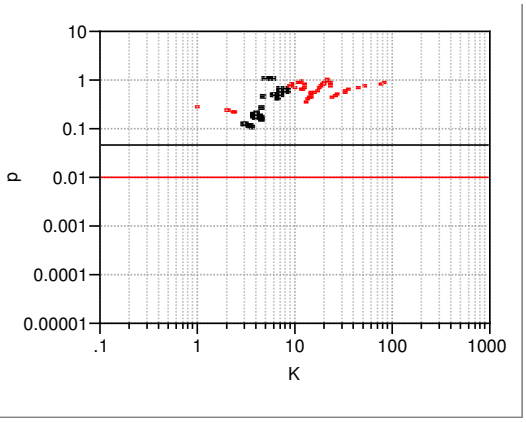
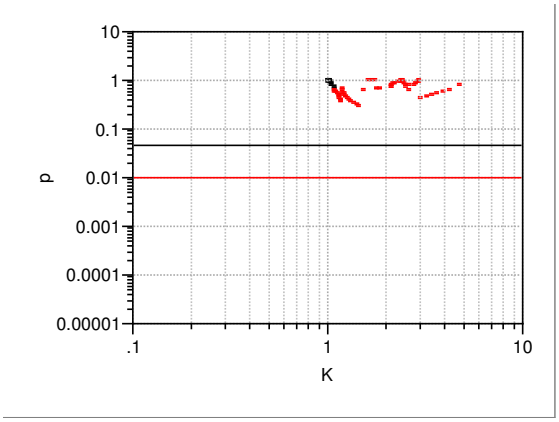
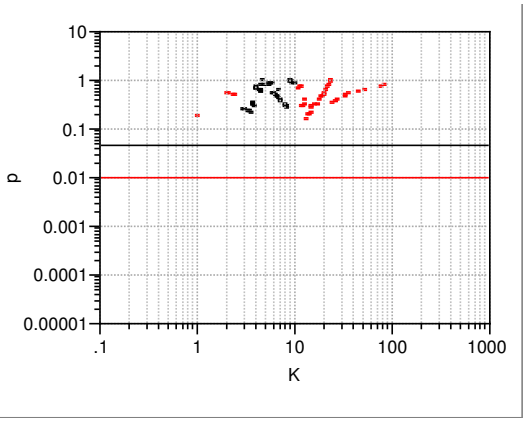
K = SOMCP / 100 ml

Exposure definition 1: "10 minutes bathing; ≥ 3 head immersions"	Exposure definition 2: "1 head immersion"
	
GE_UK	**GE_UK**
	
GE_UK-wf	**GE_UK-wf**
	
GE_NL-2	**GE_NL-2**

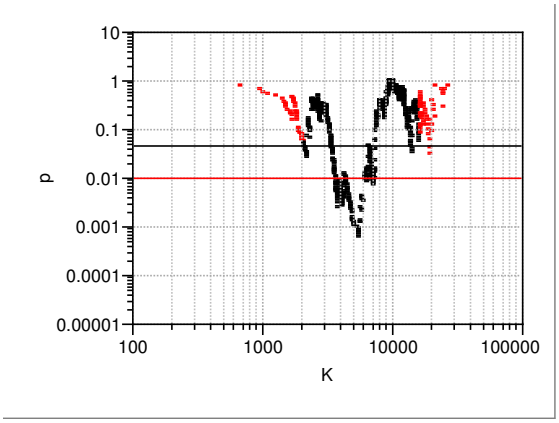
K = AE / 100 ml

Exposure definition 1: "10 minutes bathing; ≥ 3 head immersions"	Exposure definition 2: "1 head immersion"
	
GE_UK	**GE_UK**
	
GE_UK-wf	**GE_UK-wf**
	
GE_NL-2	**GE_NL-2**

K = PA / 10 ml

Exposure definition 1: "10 minutes bathing; ≥ 3 head immersions"	Exposure definition 2: "1 head immersion"
	
GE_UK	**GE_UK**
	
GE_UK-wf	**GE_UK-wf**
	
GE_NL-2	**GE_NL-2**

K = AE / 100 ml

Exposure definition 1: "10 minutes bathing; ≥ 3 head immersions"	Exposure definition 2: "1 head immersion"
	
SKIN	**not applicable**

Annex 22. Control of bias: Univariate screening for potential confounding variables

To control for bias a variety of potential confounding factors (variables) were analysed. Initially all variables were univariately screened for possible effects in the total final cohort of bathers and non-bathers (Pearson's Chi square tests). Variables showing significant univariate effects ($p<0.05$) were considered to be potential confounders of the threshold concentrations, and were further analysed using a multivariate nominal logistic regression procedure (effect likelihood ratio tests).

Table legend:

Category — Category of potential confounding variables

Variable — Variable name in computer data base

Explanation — Explanation of variable

Tested — * if one of the following disease definitions was included in the test:

AFRI	= Acute febrile respiratory infections
CC	= common cold
EAR	= Ear inflammation
EYE	= Eye inflammation
GE	= Gastroenteritis
SKIN	= Skin infection or cutireaction
UTI	= Urinary tract infection

Effect — * if the variable revealed a significant effect on the outcome of one of the following diseases in Pearson's Chi square test ($p < 0.05$):

AFRI	= Acute febrile respiratory infections
CC	= common cold
EAR	= Ear inflammation
EYE	= Eye inflammation
GE_UK	= Gastroenteritis UK definition
GE_UK-wf	= GE UK definition without consideration of stool frequency
GE_NL-2	= Gastroenteritis NL-2 definition
SKIN	= Skin infection or cutireaction
UTI	= Urinary tract infection

Category	Variable name	Explanation	Tested					Effect							
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Ambient conditions	LOCATION	study location (Kirchentellinsfurt, Uhldingen-Mühlhofen, Berlin, Lübeck, München)	*	*	*	*	*	*			*		*		*
	WEATHER_TEMP	weather conditions (cool or warm)	*	*	*	*	*	*							*
Interviewer effect	INR3	number of the interviewer in the 3rd interview	*	*	*	*	*								
Age	AGE	age in years	*	*	*	*	*								
	AGE-GROUP	age grouped by 10-years intervals	*	*	*	*	*	*							
	AGE-GROUP_2	age grouped by 10-year intervals and 60+	*	*	*	*	*	*							
Gender	SEX1	male or female	*	*	*	*	*						*		
Socio-economic status	DEGREE1	degree (none, primary school, non-classical secondary school, secondary scool 12th class, secondary school 13th class, non-classical university, classical university, other)	*	*	*	*	*	*							
	DEGREE1_2	degree (none, primary school, non-classical secondary school, secondary scool, university)	*	*	*	*	*	*							*

Category	Variable name	Explanation	Tested					Effect							
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Socio-economic status	OCCUP1	occupation (unemployed, child or pupil, student, household, practicing a profession, retired)	*	*	*	*	*	*							
	OCCUP1_2	occupation (unemployed, child or pupil, student, household, practicing a profession)	*	*	*	*	*	*							
Household	HHMEMB1	number of household members	*	*	*	*	*								
	HHMEMB1_2	number of household members (1, 2, 3, 4, 5, >5)	*	*	*	*	*			*					
	HHCHILD1	number of children < 5 years of age living in household	*	*	*	*	*								
	HHCHILD1_2	number of children < 5 years of age living in household (none, 1, >1)	*	*	*	*	*				*		*	*	
	HHCHILD1_3	children < 5 years living in household (yes, no)		*	*	*					*	*		*	
	HHVOL2	other household members participating in the study (yes, no)	*	*	*	*	*						*		
	HHCOLD1	household member with common cold in 2 weeks before 1st interview (yes, no)	*			*		*							
	HHTHRO1	household member with sore throat in 2 weeks before 1st interview (yes, no)	*												
	HHLUNG1	household member with lung infection in 2 weeks before 1st interview (yes, no)	*												

Category	Variable name	Explanation	Tested					Effect							
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Household	HHFEV1	household member with fever in 2 weeks before 1st interview (yes, no)	*			*	*						*		
	HHEAR1	household member with ear infection in 2 weeks before 1st interview (yes, no)		*											
	HHEYE1	household member with eye infection in 2 weeks before 1st interview (yes, no)			*										
	HHNAUS1	household member with nausea in 2 weeks before 1st interview (yes, no)				*									
	HHVOM1	household member with vomiting in 2 weeks before 1st interview (yes, no)				*									
	HHDIAR1	household member with diarrhoea in 2 weeks before 1st interview (yes, no)				*							*		
	HHSKIN1	household member with skin ailments in 2 weeks before 1st interview (yes, no)					*								
Chronic symptoms	CCHEST1	chronic chest ailments	*												
	CEAR1	chronic ear ailments	*	*											
	CEYE1	chronic eye ailments			*										
	CHAY1	chronic hay fever	*		*		*	*		*					
	CSTOM1	chronic stomach ailments				*						*	*	*	
	CGUT1	chronic gut ailments				*									
	ILLDIARFR1	normal frequency of diarrhoea (never, hardly ever: 1-2 per year, often: 1-2 per month)				*					*	*	*	*	

Category	Variable name	Explanation	Tested					Effect								
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Chronic symptoms	TRAVSICK4	proneness to motion sickness (always, often, hardly ever, never)				*							*	*	
	CLIV1	chronic liver ailments				*	*								
	CSKIN1	chronic skin ailments					*								*
	CREN1	chronic renal ailments					*								
	CNEUR1	chronic neural ailments					*								
	CDIAB1	chronic diabetes	*			*	*								
	ILL6MON1	any severe illness in 6 months before 1st interview (yes, no)	*	*	*	*	*								
	ILLHOSP1	stay in hospital in 6 months before 1st interview (yes, no)	*	*	*	*	*								
Symptoms or ailments	SYFEV1	fever in three weeks before 1st interview	*	*		*	*	*							
	SYFEV2	fever between 1st and 2nd interview		*			*								
	SYFEV3	fever in the week after exposure		*											
	SYHEAD1	headache in three weeks before 1st interview	*	*				*							
	SYHEAD2	headache between 1st and 2nd interview		*											
	SYHEAD3	headache in the week after exposure		*					*						
	SYNOSE1	runny nose in three weeks before 1st interview	*	*											
	SYNOSE2	runny nose between 1st and 2nd interview		*											
	SYNOSE3	runny nose in the week after exposure		*											

Category	Variable name	Explanation	Tested					Effect						
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Symptoms or ailments	SYDCOUGH1	dry cough in three weeks before 1st interview	*	*											
	SYDCOUGH2	dry cough between 1st and 2nd interview		*											
	SYDCOUGH3	dry cough in the week after exposure		*					*						
	SYPCOUGH1	productive cough in three weeks before 1st interview	*	*											
	SYPCOUGH2	productive cough between 1st and 2nd interview		*											
	SYPCOUGH3	productive cough in the week after exposure		*											
	SYTHRO1	sore throat in three weeks before 1st interview	*	*											
	SYTHRO2	sore throat between 1st and 2nd interview		*											
	SYTHRO3	sore throat in the week after exposure		*											
	SYBREATH1	breathing problems in three weeks before 1st interview	*	*											
	SYBREATH2	breathing problems between 1st and 2nd interview		*											
	SYBREATH3	breathing problems in the week after exposure		*											
	SYCHEST1	pain in the chest in three weeks before 1st interview	*												
	SYJOINT1	pain in the joints in three weeks before 1st interview	*												

Category	Variable name	Explanation	Tested					Effect						
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Symptoms or ailments	SYEAR1	ear inflammation in three weeks before 1st interview	*	*					*						
	SYEYE1	eye inflammation in three weeks before 1st interview			*										
	SYVIS1	vision disorders in three weeks before 1st interview			*										
	SYAPP1	loss of appetite in three weeks before 1st interview	*			*									
	SYINDIG1	indigestion in three weeks before 1st interview				*									
	SYNAUS1	nausea in three weeks before 1st interview				*									
	SYVOM1	vomiting in three weeks before 1st interview				*									
	SYSTOM1	stomach pains or cramps in three weeks before 1st interview				*								*	
	SYLOOSEB1	loose bowel motions in three weeks before 1st interview				*					*	*	*	*	
	SYDIAR1	diarrhoea in three weeks before 1st interview				*					*	*	*	*	
	SYANY_GI	any gastrointestinal disorders in three weeks before 1st interview				*							*	*	
	SYSKIN1	skin ailments in three weeks before 1st interview					*								
	SYITCH1	itching in three weeks before 1st interview					*								*

Category	Variable name	Explanation	Tested					Effect							
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Symptoms or ailments	SYPIN1	needles and pins in three weeks before 1st interview					*								
	SYSKINUL1	skin ulcer in three weeks before 1st interview					*								
Medicines	MED1	consumption of prescription drugs in 4 weeks before 1st interview (yes, no)	*	*	*	*	*								
	MEDANTI1	antibiotics in 4 weeks before 1st interview (yes, no)	*	*	*	*	*								
	MEDCORT1	steroids in 4 weeks before 1st interview (yes, no)	*	*	*	*	*								
	MEDLAX1	laxatives in 4 weeks before 1st interview (yes, no)				*									
Medicines	MEDSTOM1	stomach remedies in 4 weeks before 1st interview (yes, no)				*							*		
Food	FRMEAT2	consumption of raw meat in two or three days before exposure				*									
	FRMEAT3	consumption of raw meat in the week after exposure				*									
	FRMILK2	consumption of raw milk in two or three days before exposure				*							*		

Category	Variable name	Explanation	Tested					Effect						
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Food	FRMILK3	consumption of raw milk in the week after exposure				*									
	FRCHEESE2	consumption of raw milk cheese in two or three days before exposure				*									
	FRCHEESE3	consumption of raw milk cheese in the week after exposure				*									
	FEGG2	consumption of scambled eggs, omlettes in two or three days before exposure				*									
	FEGG3	consumption of scrambled eggs, omelettes in the week after exposure				*									
	FMAYO2	consumption of selfmade mayonnaise in two or three days before exposure				*									
	FMAYO3	consumption of selfmade mayonnaise in the week after exposure				*									
	FTIRA2	consumption of tiramisu in two or three days before exposure				*									
	FTIRA3	consumption of tiramisu in the week after exposure				*									
	FICE2	consumption of icecream in two or three days before exposure				*									
	FICE3	consumption of icecream in the week after exposure				*							*		

Category	Variable name	Explanation	Tested					Effect						
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Food	FCHICK2	consumption of chicken meat or poultry in two or three days before exposure				*									
	FCHICK3	consumption of chicken meat or poultry in the week after exposure				*									
	FFASTF2	consumption of fast-food in two or three days before exposure				*									
	FFASTF3	consumption of fast-food in the week after exposure				*									
	FBURGER2	consumption of hamburgers in two or three days before exposure				*									
	FBURGER3	consumption of hamburgers in the week after exposure				*									
	FHOTDOG2	consumption of hot digs in two or three days before exposure				*						*			
	FHOTDOG3	consumption of hot dogs in the week after exposure				*									
	FSAUSAGE2	consumption of grilled sausages in two or three days before exposure				*									
	FSAUSAGE3	consumption of grilled sausages in the week after exposure				*									
	FSANDW2	consumption of bought sandwiches in two or three days before exposure				*									

Category	Variable name	Explanation	Tested					Effect							
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Food	FSANDW3	consumption of bought sandwiches in the week after exposure				*					*		*	*	
	FSALAD2	consumption of salads in two or three days before exposure				*									
	FSALAD3	consumption of salads in the week after exposure				*									
	FSEAF2	consumption of sea food in two or three days before exposure				*									
	FSEAF3	consumption of sea food in the week after exposure				*									
	FBBQ2	consumption of blablab in two or three days before exposure				*									
	FBBQ3	participation in a barbecuing party in the week after exposure (yes, no)				*									
Alcohol	ALC1	consumption of alcohol (yes, no)	*	*	*	*	*								*
	ALC7D1	amount of alcohol consumed in the week before 1st interview (number of units (1 unit = 0.5 l beer, 0.25 l wine, 0.02 l spirits))	*	*	*	*	*								

Category	Variable name	Explanation	Tested					Effect							
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Alcohol	ALC7D1_2	amount of alcohol consumed in the week before 1st interview (0, 1-5, 6-10, >10 units (1 unit = 0.5 l beer, 0.25 l wine, 0.02 l spirits))	*	*	*	*	*						*		
	ALC7D1_3	amount of alcohol consumed in the week before 1st interview (0, 1-5, >5 units (1 unit = 0.5 l beer, 0.25 l wine, 0.02 l spirits))		*	*										
	ALC7D1_4	amount of alcohol consumed in the week before 1st interview (0 units, >0 units (1 unit = 0.5 l beer, 0.25 l wine, 0.02 l spirits))			*										
	ALCUS1	was this the normal amount of alcohol (yes, no)	*	*	*	*	*								
Tobacco	SMOKE1	smoking (yes, yes only pipe, no)	*	*	*	*	*								
	SMOKE1_2	smoking (yes, no)	*	*	*	*	*								
	SMOKENR1	number of cigarettes per day	*	*	*	*	*								
	SMOKENR1_2	number of cigarettes per day (0, 1-5, 6-20, >20)	*	*	*	*	*								
Leisure activities	LEPARTY1	normal frequency of party or disco visits per month (never, sometimes: 1-3, often: >3)	*	*	*	*	*	*							

Category	Variable name	Explanation	Tested					Effect							
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Leisure activities	LEPUB1	normal frequency of pub visits per month (never, sometimes: 1-3, often: >3)	*	*	*	*	*	*				*			*
	LESPORT1	normal frequency of sports activities per month (never, sometimes: 1-3, often: >3)	*	*	*	*	*	*						*	
	LECIN1	normal frequency of cinema visits per month (never, sometimes: 1-3, often: >3)	*												
	LEOTHER1	normal frequency of other activities involving contact to large groups of people (never, sometimes: 1-3, often: >3)	*												
Travel history	TRAVEL1	overnight stays outside household in 4 weeks before 1st interview (yes, no)	*	*	*	*	*	*							
	TRAVPL1	travelling abroad in 4 weeks before 1st interview (yes, no)	*	*	*	*	*								
Water related	BP_WATER	exposed and swallowed water during trial (no, tea-spoon, table-spoon, cup)	*			*									
	BP_Water_B-nein_B-ja	exposed and swallowed water during trial (yes, no)	*			*						*			
	BP_Water_NB=0_B=1-4	swallowed water during trial (unexposed or exposed and no, tea-spoon, table-spoon, cup)	*			*					*	*			

Category	Variable name	Explanation	Tested					Effect							
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Water related	BP_Water_NB_B-nein_B-ja	swallowed water during trial (unexposed or exposed and no, exposed and yes)	*			*					*	*		*	
	BATH1	normal frequency of bathing in natural recreational waters (fresh or sea water) per month during summer (never; sometimes: 1-3; often: >3)	*	*	*	*	*								
	BATH2	swimming or bathing in the two or three days between 1st and 2nd interview (yes, no)	*	*	*	*	*								
	BATH3	additional swimming or bathing in the week after the trial day (yes, no)	*	*	*	*	*						*		
	BATHFR1	frequency of bathing in 4 weeks before 1st interview	*	*	*	*	*								
	BATHFR1_2	frequency of bathing in 4 weeks before 1st interview (0, 1-5, >5)	*	*	*	*	*								
	HBEACH1	normal frequency of going to a beach without entering the water per month (never, sometimes: 1-3, often: >3)	*	*	*	*	*	*							
	HBEACHFR1	number of beach visits without entering the water in the 4 weeks before 1st interview	*	*	*	*	*								
	HBEACHFR1_2	number of beach visits without entering the water in 4 weeks before 1st interview (0, 1-5, >5)	*	*	*	*	*								

Category	Variable name	Explanation	Tested					Effect							
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Water related	HBEACHFR1_3	beach visits without entering the water in 4 weeks before 1st interview (yes, no)	*	*	*					*					
	HPUPOOL1	normal frequency of using a public pool per month (never, sometimes: 1-3, often: >3)	*	*	*	*	*	*							
	HPRPOOL1	normal frequency of using a private pool per month during sumer (never; sometimes: 1-3; often: >3)	*	*	*	*	*								
	HPRPOOL1_2	utilisation of private pools (yes, no)	*	*	*	*	*	*							
	HLEPARK1	visit to a leasure park with water activities in 4 weeks before 1st interview (yes, no)	*	*	*	*	*								*
	BASWIMDIS1	maximal swimming distance (number of meters)	*	*	*	*	*								
	BASWIMDIS1_2	maximal swimming distance (-50, >50 m)	*	*	*	*	*								
	BASWIMDIS1_3	maximal swimming distance (-50, -500, -5000 m)		*	*										
	BASWIMDUR1	maximal swimming duration (number of minutes)	*	*	*	*	*								
	BASWIMDUR1_2	maximal swimming duration (10 min, 20 min, 30 min, 1 h, 2 h)	*	*	*	*	*								
	BASWIMDUR1_3	maximal swimming duration (10 min, >10 min)		*											

Category	Variable name	Explanation	Tested					Effect							
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Water related	WASURF1	normal frequency of surfing during summer (never, sometimes: 1-3, often: >3)	*	*	*	*	*	*							
	WASURF1_2	normal frequency of surfing during summer (never, once or more)		*	*										
	WAPAD1	normal frequency of paddling during summer (never, sometimes: 1-3, often: >3)	*	*	*	*	*								
	WAPAD1_2	normal frequency of paddling during summer (never, once or more)		*	*										
	WACANOE1	normal frequency of canoeing during summer (never, sometimes: 1-3, often: >3)	*	*	*	*	*	*		*					
	WACANOE1_2	normal frequency of canoeing during summer (never, once or more)		*	*										
	WAMOTOR1	normal frequency of motorboating during summer (never, once or more)	*	*	*	*	*	*							
	WAMOTOR1_2	normal frequency of motorboating during summer (never, sometimes: 1-3, often: >3)		*	*										
	WADIV1	normal frequency of diving during summer (never, once or more)	*	*	*	*	*	*							
	WADIV1_2	normal frequency of diving during summer (never, sometimes: 1-3, often: >3)		*	*										
	WAFISH1	normal frequency of fishing during summer (never, once or more)	*	*	*	*	*								

Category	Variable name	Explanation	Tested					Effect							
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
	WAFISH1_2	normal frequency of fishing during summer (never, once or more)		*	*										
Risk perception	WADA4	are water related activities considered to be dangerous (yes, no)	*	*	*	*	*								
	BBILL4	ever gone to a beach while feeling ill (yes, no)	*	*	*	*	*	*		*					
	BBILLWAT4	did feeling ill prevent from entering the water (yes, no)	*	*	*	*	*								
	WQNBBEACH4	ever refused to go bathing because beach was too dirty (yes, no)	*	*	*	*	*				*	*		*	
	WQNBWATER4	ever refused to go bathing because water was too dirty (yes, no)	*	*	*	*	*					*			
	WQNBWAVES4	ever refused to go bathing because waves were too rough (yes, no)	*	*	*	*	*								
	WQNBHRISK4	ever refused to go bathing because of fear to become ill (yes, no)	*	*	*	*	*								*
	SUNBFR4	frequency of sunburns after beach visits (always, often, hardly ever, never)					*								
	SUNBMED4	are sunburns treated (always, often, hardly ever, never)					*								

Category	Variable name	Explanation	Tested					Effect							
			CC	EAR	EYE	GE	SKIN	CC	EAR	EYE	GE_UK-wf	GE_UK	GE_UK-3w	GE_NL-2	SKIN
Information	IRECRUIT4	initial source of information about this study (partner, recruitment team, television, newspaper, other)	*	*	*	*	*						*		
	IMEDIA4	having seen or heard news about this study (yes, no)	*	*	*	*	*								
	INEWSPAP4	regular reading of a newspaper (yes, no)	*	*	*	*	*								
	IENVORG4	member of an environmental organisation (yes, no)	*	*	*	*	*						*		
	WQCONTR4	informed about the control of beaches in Germany (yes, no)	*	*	*	*	*	*							
	WQCONTRPN4	quality of information on control of beaches in Germany (positive or negative)	*	*	*	*	*	*							*
	WQCONTRWO4	worried about information on the control of beaches in Germany no, a bit, very much)	*	*	*	*	*								
	WQCONTRWO4_2	worried about information on the control of beaches in Germany (yes, no)	*	*	*										
	WQCLEAN4	information on the cleanliness of recreational waters in Germany (yes, no)	*	*	*	*	*	*			*			*	
	WQCLEANPN4	quality of information on cleanliness of German recreational waters (positive or negative)	*	*	*	*	*	*							

Annex 23. Control of bias: Analysis of potential threshold concentrations and potential confounding variables by multiple logistic regression (Effect Likelihood Ratio Tests)

To control for bias a variety of potential confounding factors (variables) were analysed. Initially all variables were univariately screened for possible effects in the total final cohort of bathers and non-bathers (Pearson's Chi square tests). Variables showing significant univariate effects ($p<0.05$) were considered to be potential confounders of the threshold concentrations, and were further analysed using a multivariate nominal logistic regression procedure (effect likelihood ratio tests). If microbiological threshold concentrations remained significant effects ($p<0.05$) in a model consisting of the disease as response variable and the threshold and the potential confounder as model effects, thresholds were considered to be unbiased and were accepted, and the potential confounder was considered to be an independent predictor of disease. In addition all models were analysed for possible interaction effects between threshold values and potential confounding variables by crossing both effects in separate effect likelihood ratio tests. Significant interaction effects ($p<0.05$) were recorded but the thresholds were not rejected.

Table legend:

Parameter	Microorganism:	
	CP	= Clostridium perfringens
	EC	= Escherichia coli
	IE	= Intestinal enterococci
	SOMCP	= Somatic coliphages
	AE	= Aeromonads
	PA	= Pseudomonas aeruginosa
Unit	MPN	= Most Probable Number
	CFP	= Colony Forming Particle

Table legend, continued

Disease	AFRI	= Acute febrile respiratory infections
	CC	= common cold
	EAR	= Ear inflammation
	EYE	= Eye inflammation
	GE_UK	= Gastroenteritis UK definition
	GE_UK-wf	= GE UK definition without consideration of stool frequency
	GE_NL-2	= Gastroenteritis NL-2 definition
	SKIN	= Skin infection or cutireaction
	UTI	= Urinary tract infection
Threshold	Potential threshold concentration --- no concentration fulfilling the criteria for a potential threshold yellow = Chi square test result not suspect and threshold unbiased	
p ChiSq	Pearson's Chi square p value in a comparison of the incidence rates above and below the threshold concentration suspect = suspect p value due to an expected cell value of less than 5 in Pearson's Chi square test	
p Fisher	p value in Fisher's Exact Test	
Status	o.k.	no confounding and no interaction with any of the tested potential confounding variables
	o.k. (i)	no confounding but interaction with at least one other independent predictor of disease
	biased	potential threshold confounded by at least one other predictor of disease
fn	footnote number	
sign. better	microbiological parameters which are significantly better predictors of the outcome (disease)	

Table footnotes:

No.	Number of footnote	
Parameter	Microorganism:	
	CP	= *Clostridium perfringens*
	EC	= *Escherichia coli*
	IE	= Intestinal enterococci
	SOMCP	= Somatic coliphages
	AE	= Aeromonads
	PA	= *Pseudomonas aeruginosa*
Variable	Variable name in computer data base	
p	Chi Square p value in Effect Likely Ratio Test	
Effect	b = bias i = significant interaction between "variable" and "threshold"	
Explanation	Explanation of variable	

Parameter	Unit	Disease	Exposure Definition 1 "10 minutes bathing; ≥ 3 head immersions"						Exposure Definition 2 "1 head immersion"					
			Threshold	p ChiSq	p Fisher	status	fn	sign. better	Threshold	p ChiSq	p Fisher	status	fn	sign. better
EC	MPN/100ml	AFRI	---						---					
IE	MPN/100ml	AFRI	---						---					
CP	CFP/10ml	AFRI	---						---					
SOMCP	PFP/100ml	AFRI	---						---					
AE	CFP/100ml	AFRI	---						---					
PA	CFP/10ml	AFRI	---						---					
EC	MPN/100ml	CC	261	0.0007		biased	1		936.3	0.0130		biased	16	
IE	MPN/100ml	CC	132	0.0010		biased	2		---					
CP	CFP/10ml	CC	3	0.0220		biased	3		---					
SOMCP	PFP/100ml	CC	128	0.0012		biased	4		247.5	0.0052		biased	17	
AE	CFP/100ml	CC	25580	suspect	0.0230	biased	5		---					
PA	CFP/10ml	CC	---						4	0.0500		biased	18	
EC	MPN/100ml	EAR	---						---					
IE	MPN/100ml	EAR	---						9467	suspect	0.0160	biased	19	
CP	CFP/10ml	EAR	---						---					
SOMCP	PFP/100ml	EAR	---						---					
AE	CFP/100ml	EAR	---						542835	suspect	0.0480	biased	20	
PA	CFP/10ml	EAR	---						---					
EC	MPN/100ml	EYE	---						18112	suspect	0.0160	biased	21	
IE	MPN/100ml	EYE	---						9467	suspect	0.0170	biased	22	
CP	CFP/10ml	EYE	---						136	suspect	0.0026	biased	23	
SOMCP	PFP/100ml	EYE	---						---					
AE	CFP/100ml	EYE	16612	suspect	0.0320	biased	6		542835	suspect	0.0008	o.k. (i)	24	
PA	CFP/10ml	EYE	---						---					

Parameter	Unit	Disease	Exposure Definition 1 "10 minutes bathing; ≥ 3 head immersions"						Exposure Definition 2 "1 head immersion"					
			Threshold	p ChiSq	p Fisher	status	fn	sign. better	Threshold	p ChiSq	p Fisher	status	fn	sign. better
EC	MPN/100ml	GE_UK	180	0.0086		o.k.			1453	0.0015		o.k.		
		GE_UK-wf	78	0.0025		o.k.			---					
		GE_NL-2	167	0.0019		o.k.			2163	0.0030		o.k.		CP, EN
IE	MPN/100ml	GE_UK	24	0.0220		o.k.			123	0.0075		o.k.		
		GE_UK-wf	21	0.0006		o.k.			123	0.0005		o.k.		
		GE_NL-2	24	0.0010		o.k. (i)	7		145	0.0018		o.k. (i)	25	
CP	CFP/10ml	GE_UK	1	0.0058		o.k.			4	0.0080		o.k. (i)	26	EC
		GE_UK-wf	1	0.0019		o.k.		EN	4	0.0043		o.k.		EC, EN
		GE_NL-2	1	0.0018		o.k. (i)	8		4	0.0032		o.k. (i)	27	
SOMCP	PFP/100ml	GE_UK	150	0.0080		o.k. (i)	9	CP, EC, EN	330	0.0058		o.k.		EC
		GE_UK-wf	10	0.0035		o.k. (i)	10		50	0.0049		o.k. (i)	28	
		GE_NL-2	10	0.0025		o.k. (i)	11		119	0.0180		o.k. (i)	29	
AE	CFP/100ml	GE_UK	---						---					
		GE_UK-wf	---						13600	0.0370		o.k.		CP, EC, EN
		GE_NL-2	---						5950	suspect	0.0170	biased	30	
PA	CFP/10ml	GE_UK	1	suspect	0.0310	biased	12		---					
		GE_UK-wf	---						---					
		GE_NL-2	---						---					

Parameter	Unit	Disease	Exposure Definition 1 "10 minutes bathing; ≥ 3 head immersions"						Exposure Definition 2 "1 head immersion"					
			Threshold	p ChiSq	p Fisher	status	fn	sign. better	Threshold	p ChiSq	p Fisher	status	fn	sign. better
EC	MPN/100ml	SKIN	---											
IE	MPN/100ml	SKIN	356	suspect	0.0070	o.k. (i)	13							
CP	CFP/10ml	SKIN	5	suspect	0.0440	biased	14							
SOMCP	PFP/100ml	SKIN	---											
AE	CFP/100ml	SKIN	5622	0.0006		o.k. (i)	15							
PA	CFP/10ml	SKIN	---											
EC	MPN/100ml	UTI	---											
IE	MPN/100ml	UTI	---											
CP	CFP/10ml	UTI	---											
SOMCP	PFP/100ml	UTI	---											
AE	CFP/100ml	UTI	---											
PA	CFP/10ml	UTI	---											

not applicable

not applicable

--- no concentration fulfilling the criteria for a potential threshold

threshold concentration fulfilling all criteria of validity

Footnotes:

No.	Parameter	Disease	Variable	p	Effect	Explanation
Exposure Definition 1						
1	EC	CC	WQCONTRPN4	0.0067	b	quality of information on control of beaches in Germany (positive or negative)
			WQCLEANPN4	0.0184	b	quality of information on cleanliness of German recreational waters (positive or negative)
2	IE	CC	WQCONTRPN4	0.0073	b	quality of information on control of beaches in Germany (positive or negative)
			SYHEAD1	0.0100	i	headache in three weeks before 1st interview
			BBILL4	0.0417	i	ever gone to a beach while feeling ill (yes, no)
3	CP	CC	LOCATION	0.0014	b	study location (Kirchentellinsfurt, Uhldingen-Mühlhofen, Berlin, Lübeck, München)
			WEATHER_TEMP	0.0001	b	weather conditions (cool or warm)
			AGE-GROUP	0.0000	b	age grouped by 10-years intervals
			AGE-GROUP_2	0.0000	b	age grouped by 10-year intervals and 60+
			DEGREE1	0.0037	b	degree (none, primary school, non-classical secondary school, secondary scool 12th class, secondary school 13th class, non-classical university, classical university, other)
			DEGREE1_2	0.0013	b	degree (none, primary school, non-classical secondary school, secondary scool, university)
			OCCUP1	0.0005	b	occupation (unemployed, child or pupil, student, household, practicing a profession, retired)
			OCCUP1_2	0.0002	b	occupation (unemployed, child or pupil, student, household, practicing a profession)
			WQCONTRPN4	0.0114	b	quality of information on control of beaches in Germany (positive or negative)
			WQCLEANPN4	0.0190	b	quality of information on control of beaches in Germany (positive or negative)
			BBILL4	0.0006	i	ever gone to a beach while feeling ill (yes, no)
4	SOMCP	CC	LOCATION	0.0125	b	study location (Kirchentellinsfurt, Uhldingen-Mühlhofen, Berlin, Lübeck, München)
			WEATHER_TEMP	0.0007	b	weather conditions (cool or warm)
			WQCONTRPN4	0.0082	b	quality of information on control of beaches in Germany (positive or negative)
			WQCLEANPN4	0.0195	b	quality of information on control of beaches in Germany (positive or negative)
			TRAVEL1	0.0014	i	overnight stays outside household in 4 weeks before 1st interview (yes, no)
5	AE	CC	AGE-GROUP	0.0000	b	age grouped by 10-years intervals
			AGE-GROUP_2	0.0000	b	age grouped by 10-year intervals and 60+

No.	Parameter	Disease	Variable	p	Effect	Explanation
			DEGREE1	0.0019	b	degree (none, primary school, non-classical secondary school, secondary scool 12th class, secondary school 13th class, non-classical university, classical university, other)
			DEGREE1_2	0.0006	b	degree (none, primary school, non-classical secondary school, secondary scool, university)
			OCCUP1	0.0006	b	occupation (unemployed, child or pupil, student, household, practicing a profession, retired)
			OCCUP1_2	0.0003	b	occupation (unemployed, child or pupil, student, household, practicing a profession)
6	AE	EYE	CHAY1	0.0423	b	chronic hay fever
			WACANOE1	0.0166	b	normal frequency of canoeing during summer (never, sometimes: 1-3, often: >3)
			WACANOE1_2	0.0062	b	normal frequency of canoeing during summer (never, once or more)
			HBEACHFR1_3	0.0301	b	beach visits without entering the water in 4 weeks before 1st interview (yes, no)
			BBILL4	0.0067	b	ever gone to a beach while feeling ill (yes, no)
7	IE	GE_NL-2	SYSTOM1	0.0081	i	stomach pains or cramps in three weeks before 1st interview
			SYANY_GI	0.0446	i	any gastrointestinal disorders in three weeks before 1st interview
8	CP	GE_NL-3	SYSTOM1	0.0089	i	stomach pains or cramps in three weeks before 1st interview
9	SOMCP	GE_UK	SYLOOSEB1	0.0354	i	loose bowel motions in three weeks before 1st interview
10	SOMCP	GE_UK-wf	SYLOOSEB1	0.0173	i	loose bowel motions in three weeks before 1st interview
11	SOMCP	GE_NL-2	SYSTOM1	0.0104	i	stomach pains or cramps in three weeks before 1st interview
			SYANY_GI	0.0172	i	any gastrointestinal disorders in three weeks before 1st interview
12	PA	GE_UK	HHCHILD1_3	0.0076	b	children < 5 years living in household (yes, no)
			SYLOOSEB1	0.0000	i	loose bowel motions in three weeks before 1st interview
			FHOTDOG2	0.0161	i	consumption of hot digs in two or three days before exposure
13	IE	SKIN	LOCATION	0.0000	i	study location (Kirchentellinsfurt, Uhldingen-Mühlhofen, Berlin, Lübeck, München)
			WEATHER_TEMP	0.0000	i	weather conditions (cool or warm)
			CSKIN1	0.0000	i	chronic skin ailments
			SYITCH1	0.0000	i	itching in three weeks before 1st interview

No.	Parameter	Disease	Variable	p	Effect	Explanation
			HLEPARK1	0.0000	i	visit to a leisure park with water activities in 4 weeks before 1st interview (yes, no)
			WQNBHRISK4	0.0000	i	ever refused to go bathing because of fear to become ill (yes, no)
14	CP	SKIN	WQNBHRISK4	0.0375	b	ever refused to go bathing because of fear to become ill (yes, no)
			WEATHER_TEMP	0.0159	i	weather conditions (cool or warm)
15	AE	SKIN	WEATHER_TEMP	0.0022	i	weather conditions (cool or warm)
			CSKIN1	0.0000	i	chronic skin ailments
			SYITCH1	0.0012	i	itching in three weeks before 1st interview
			ALC1	0.0031	i	consumption of alcohol (yes, no)
Exposure Definition 2						
16	EC	CC	LOCATION	0.0019	b	study location (Kirchentellinsfurt, Uhldingen-Mühlhofen, Berlin, Lübeck, München)
			WEATHER_TEMP	0.0001	b	weather conditions (cool or warm)
			AGE-GROUP	0.0000	b	age grouped by 10-years intervals
			AGE-GROUP_2	0.0000	b	age grouped by 10-year intervals and 60+
			DEGREE1	0.0058	b	degree (none, primary school, non-classical secondary school, secondary scool 12th class, secondary school 13th class, non-classical university, classical university, other)
			DEGREE1_2	0.0023	b	degree (none, primary school, non-classical secondary school, secondary scool, university)
			OCCUP1	0.0011	b	occupation (unemployed, child or pupil, student, household, practicing a profession, retired)
			LESPORT1	0.0174	b	normal frequency of sports activities per month (never, sometimes: 1-3, often: >3)
			WQCONTRPN4	0.0079	b	quality of information on control of beaches in Germany (positive or negative)
			WQCLEANPN4	0.0211	b	quality of information on control of beaches in Germany (positive or negative)
			BBILL4	0.0091	i	ever gone to a beach while feeling ill (yes, no)
17	SOMCP	CC	LOCATION	0.0068	b	study location (Kirchentellinsfurt, Uhldingen-Mühlhofen, Berlin, Lübeck, München)
			WEATHER_TEMP	0.0005	b	weather conditions (cool or warm)
			DEGREE1	0.0054	b	degree (none, primary school, non-classical secondary school, secondary scool 12th class, secondary school 13th class, non-classical university, classical university, other)
			DEGREE1_2	0.0021	b	degree (none, primary school, non-classical secondary school, secondary scool, university)

No.	Parameter	Disease	Variable	p	Effect	Explanation
			WQCONTRPN4	0.0076	b	quality of information on control of beaches in Germany (positive or negative)
			WQCLEANPN4	0.0226	b	quality of information on control of beaches in Germany (positive or negative)
			TRAVEL1	0.0188	i	overnight stays outside household in 4 weeks before 1st interview (yes, no)
			WADIV1	0.0208	i	normal frequency of diving during summer (never, once or more)
18	PA	CC	LOCATION	0.0012	b	study location (Kirchentellinsfurt, Uhldingen-Mühlhofen, Berlin, Lübeck, München)
			WEATHER_TEMP	0.0001	b	weather conditions (cool or warm)
			AGE-GROUP	0.0000	b	age grouped by 10-years intervals
			AGE-GROUP_2	0.0000	b	age grouped by 10-year intervals and 60+
			OCCUP1	0.0005	b	occupation (unemployed, child or pupil, student, household, practicing a profession, retired)
			OCCUP1_2	0.0002	b	occupation (unemployed, child or pupil, student, household, practicing a profession)
			HHCOLD1	0.0170	b	household member with common cold in 2 weeks before 1st interview (yes, no)
			WACANOE1	0.0237	b	normal frequency of canoeing during summer (never, sometimes: 1-3, often: >3)
			HPUPOOL1	0.0132	b	normal frequency of using a private pool per month during sumer: never; sometimes: 1-3; often: >3)
			WQCONTRPN4	0.0099	b	quality of information on control of beaches in Germany (positive or negative)
			WQCLEANPN4	0.0136	b	quality of information on control of beaches in Germany (positive or negative)
			DEGREE1	0.0441	i	degree (none, primary school, non-classical secondary school, secondary scool 12th class, secondary school 13th class, non-classical university, classical university, other)
			DEGREE1_2	0.0003	i	degree (none, primary school, non-classical secondary school, secondary scool, university)
			BBILL4	0.0084	i	ever gone to a beach while feeling ill (yes, no)
			WASURF1	0.0076	i	normal frequency of surfing during summer (never, sometimes: 1-3, often: >3)
19	IE	EAR	SYEAR1	0.0031	b	ear inflammation in three weeks before 1st interview
			SYHEAD1	0.0000	i	headache in three weeks before 1st interview
			SYDCOUGH3	0.0000	i	dry cough in the week after exposure
20	AE	EAR	SYEAR1	0.0044	b	ear inflammation in three weeks before 1st interview
			SYHEAD1	0.0000	i	headache in three weeks before 1st interview
			SYDCOUGH3	0.0000	i	dry cough in the week after exposure
21	EC	EYE	CHAY1	0.0418	b	chronic hay fever
			HBEACHFR1_3	0.0267	b	beach visits without entering the water in 4 weeks before 1st interview (yes, no)

No.	Parameter	Disease	Variable	p	Effect	Explanation
			HHMEMB1_2	0.0201	i	number of household members (1, 2, 3, 4, 5, >5)
			BBILL4	0.0353	i	ever gone to a beach while feeling ill (yes, no)
22	IE	EYE	CHAY1	0.0453	b	chronic hay fever
			HHMEMB1_2	0.0000	i	number of household members (1, 2, 3, 4, 5, >5)
			WACANOE1	0.0000	i	normal frequency of canoeing during summer (never, sometimes: 1-3, often: >3)
			WACANOE1_2	0.0000	i	normal frequency of canoeing during summer (never, once or more)
			WAPAD1_2	0.0000	i	normal frequency of paddling during summer (never, once or more)
			HBEACHFR1_3	0.0000	i	beach visits without entering the water in 4 weeks before 1st interview (yes, no)
23	CP	EYE	CHAY1	0.0458	b	chronic hay fever
			HBEACHFR1_3	0.0280	b	beach visits without entering the water in 4 weeks before 1st interview (yes, no)
			BBILL4	0.0104	i	ever gone to a beach while feeling ill (yes, no)
24	AE	EYE	CHAY1	0.0000	i	chronic hay fever
			HBEACHFR1_3	0.0000	i	beach visits without entering the water in 4 weeks before 1st interview (yes, no)
25	IE	GE_NL-2	SYSTOM1	0.0084	i	stomach pains or cramps in three weeks before 1st interview
			TRAVSICK4	0.0152	i	proneness to motion sickness (always, often, hardly ever, never)
26	CP	GE_UK	SYLOOSEB1	0.0354	i	loose bowel motions in three weeks before 1st interview
27	CP	GE_NL-2	SYSTOM1	0.0234	i	stomach pains or cramps in three weeks before 1st interview
28	SOMCP	GE_UK-wf	SYLOOSEB1	0.0189	i	loose bowel motions in three weeks before 1st interview
29	SOMCP	GE_NL-2	SYSTOM1	0.0319	i	stomach pains or cramps in three weeks before 1st interview
30	AE	GE_NL-2	HHCHILD1_2	0.0865	b	number of children < 5 years of age living in household (none, 1, >1)
			HHCHILD1_3	0.0722	b	children < 5 years living in household (yes, no)
			CSTOM1	0.0042	i	chronic stomach ailments

Annex 24. "Which indicator is the best?": Comparison of the indicator organisms by multiple logistic regression (Effect Likelihood Ratio Tests)

To evaluate differences in the power of the various groups of microorganisms to indicate the risk of acquiring gastroenteritis, effect likelihood ratio tests were performed for all combinations of indicator organisms, exposure definitions and definitions of gastroenteritis one by one. Threshold concentrations determined for the various combinations of indicator organisms, exposure definitions and definitions of gastroenteritis were modelled as effect source variables, and gastroenteritis was modelled as outcome variable. The likelihood-ratio Chi square tests were calculated as twice the difference of the log-likelihoods between the full model and the model constrained by the hypothesis to be tested (the model without the effect). Chi square p values were used to decide which of the two indicator organisms in the model was superior. The results demonstrated that intestinal enterococci and *Escherichia coli* are the two indicators which worked well in most cases. *Clostridium perfringens* and somatic coliphages would also work but would probably be less effective. Compared to the faecal indicator organisms aeromonads are of no use as potential predictors for the risk of acquiring gastroenteritis.

Legend

Parameter:	
CP	*Clostridium perfringens*
EC	*Escherichia coli*
IE	Intestinal enterococci
SOMCP	Somatic coliphages
AE	Aeromonads

Disease definition:	
GE_UK	Gastroenteritis, UK definition
GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis, NL-2 definition

Miscellaneous:	
(b)	number of tests in which the parameter was superior; i. e. a better predictor of the disease
p	Chi square p value in effect likelihood ratio test
s	significant difference * if $p < 0.05$

		Exposure definition 1: ("10 min bathing; 3 head immersions")						Exposure definition 2: ("1 head immersion")					
Parameter	(b)	GE_UK p	s	GE_UK-wf p	s	GE_NL-2 p	s	GE_UK p	s	GE_UK-wf p	s	GE_NL-2 p	s
CP	2	0.0896		0.1058		0.1869		0.1443				0.0469	*
EC	3	0.1983		0.0926		0.1362		0.0242	*			0.0509	
CP	1	0.0778		0.3041		0.3279		0.3757		0.7288		0.3430	
EN	5	0.4363		0.0497	*	0.1339		0.0878		0.0120	*	0.0783	
CP	3	0.0085	*	0.1235		0.2311		0.1637		0.4922		0.1254	
SOMCP	3	0.1110		0.1476		0.1868		0.0645		0.1078		0.3211	
EC	2	0.1770		0.5314		0.3824		0.0794				0.1673	
EN	3	0.4602		0.0846		0.2097		0.1328				0.0394	*
EC	4	0.0244	*	0.2061		0.3254		0.0352	*			0.1012	
SOMCP	1	0.1499		0.2591		0.2584		0.3743				0.1872	
EN	6	0.0336	*	0.0974		0.3680		0.1384		0.0561		0.0750	
SOMCP	0	0.1006		0.9567		0.6749		0.2075		0.7867		0.9470	
AE	0											0.7416	
CP	1											0.0241	*
AE	0											0.2091	
EC	1											0.0114	*
AE	0											0.8223	
EN	1											0.0078	*
AE	0											0.4924	
SOMCP	1											0.0584	

Total

EN	15
EC	10
CP	7
SOMCP	5
AE	0

Annex 25. Dependence of threshold concentrations and incidence rates of gastroenteritis below and above threshold concentrations on disease definition, exposure definition and faecal indicator

Threshold concentrations for increased risk of gastroenteritis from bathing in faecally contaminated water could be determined using a step model approach for 23 of the 24 possible combinations of four faecal indicator parameters (EC, IE, CP, SOMCP), three definitions of gastroenteritis (GE_UK, GE_UK-wf, GE_NL-2) and two exposure definitions ("10 minutes bathing, ≥ 3 head immersions", "1 head immersion"). ANOVA tests were used to examine the dependence of threshold concentrations and incidence rates of gastroenteritis below and above threshold concentrations on disease definition, exposure definition and faecal indicator parameters.

The analyses suggest that:

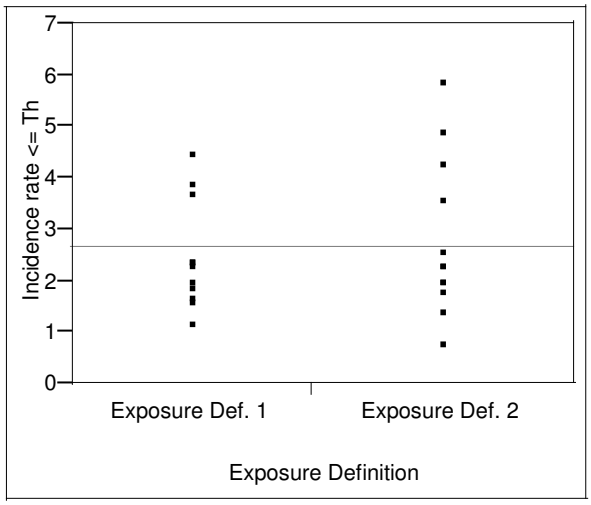
- Threshold concentrations depend on the definition of exposure (high exposure intensities yielding lower threshold levels than low exposure intensities), and on the choice of the faecal indicator parameters (faecal indicator parameters which are generally less abundant in faecal material, e. g. CP, yielding lower threshold levels than parameters which are generally more abundant, e. g. EC). Threshold concentrations, however, are independent of the definition of gastroenteritis, i. e. is makes no difference whether gastroenteritis is defined in a more or less stringent way.

- The incidence rate of gastroenteritis below threshold concentrations depends only on the definition of gastroenteritis, the more stringent definitions yielding lower incidence rates than the less stringent definitions. It is, however, independent of the choice of the faecal indicator parameter (EC, IE, CP or SOMCP). It is also independent of the definition of exposure.

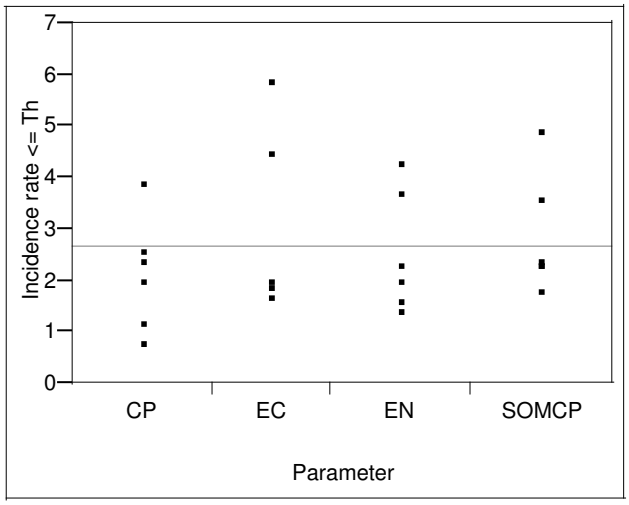
- The incidence rate of gastroenteritis above threshold concentrations (maximum incidence rate or "ceiling" of the dose response relationship) depends only on the definition of gastroenteritis as well, the more stringent definitions yielding lower maximum levels than the less stringent definitions. Like the incidence rate below threshold concentrations it is independent of the choice of the faecal indicator parameter and independent of the definition of exposure.

Legend

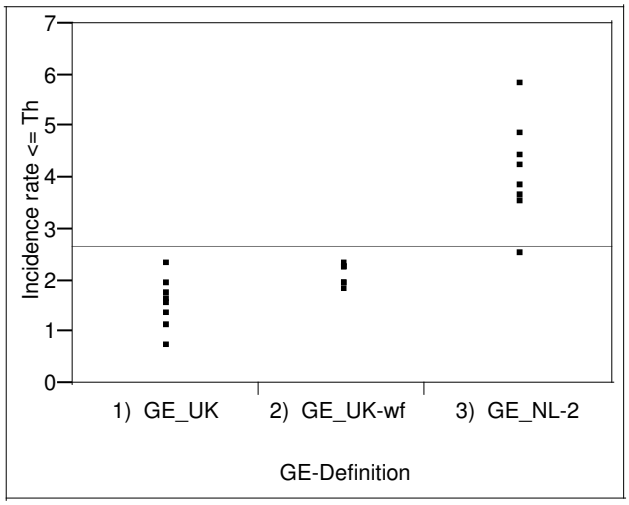
GE_UK	Gastroenteritis, UK definition
GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis, NL-2 definition
CP	*Clostridium perfringens*
EC	*Escherichia coli*
IE	Intestinal enterococci
SOMCP	Somatic coliphages
Exposure Def. 1	"10 minutes bathing, ≥ 3 head immersions"
Exposure Def. 2	"1 head immersion"
Th	Threshold concentration



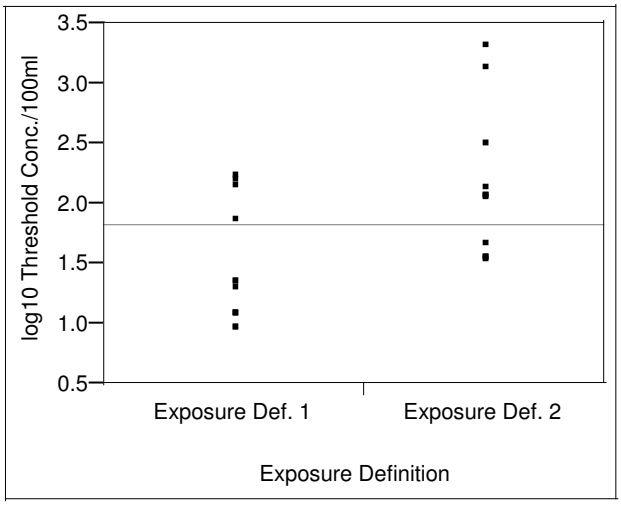
ANOVA p = 0.5741



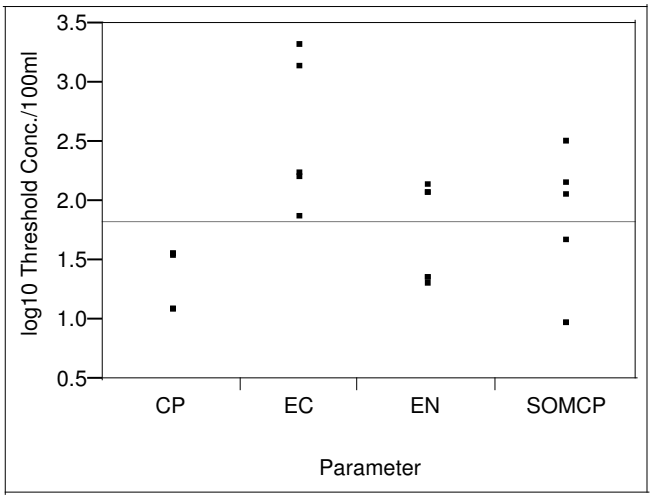
ANOVA p = 0.6050



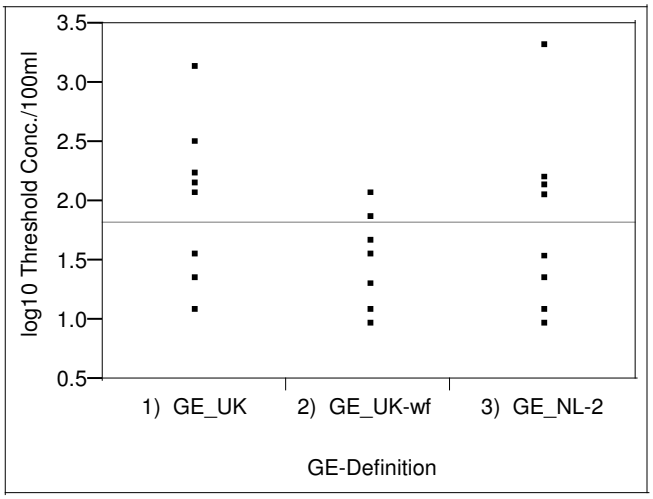
ANOVA p < 0.0001



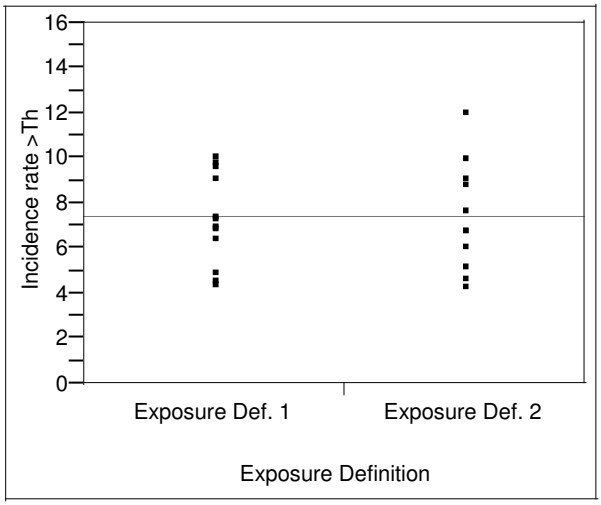
ANOVA p = 0.0088



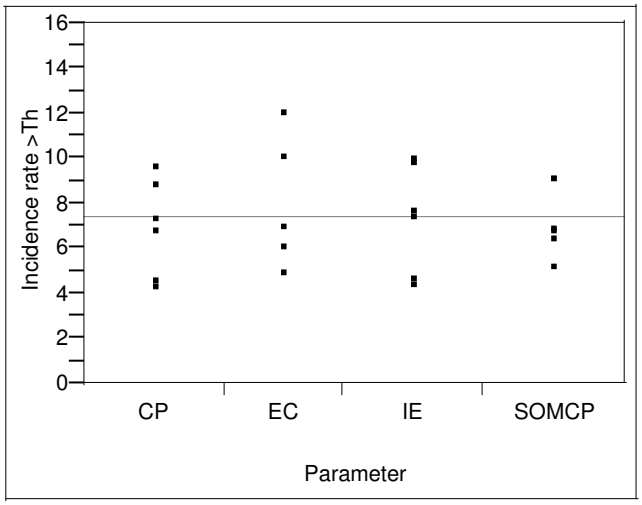
ANOVA p = 0.0061



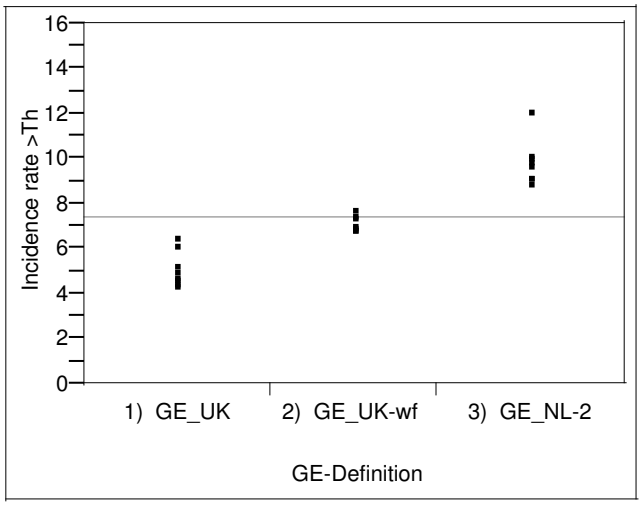
ANOVA p = 0.3209



ANOVA p = 0.8997

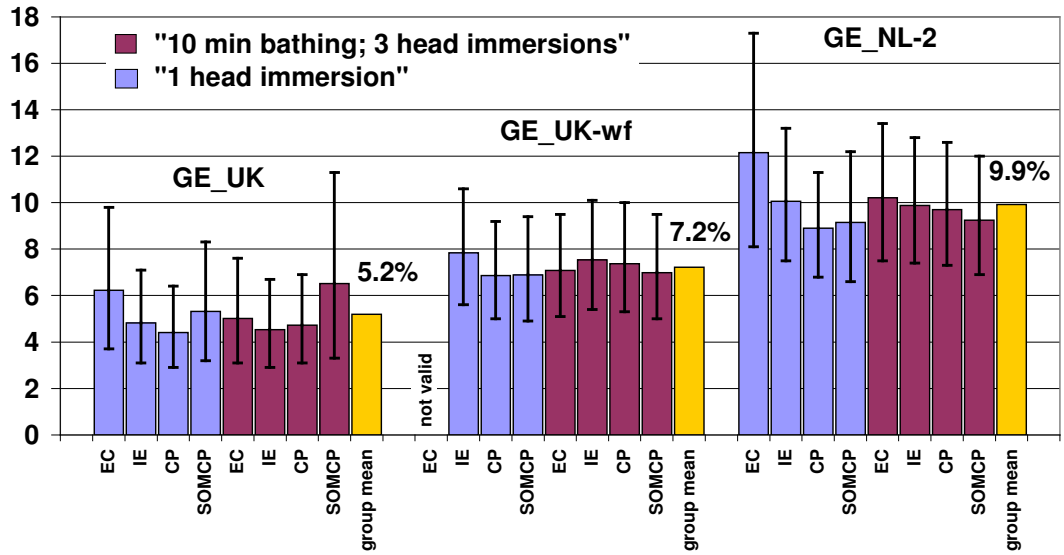


ANOVA p = 0.8772



ANOVA p < 0.0001

Incidence rate of gastroenteritis in bathers above threshold



I = **95% Confidence interval**

Legend

GE_UK	Gastroenteritis, UK definition
GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis, NL-2 definition
CP	*Clostridium perfringens*
EC	*Escherichia coli*
IE	Intestinal enterococci
SOMCP	Somatic coliphages

Annex 26. Incidence rates of gastroenteritis in quartile categories

In the tables and figures in this annex the incidence rates among non-bathers were compared to the incidence rates among bathers exposed in quartile categories of microbiological exposure concentrations. Pearson's Chi square tests were separately performed for each of the four categories. Significant differences between bathers and non-bathers ($p<0.05$) were marked by an asterisk.

Legend

Disease definitions:

GE_UK	Gastroenteritis, UK definition
GE_UK-3-weeks	Gastroenteritis, UK definition, 3 weeks after exposure
GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis, NL-2 definition

Faecal indicator parameters:

CP	*Clostridium perfringens*	(concentration per 10 ml)
EC	*Escherichia coli*	(concentration per 100 ml)
IE	Intestinal enterococci	(concentration per 100 ml)
SOMCP	Somatic coliphages	(concentration per 100 ml)

Exposure definitions:

Exposure Def. 1	"10 minutes bathing, ≥ 3 head immersions"
Exposure Def. 2	"1 head immersion"

Exposure:

0	Non-bathers
xy	quartile cut points

Disease:

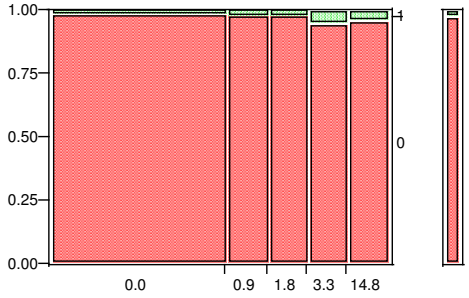
0	Participants without gastroenteritis according to specified definition
1	Participants with gastroenteritis according to specified definition

Miscellaneous:

n	Total number of participants in this analysis
p Chi^2	Probability of error in Pearson's Chi Square test
*	p $Chi^2 < 0.05$
s	"suspect"; if an expected cell value was less than 5

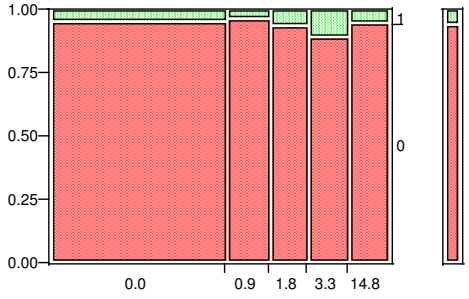
Contingency Table
Quartiles_Exposure Def. 1_CP By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
0.9	218	4	222	1.8	0.6677		s
1.8	195	4	199	2.0	0.5324		s
3.3	198	11	209	5.3	0.0005	*	s
14.8	198	9	207	4.3	0.0058	*	s
	1716	41	1757				



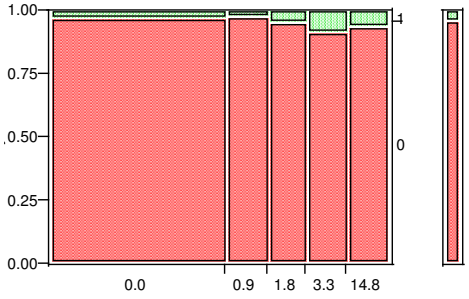
Contingency Table
Quartiles_Exposure Def. 1_CP By GE_UK-3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
0.9	210	8	218	3.7	0.4855		
1.8	183	13	196	6.6	0.2819		
3.3	183	22	205	10.7	0.0010	*	
14.8	188	11	199	5.5	0.6530		
	1623	97	1720				



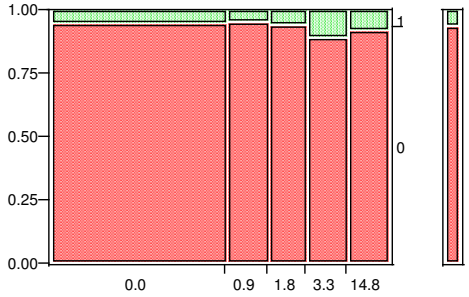
Contingency Table
Quartiles_Exposure Def. 1_CP By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
0.9	218	6	224	2.7	0.9064		
1.8	193	9	202	4.5	0.2266		
3.3	193	18	211	8.5	0.0001	*	
14.8	197	13	210	6.2	0.0158	*	
	1696	72	1768				



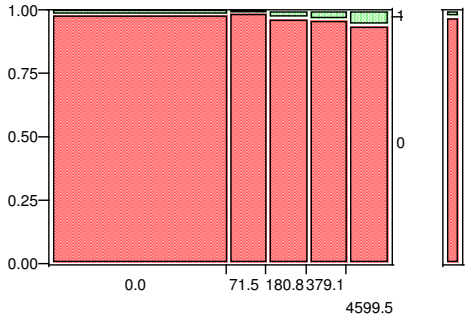
Quartiles_Exposure Def. 1_CP By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
0.9	213	11	224	4.9	0.8550		
1.8	190	12	202	5.9	0.6766		
3.3	188	23	211	10.9	0.0021	*	
14.8	192	17	209	8.1	0.1014		
	1656	111	1767				



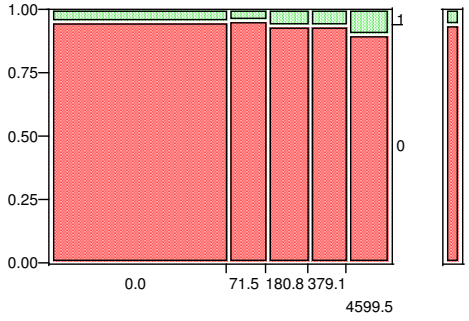
Contingency Table
Quartiles_Exposure Def. 1_EC By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
71.5	203	2	205	1.0	0.6214		s
180.8	203	6	209	2.9	0.1391		s
379.1	199	8	207	3.9	0.0184	*	s
4599.5	195	12	207	5.8	0.0001	*	s
	1707	41	1748				



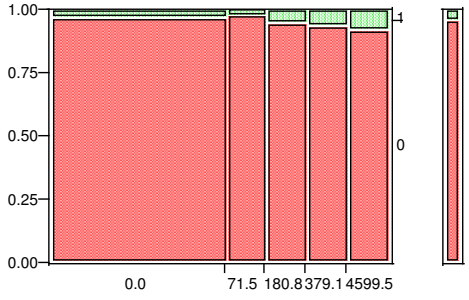
Contingency Table
Quartiles_Exposure Def. 1_EC By GE_UK-3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
71.5	194	9	203	4.4	0.8393		
180.8	195	13	208	6.3	0.3784		
379.1	186	13	199	6.5	0.3049		
4599.5	181	19	200	9.5	0.0086	*	
	1615	97	1712				



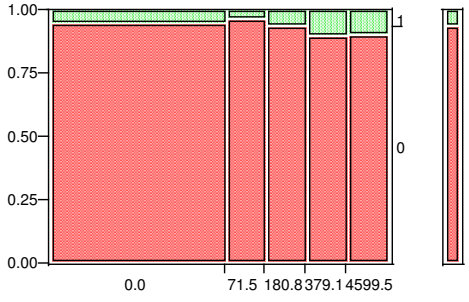
Contingency Table
Quartiles_Exposure Def. 1_EC By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
71.5	203	4	207	1.9	0.4717		
180.8	201	11	212	5.2	0.0806		
379.1	197	14	211	6.6	0.0068	*	
4599.5	191	17	208	8.2	0.0003	*	
	1687	72	1759				



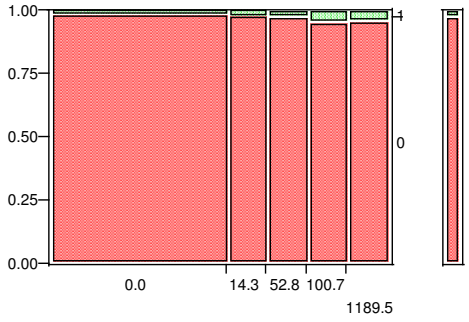
Contingency Table
Quartiles_Exposure Def. 1_EC By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
71.5	200	7	207	3.4	0.2693		
180.8	197	14	211	6.6	0.4124		
379.1	189	22	211	10.4	0.0046	*	
4599.5	188	20	208	9.6	0.0159	*	
	1647	111	1758				



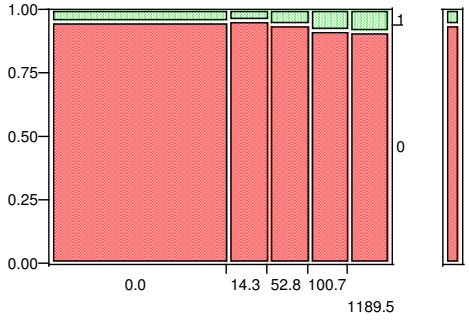
Contingency Table
Quartiles_Exposure Def. 1_EN By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
14.3	203	4	207	1.9	0.5797		s
52.8	205	5	210	2.4	0.3121		s
100.7	197	10	207	4.8	0.0017	*	s
1189.5	195	9	204	4.4	0.0052	*	s
	1707	41	1748				



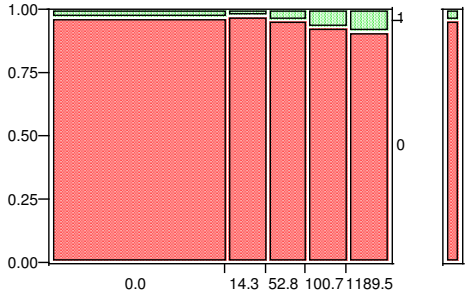
Contingency Table
Quartiles_Exposure Def. 1_EN By GE_UK-3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
14.3	195	9	204	4.4	0.8285		
52.8	197	12	209	5.7	0.5584		
100.7	185	16	201	8.0	0.0689		
1189.5	179	17	196	8.7	0.0292	*	
	1615	97	1712				



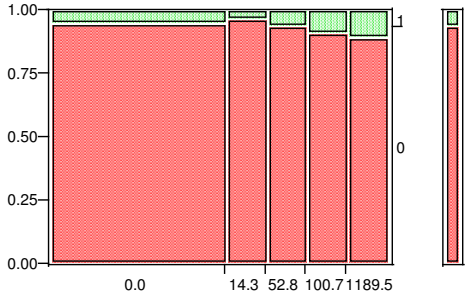
Contingency Table
Quartiles_Exposure Def. 1_EN By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
14.3	203	5	208	2.4	0.7383		
52.8	203	9	212	4.2	0.2805		
100.7	196	14	210	6.7	0.0065	*	
1189.5	190	18	208	8.7	0.0001	*	
	1687	72	1759				



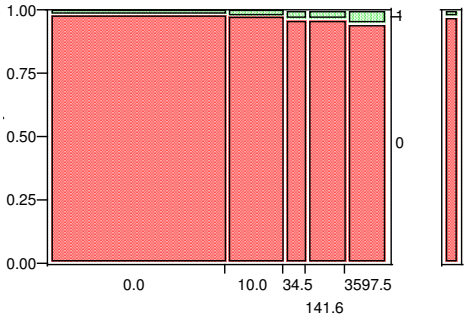
Contingency Table
Quartiles_Exposure Def. 1_EN By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
14.3	200	8	208	3.8	0.4126		
52.8	198	13	211	6.2	0.5817		
100.7	191	19	210	9.0	0.0336	*	
1189.5	185	23	208	11.1	0.0017	*	
	1647	111	1758				



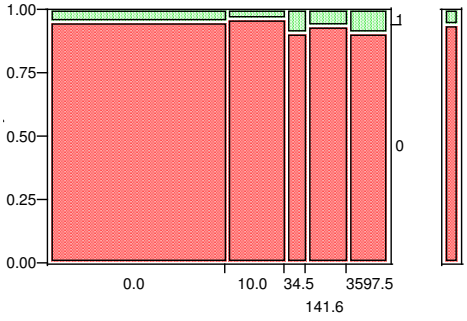
Contingency Table
Quartiles_Exposure Def. 1_SOMCP By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
10	295	5	300	1.7	0.7517		s
34.5	107	4	111	3.6	0.0869		s
141.6	202	7	209	3.3	0.0554		s
3597.5	194	11	205	5.4	0.0004	*	s
	1705	40	1745				



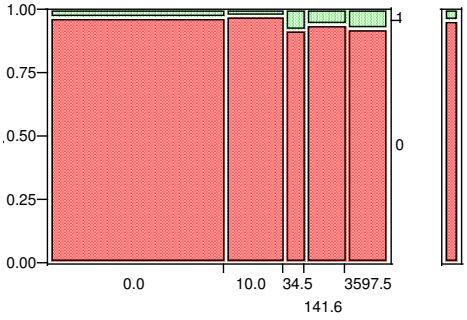
Contingency Table
Quartiles_Exposure Def. 1_SOMCP By GE_UK-3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
10	286	11	297	3.7	0.4434		
34.5	100	10	110	9.1	0.0546		
141.6	191	13	204	6.4	0.3449		
3597.5	177	18	195	9.2	0.0136	*	
	1613	95	1708				



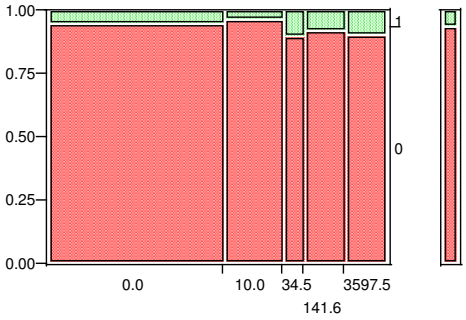
Contingency Table
Quartiles_Exposure Def. 1_SOMCP By GE_UK-wf

	Disease						
Count	0	1		%	p Chi²	*	s
0	895	26	921	2.8			
10	295	7	302	2.3	0.6383		
34.5	106	9	115	7.8	0.0051	*	s
141.6	200	12	212	5.7	0.0386	*	
3597.5	190	16	206	7.8	0.0007	*	
	1686	70	1756				



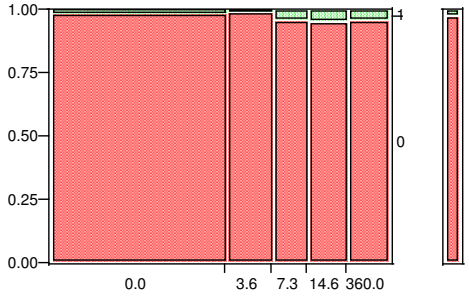
Contingency Table
Quartiles_Exposure Def. 1_SOMCP By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
10	290	11	301	3.7	0.2739		
34.5	103	12	115	10.4	0.0238	*	
141.6	195	17	212	8.0	0.1130		
3597.5	186	20	206	9.7	0.0143	*	
	1647	108	1755				



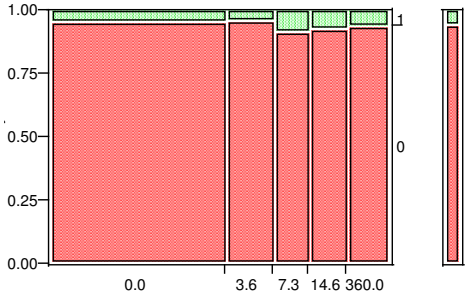
Contingency Table
Quartiles_Exposure Def. 2_CP By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
3.6	240	2	242	0.8	0.4719		s
7.3	177	8	185	4.3	0.0081	*	s
14.6	192	9	201	4.5	0.0045	*	s
360	200	9	209	4.3	0.0063	*	s
	1716	41	1757				



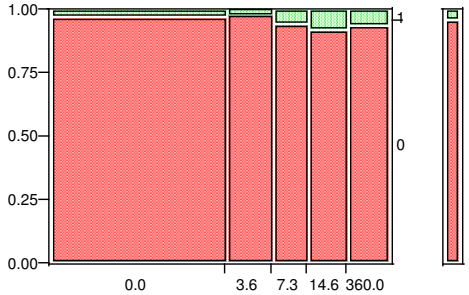
Contingency Table
Quartiles_Exposure Def. 2_CP By GE_UK-3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
3.6	230	10	240	4.2	0.6943		
7.3	166	16	182	8.8	0.0290	*	
14.6	180	15	195	7.7	0.0979		
360	188	13	201	6.5	0.3207		
	1623	97	1720				



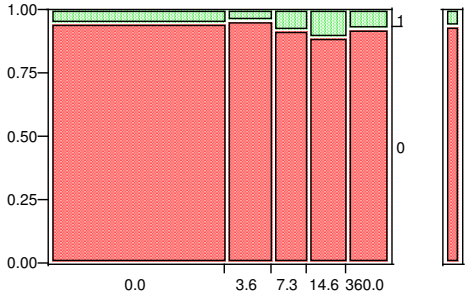
Contingency Table
Quartiles_Exposure Def. 2_CP By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
3.6	239	5	244	2.0	0.5043		
7.3	175	11	186	5.9	0.0324	*	
14.6	188	17	205	8.3	0.0002	*	
360	199	13	212	6.1	0.0172	*	
	1696	72	1768				



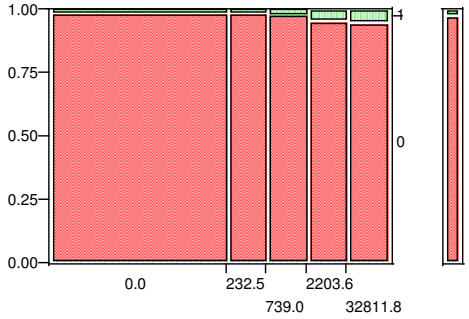
Contingency Table
Quartiles_Exposure Def. 2_CP By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
3.6	234	10	244	4.1	0.4771		
7.3	171	15	186	8.1	0.1256		
14.6	183	22	205	10.7	0.0031	*	
360	195	16	211	7.6	0.1786		
	1656	111	1767				



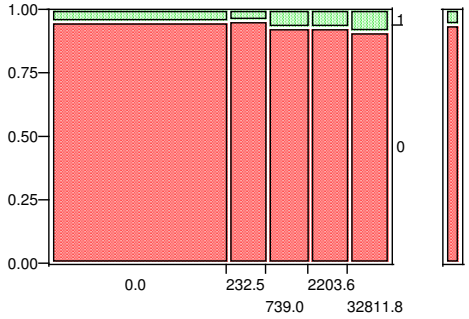
Contingency Table
Quartiles_Exposure Def. 2_EC By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
232.5	202	3	205	1.5	0.9561		s
739	205	4	209	1.9	0.5915		s
2203.6	198	10	208	4.8	0.0018	*	s
32811.8	195	11	206	5.3	0.0004	*	s
	1707	41	1748				



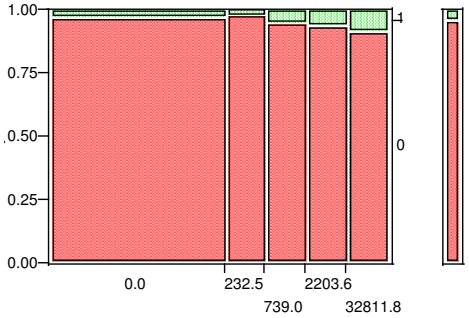
Contingency Table
Quartiles_Exposure Def. 2_EC By GE_UK-3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
232.5	194	9	203	4.4	0.8393		
739	194	14	208	6.7	0.2474		
2203.6	189	14	203	6.9	0.2152		
32811.8	179	17	196	8.7	0.0292	*	
	1615	97	1712				



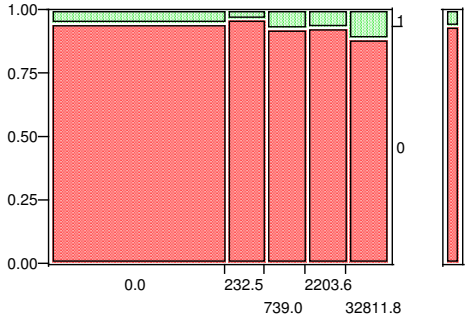
Contingency Table
Quartiles_Exposure Def. 2_EC By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
232.5	202	4	206	1.9	0.4775		
739	202	11	213	5.2	0.0830		
2203.6	197	13	210	6.2	0.0158	*	
32811.8	191	18	209	8.6	0.0001	*	
	1687	72	1759				



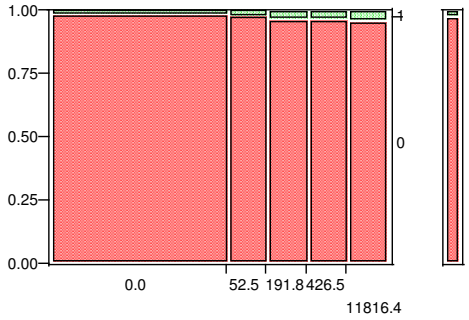
Contingency Table
Quartiles_Exposure Def. 2_EC By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
232.5	198	8	206	3.9	0.4277		
739	196	16	212	7.5	0.1842		
2203.6	195	15	210	7.1	0.2709		
32811.8	185	24	209	11.5	0.0008	*	
	1647	111	1758				



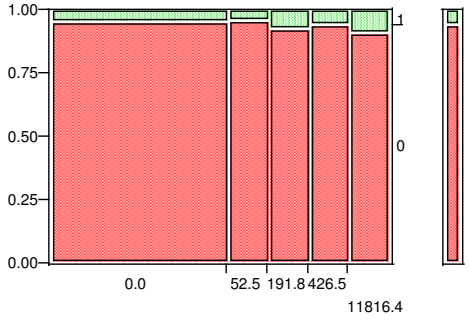
Contingency Table
Quartiles_Exposure Def. 2_EN By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
52.5	204	4	208	1.9	0.5856		s
191.8	199	8	207	3.9	0.0184	*	s
426.5	202	7	209	3.3	0.0554		s
11816.4	195	9	204	4.4	0.0052	*	s
	1707	41	1748				



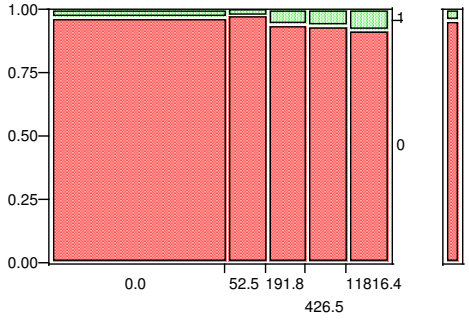
Contingency Table
Quartiles_Exposure Def. 2_EN By GE_UK-3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
52.5	196	9	205	4.4	0.8179		
191.8	192	15	207	7.2	0.1485		
426.5	192	12	204	5.9	0.5082		
11816.4	176	18	194	9.3	0.0129	*	
	1615	97	1712				



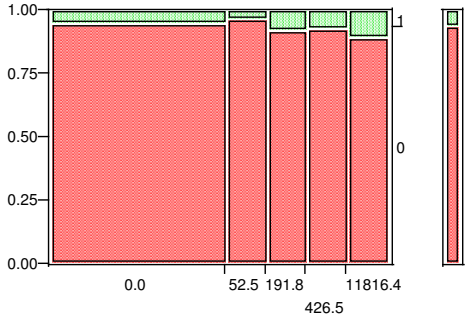
Contingency Table
Quartiles_Exposure Def. 2_EN By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
52.5	204	4	208	1.9	0.4661		
191.8	198	12	210	5.7	0.0359	*	
426.5	199	13	212	6.1	0.0172	*	
11816.4	191	17	208	8.2	0.0003	*	
	1687	72	1759				



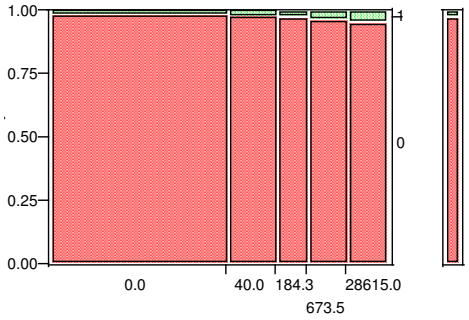
Contingency Table
Quartiles_Exposure Def. 2_EN By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
52.5	201	7	208	3.4	0.2639		
191.8	192	17	209	8.1	0.1014		
426.5	196	16	212	7.5	0.1842		
11816.4	185	23	208	11.1	0.0017	*	
	1647	111	1758				



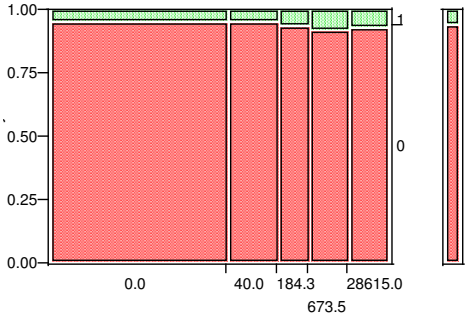
Contingency Table
Quartiles_Exposure Def. 2_SOMCP By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
40	247	5	252	2.0	0.5137		s
184.3	155	4	159	2.5	0.3025		s
673.5	198	8	206	3.9	0.0178	*	s
28615	198	10	208	4.8	0.0018	*	s
	1705	40	1745				



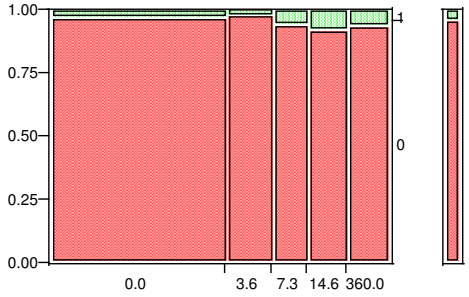
Contingency Table
Quartiles_Exposure Def. 2_SOMCP By GE_UK-3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
40	239	12	251	4.8	0.9928		
184.3	147	10	157	6.4	0.3955		
673.5	185	16	201	8.0	0.0689		
28615	183	14	197	7.1	0.1798		
	1613	95	1708				



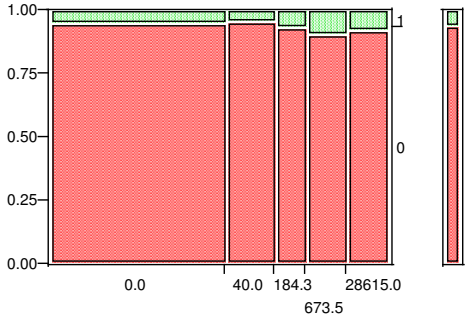
Contingency Table
Quartiles_Exposure Def. 2_SOMCP By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
40	247	7	254	2.8	0.9543		
184.3	153	9	162	5.6	0.0697		
673.5	195	15	210	7.1	0.0025	*	
28615	196	13	209	6.2	0.0151	*	
	1686	70	1756				



Contingency Table
Quartiles_Exposure Def. 2_SOMCP By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
40	242	12	254	4.7	0.7548		
184.3	150	11	161	6.8	0.4034		
673.5	190	20	210	9.5	0.0177	*	
28615	192	17	209	8.1	0.1014		
	1647	108	1755				



Annex 27. Incidence rates of gastroenteritis in quintile categories

In the tables and figures in this annex the incidence rates among non-bathers were compared to the incidence rates among bathers exposed in quintile categories of microbiological exposure concentrations. Pearson's Chi square tests were separately performed for each of the five categories. Significant differences between bathers and non-bathers ($p<0.05$) were marked by an asterisk.

Legend

Disease definitions:

GE_UK	Gastroenteritis, UK definition
GE_UK-3-weeks	Gastroenteritis, UK definition, 3 weeks after exposure
GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis, NL-2 definition

Faecal indicator parameters:

CP	*Clostridium perfringens*	(concentration per 10 ml)
EC	*Escherichia coli*	(concentration per 100 ml)
IE	Intestinal enterococci	(concentration per 100 ml)
SOMCP	Somatic coliphages	(concentration per 100 ml)

Exposure definitions:

Exposure Def. 1	"10 minutes bathing, ≥ 3 head immersions"
Exposure Def. 2	"1 head immersion"

Exposure:

0	Non-bathers
xy	quintile cut points

Disease:

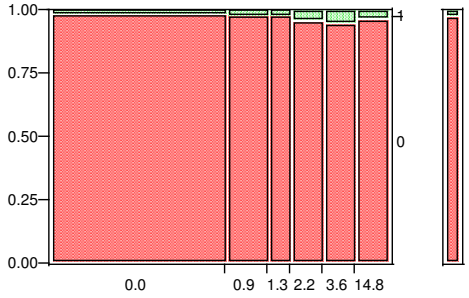
0	Participants without gastroenteritis according to specified definition
1	Participants with gastroenteritis according to specified definition

Miscellaneous:

n	Total number of participants in this analysis
p Chi^2	Probability of error in Pearson's Chi Square test
*	p $Chi^2 < 0.05$
s	"suspect"; if an expected cell value was less than 5

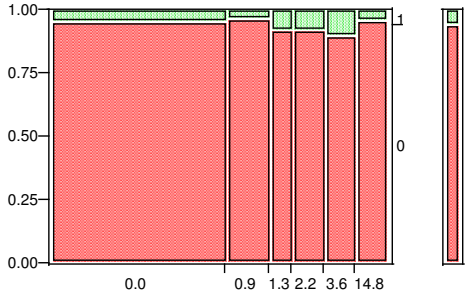
Contingency Table
Quintiles_Exposure Def. 1_CP By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
0.9	218	4	222	1.8	0.6677		s
1.3	113	2	115	1.7	0.7826		s
2.2	162	7	169	4.1	0.0152	*	s
3.6	155	9	164	5.5	0.0007	*	s
14.8	161	6	167	3.6	0.0480	*	s
	1716	41	1757				



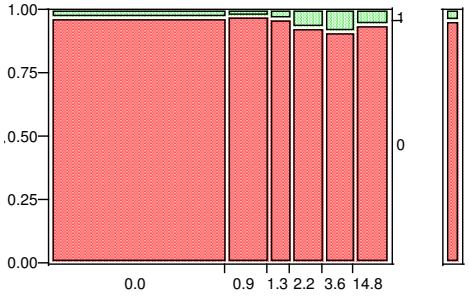
Contingency Table
Quintiles_Exposure Def. 1_CP By GE_UK-3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
0.9	210	8	218	3.7	0.4855		
1.3	104	9	113	8.0	0.1461		
2.2	154	14	168	8.3	0.0588		
3.6	141	16	157	10.2	0.0062	*	
14.8	155	7	162	4.3	0.8048		
	1623	97	1720				



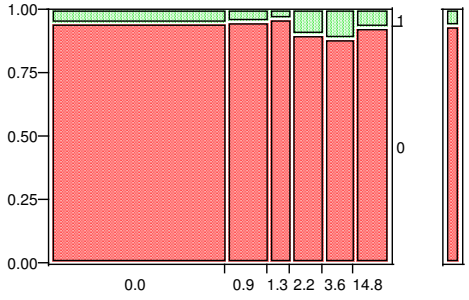
Contingency Table
Quintiles_Exposure Def. 1_CP By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
0.9	218	6	224	2.7	0.9064		
1.3	111	4	115	3.5	0.6928		s
2.2	160	12	172	7.0	0.0063	*	
3.6	152	14	166	8.4	0.0004	*	
14.8	160	10	170	5.9	0.0402	*	
	1696	72	1768				

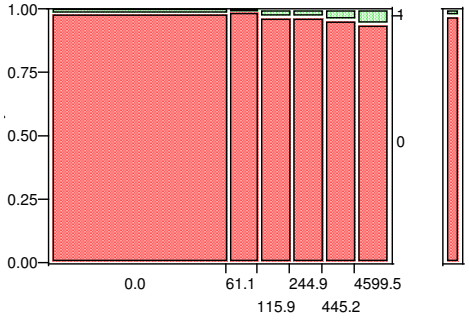


C
Quintiles_Exposure Def. 1_CP By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
0.9	213	11	224	4.9	0.8550		
1.3	111	4	115	3.5	0.4221		
2.2	155	17	172	9.9	0.0174	*	
3.6	146	19	165	11.5	0.0019	*	
14.8	158	12	170	7.1	0.3317		
	1656	111	1767				

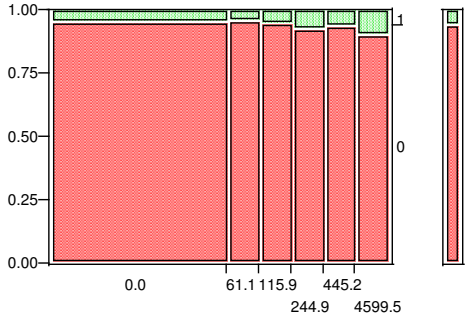


Contingency Table
Quintiles_Exposure Def. 1_EC By GE_UK



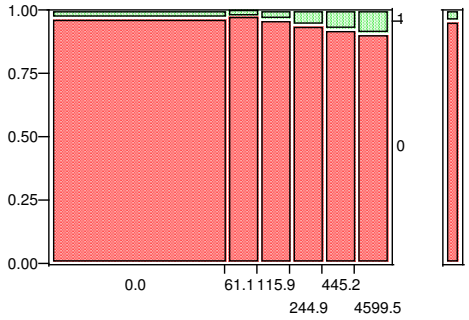
	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
61.1	163	1	164	0.6	0.4013		s
115.9	162	5	167	3.0	0.1408		s
244.9	162	5	167	3.0	0.1408		s
445.2	156	7	163	4.3	0.0118	*	s
4599.5	157	10	167	6.0	0.0002	*	s
	1707	41	1748				

Contingency Table
Quintiles_Exposure Def. 1_EC By GE_UK-3-weeks



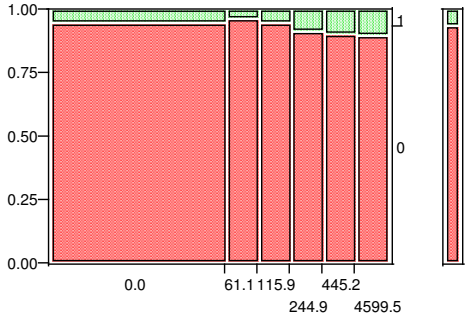
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
61.1	156	7	163	4.3	0.7929		
115.9	156	9	165	5.5	0.7062		
244.9	153	12	165	7.3	0.1808		
445.2	147	10	157	6.4	0.3955		
4599.5	144	16	160	10.0	0.0077	*	
	1615	97	1712				

Contingency Table
Quintiles_Exposure Def. 1_EC By GE_UK-wf



Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
61.1	163	3	166	1.8	0.4547		s
115.9	162	6	168	3.6	0.5973		s
244.9	160	10	170	5.9	0.0402	*	
445.2	154	12	166	7.2	0.0044	*	
4599.5	153	15	168	8.9	0.0001	*	
	1687	72	1759				

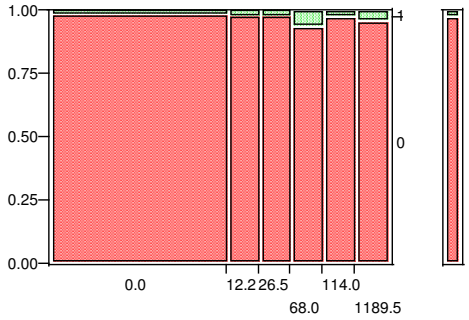
Contingency Table
Quintiles_Exposure Def. 1_EC By GE_NL-2



Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
61.1	160	6	166	3.6	0.3833		
115.9	158	9	167	5.4	0.9245		
244.9	155	15	170	8.8	0.0636		
445.2	150	16	166	9.6	0.0257	*	
4599.5	151	17	168	10.1	0.0135	*	
	1647	111	1758				

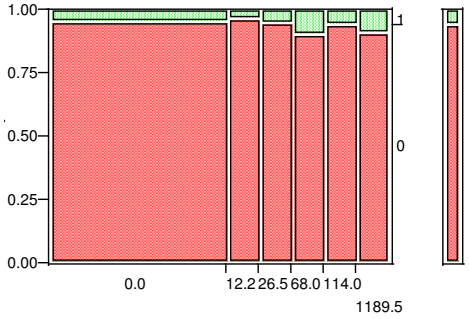
Contingency Table
Quintiles_Exposure Def. 1_EN By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
12.2	164	3	167	1.8	0.7051		s
26.5	162	3	165	1.8	0.6910		s
68	156	11	167	6.6	0.0000	*	s
114	162	4	166	2.4	0.3411		s
1189.5	156	7	163	4.3	0.0118	*	s
	1707	41	1748				



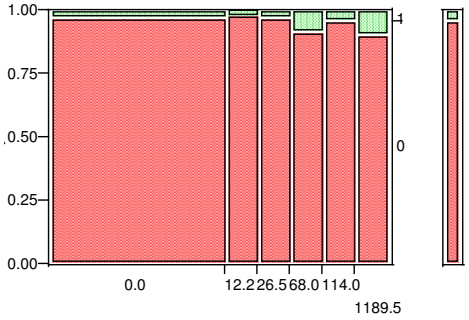
Contingency Table
Quintiles_Exposure Def. 1_EN By GE_UK-3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
12.2	158	6	164	3.7	0.5329		
26.5	156	9	165	5.5	0.7062		
68	148	16	164	9.8	0.0102	*	
114	152	9	161	5.6	0.6557		
1189.5	142	14	156	9.0	0.0316	*	
	1615	97	1712				



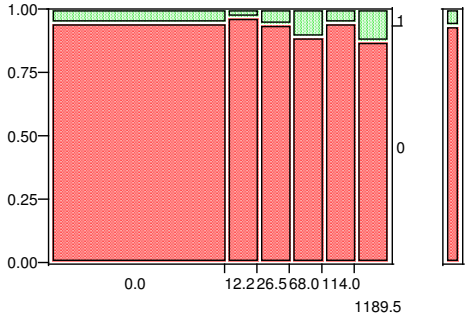
Contingency Table
Quintiles_Exposure Def. 1_EN By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
12.2	164	3	167	1.8	0.4486		s
26.5	162	5	167	3.0	0.9027		s
68	154	15	169	8.9	0.0001	*	
114	161	7	168	4.2	0.3501		
1189.5	151	16	167	9.6	0.0000	*	
	1687	72	1759				



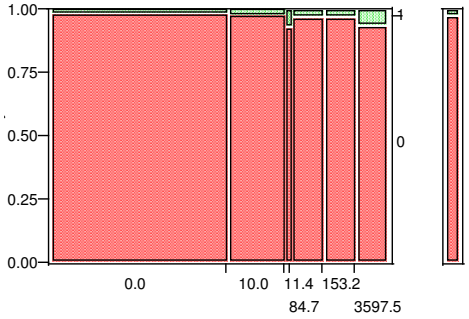
Contingency Table
Quintiles_Exposure Def. 1_EN By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
12.2	162	5	167	3.0	0.2206		
26.5	156	10	166	6.0	0.6682		
68	151	18	169	10.7	0.0064	*	
114	159	9	168	5.4	0.9380		
1189.5	146	21	167	12.6	0.0003	*	
	1647	111	1758				



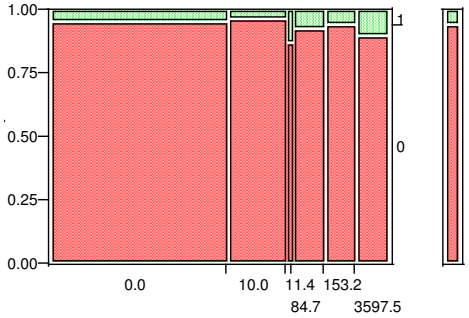
Contingency Table
Quintiles_Exposure Def. 1_SOMCP By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
10	295	5	300	1.7	0.7517		s
11.4	28	2	30	6.7	0.0231	*	s
84.7	158	5	163	3.1	0.1278		s
153.2	164	5	169	3.0	0.1475		s
3597.5	153	10	163	6.1	0.0001	*	s
	1705	40	1745				



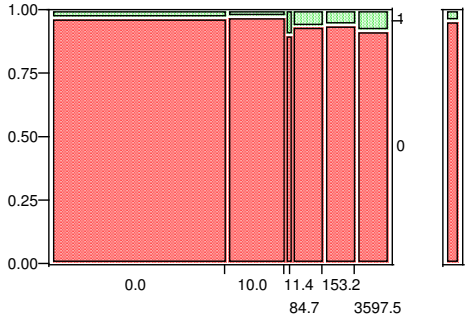
Contingency Table
Quintiles_Exposure Def. 1_SOMCP By GE_UK-3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
10	286	11	297	3.7	0.4434		
11.4	26	4	30	13.3	0.0349	*	s
84.7	148	12	160	7.5	0.1505		
153.2	153	9	162	5.6	0.6683		
3597.5	141	16	157	10.2	0.0062	*	
	1613	95	1708				



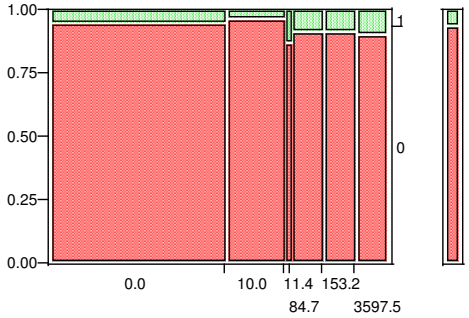
Contingency Table
Quintiles_Exposure Def. 1_SOMCP By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
10	295	7	302	2.3	0.6383		
11.4	28	3	31	9.7	0.0289	*	s
84.7	156	11	167	6.6	0.0135	*	
153.2	161	10	171	5.8	0.0419	*	
3597.5	151	13	164	7.9	0.0012	*	
	1686	70	1756				



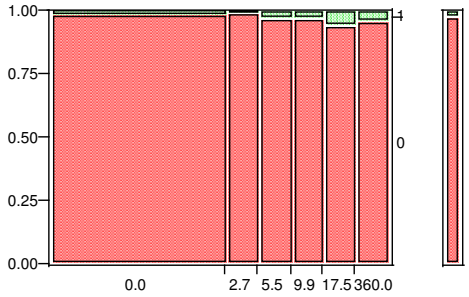
Contingency Table
Quintiles_Exposure Def. 1_SOMCP By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
10	290	11	301	3.7	0.2739		
11.4	27	4	31	12.9	0.0638		s
84.7	153	14	167	8.4	0.1038		
153.2	156	15	171	8.8	0.0667		
3597.5	148	16	164	9.8	0.0229	*	
	1647	108	1755				



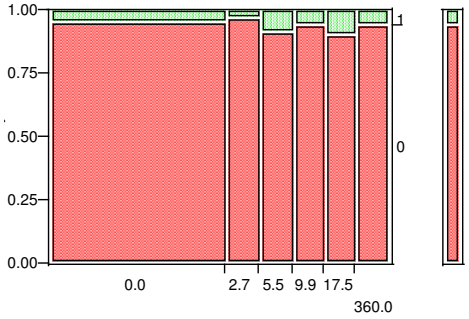
Contingency Table
Quintiles_Exposure Def. 2_CP By GE_UK

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
2.7	167	1	168	0.6	0.3871		s
5.5	169	5	174	2.9	0.1649		s
9.9	156	5	161	3.1	0.1215		s
17.5	158	10	168	6.0	0.0002	*	s
360	159	7	166	4.2	0.0134	*	s
	1716	41	1757				



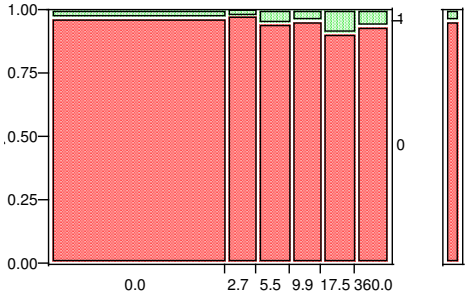
Contingency Table
Quintiles_Exposure Def. 2_CP By GE_UK-3-weeks

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
2.7	161	5	166	3.0	0.3158		
5.5	159	15	174	8.6	0.0393	*	
9.9	147	9	156	5.8	0.5930		
17.5	146	16	162	9.9	0.0089	*	
360	151	9	160	5.6	0.6431		
	1623	97	1720				



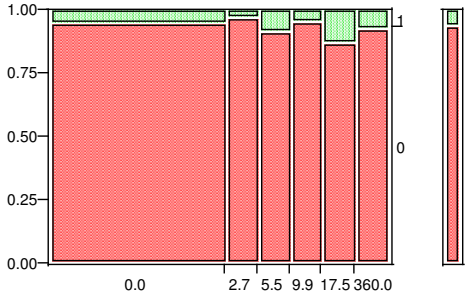
Contingency Table
Quintiles_Exposure Def. 2_CP By GE_UK-wf

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
2.7	166	3	169	1.8	0.4365		s
5.5	167	9	176	5.1	0.1131		
9.9	155	7	162	4.3	0.3063		s
17.5	155	16	171	9.4	0.0000	*	
360	158	11	169	6.5	0.0150	*	
	1696	72	1768				



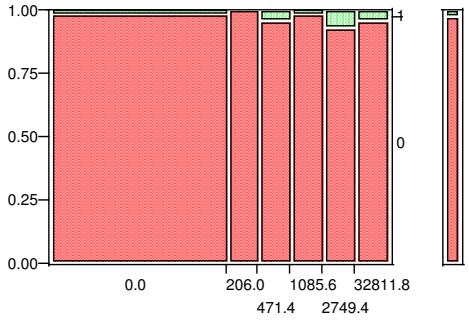
Contingency Table
Quintiles_Exposure Def. 2_CP By GE_NL-2

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
2.7	164	5	169	3.0	0.2106		
5.5	161	15	176	8.5	0.0837		
9.9	154	8	162	4.9	0.8848		
17.5	148	22	170	12.9	0.0002	*	
360	156	13	169	7.7	0.1972		
	1656	111	1767				



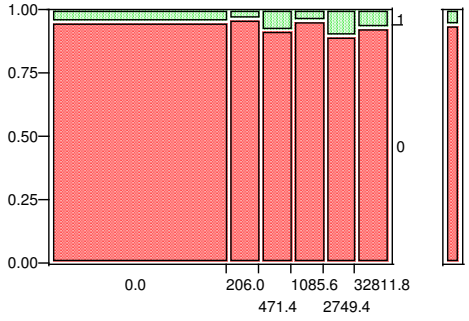
Contingency Table
Quintiles_Exposure Def. 2_EC By GE_UK

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
206	164	0	164	0.0	0.1256		s
471.4	159	7	166	4.2	0.0134	*	s
1085.6	163	2	165	1.2	0.8387		s
2749.4	155	12	167	7.2	0.0000	*	s
32811.8	159	7	166	4.2	0.0134	*	s
	1707	41	1748				



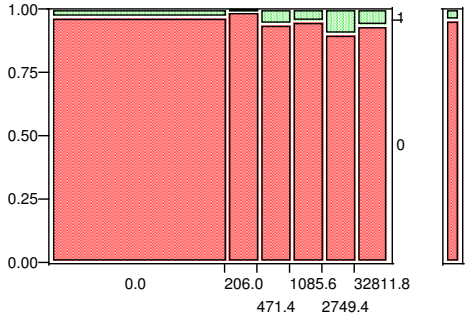
Contingency Table
Quintiles_Exposure Def. 2_EC By GE_UK-3-weeks

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
206	156	6	162	3.7	0.5521		
471.4	154	13	167	7.8	0.1079		
1085.6	154	7	161	4.3	0.8169		
2749.4	145	17	162	10.5	0.0036	*	
32811.8	147	11	158	7.0	0.2471		
	1615	97	1712				



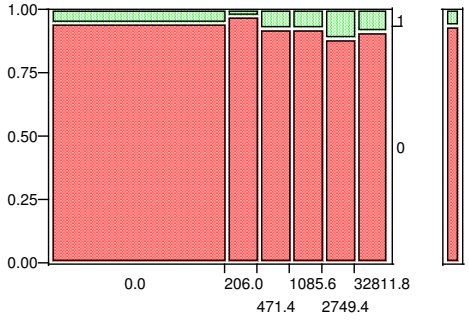
Contingency Table
Quintiles_Exposure Def. 2_EC By GE_UK-wf

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
206	164	1	165	0.6	0.0921		s
471.4	159	10	169	5.9	0.0385	*	
1085.6	160	8	168	4.8	0.1839		
2749.4	153	16	169	9.5	0.0000	*	
32811.8	156	11	167	6.6	0.0135	*	
	1687	72	1759				



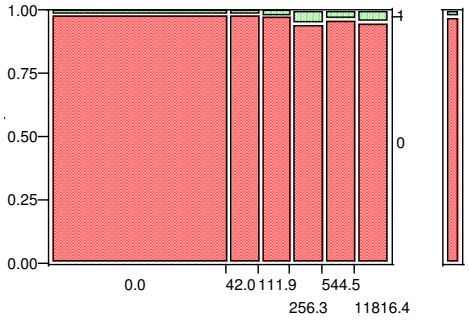
Contingency Table
Quintiles_Exposure Def. 2_EC By GE_NL-2

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
206	161	4	165	2.4	0.1225		
471.4	155	13	168	7.7	0.1903		
1085.6	155	13	168	7.7	0.1903		
2749.4	150	19	169	11.2	0.0027	*	
32811.8	153	14	167	8.4	0.1038		
	1647	111	1758				



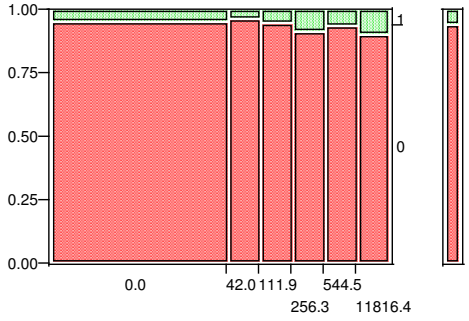
Contingency Table
Quintiles_Exposure Def. 2_EN By GE_UK

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
42	166	2	168	1.2	0.8200		s
111.9	161	3	164	1.8	0.6839		s
256.3	158	9	167	5.4	0.0008	*	s
544.5	160	6	166	3.6	0.0465	*	s
11816.4	155	8	163	4.9	0.0029	*	s
	1707	41	1748				



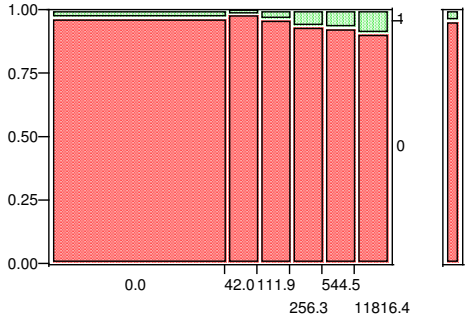
Contingency Table
Quintiles_Exposure Def. 2_EN By GE_UK-3-weeks

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
42	160	6	166	3.6	0.5142		
111.9	155	9	164	5.5	0.6935		
256.3	149	14	163	8.6	0.0460	*	
544.5	152	10	162	6.2	0.4489		
11816.4	140	15	155	9.7	0.0131	*	
	1615	97	1712				



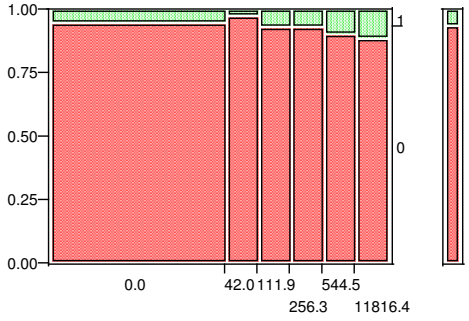
Contingency Table
Quintiles_Exposure Def. 2_EN By GE_UK-wf

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
42	166	2	168	1.2	0.2189		s
111.9	160	6	166	3.6	0.5787		s
256.3	157	11	168	6.5	0.0143	*	
544.5	158	12	170	7.1	0.0056	*	
11816.4	151	15	166	9.0	0.0001	*	
	1687	72	1759				



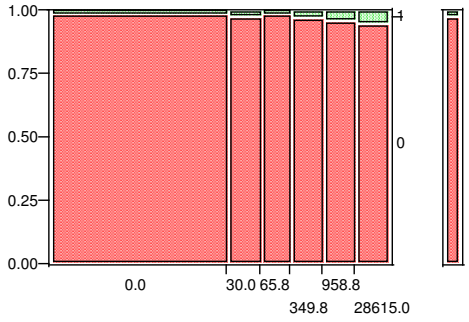
Contingency Table
Quintiles_Exposure Def. 2_EN By GE_NL-2

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
42	164	4	168	2.4	0.1136		
111.9	154	11	165	6.7	0.4477		
256.3	156	12	168	7.1	0.3130		
544.5	153	17	170	10.0	0.0154	*	
11816.4	147	19	166	11.4	0.0021	*	
	1647	111	1758				



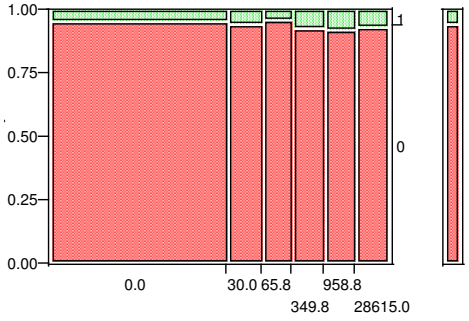
Contingency Table
Quintiles_Exposure Def. 2_SOMCP By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
30	173	4	177	2.3	0.4035		s
65.8	149	2	151	1.3	0.9316		s
349.8	160	5	165	3.0	0.1342		s
958.8	159	7	166	4.2	0.0134	*	s
28615	157	9	166	5.4	0.0007	*	s
	1705	40	1745				



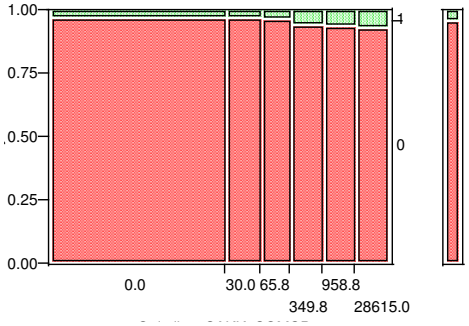
Contingency Table
Quintiles_Exposure Def. 2_SOMCP By GE_UK-3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
30	165	10	175	5.7	0.5961		
65.8	145	6	151	4.0	0.6683		
349.8	148	12	160	7.5	0.1505		
958.8	150	13	163	8.0	0.0912		
28615	146	11	157	7.0	0.2392		
	1613	95	1708				



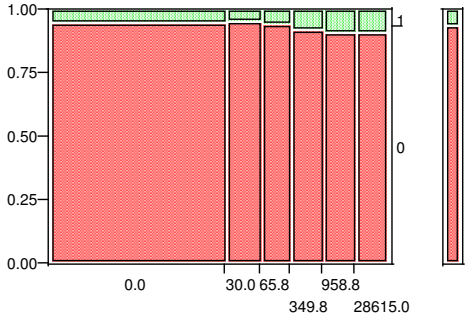
Contingency Table
Quintiles_Exposure Def. 2_SOMCP By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
30	173	5	178	2.8	0.9917		
65.8	149	6	155	3.9	0.4773		s
349.8	157	10	167	6.0	0.0354	*	
958.8	157	11	168	6.5	0.0143	*	
28615	155	12	167	7.2	0.0047	*	
	1686	70	1756				



Contingency Table
Quintiles_Exposure Def. 2_SOMCP By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
30	170	8	178	4.5	0.6903		
65.8	145	9	154	5.8	0.7458		
349.8	154	13	167	7.8	0.1836		
958.8	153	15	168	8.9	0.0577		
28615	152	15	167	9.0	0.0549		
	1647	108	1755				



Annex 28. Incidence rates of gastroenteritis in arithmetic concentration categories and in categories according to Kay et al., 1994

In the tables and figures in this annex the incidence rates among non-bathers were compared to the incidence rates among bathers exposed in arithmetic categories of microbiological exposure concentrations. 20 unit intervals were chosen for IE exposure, and 100 unit intervals were chosen for EC exposure. For the purpose of direct comparability to the results reported for sea water exposure (Kay et al., 1994) a category "80 to 158" was formed for IE exposure, as 158 IE/100ml was the maximum concentration found in these studies. In addition data were evaluated using the quartile cut points of IE exposure in the sea water studies. Pearson's Chi square test were performed separately for each of the categories. Significant differences between bathers and non-bathers ($p<0.05$) were marked by an asterisk. The results from sea water exposure were reported as incidence rates of gastroenteritis (definition GE_UK) within a time period of 3 weeks after exposure. The results from fresh water exposure are therefore also displayed for the 3-week evaluation period (definition GE_UK-3-weeks), although an evaluation interval of 3 weeks was found to be less appropriate in this study (see results section).

Legend

Disease definitions:

GE_UK	Gastroenteritis, UK definition
GE_UK-3-weeks	Gastroenteritis, UK definition, 3 weeks after exposure
GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis, NL-2 definition

Faecal indicator parameters:

CP	*Clostridium perfringens*
EC	*Escherichia coli*
IE	Intestinal enterococci
SOMCP	Somatic coliphages

Exposure definitions:

Exposure Def. 1	"10 minutes bathing, ≥ 3 head immersions"
Exposure Def. 2	"1 head immersion"

Exposure:

0	Non-bathers
xy	cut points of exposure concentration categories: Kay_quantiles (20 unit intervals from 0 to 80; 80 to 158, and >158) Kay_quartiles (quartile cut points in the sea water studies, Kay et al. 1994) 100 (100 unit intervals from 0 to 500, and >500)

Disease:

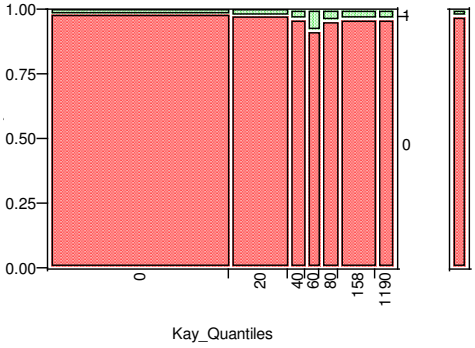
0	Participants without gastroenteritis according to specified definition
1	Participants with gastroenteritis according to specified definition

Miscellaneous:

n	Total number of participants in this analysis
p Chi^2	Probability of error in Pearson's Chi Square test
*	p $Chi^2 < 0.05$
s	"suspect"; if an expected cell value was less than 5

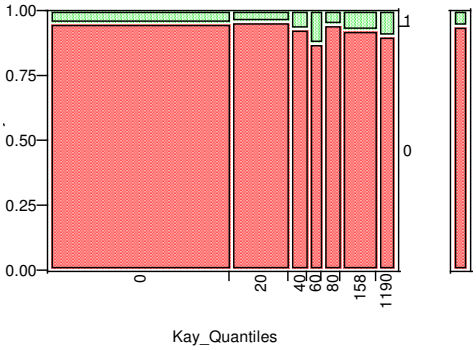
IE_Kay_Quantiles By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
20	292	5	297	1.7	0.7371		s
40	86	3	89	3.4	0.1580		s
60	69	6	75	8.0	0.0001	*	s
80	90	4	94	4.3	0.0409	*	s
158	180	7	187	3.7	0.0292	*	s
1190	83	3	86	3.5	0.1413		s
	1707	41	1748				



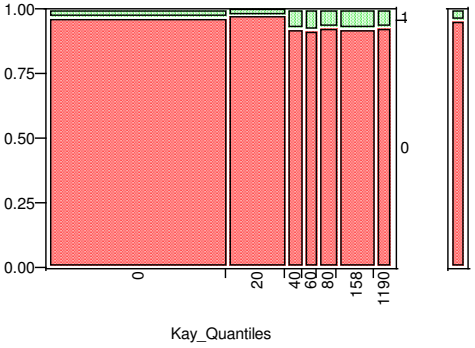
IE_Kay_Quantiles By GE_UK_3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
20	281	13	294	4.4	0.8076		
40	83	6	89	6.7	0.4124		s
60	63	9	72	12.5	0.0050	*	s
80	88	5	93	5.4	0.7941		s
158	166	13	179	7.3	0.1688		
1190	75	8	83	9.6	0.0553		s
	1615	97	1712				



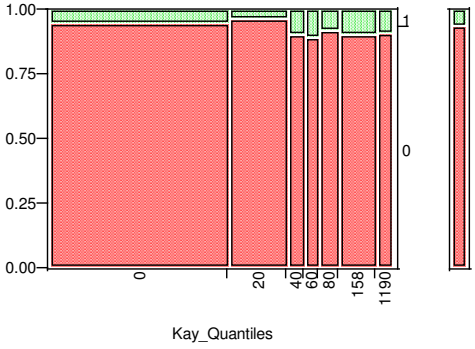
IE_Kay_Quantiles By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
20	292	6	298	2.0	0.4474		
40	84	7	91	7.7	0.0126	*	s
60	69	6	75	8.0	0.0145	*	s
80	90	7	97	7.2	0.0201	*	s
158	176	14	190	7.4	0.0022	*	
1190	81	6	87	6.9	0.0383	*	s
	1687	72	1759				



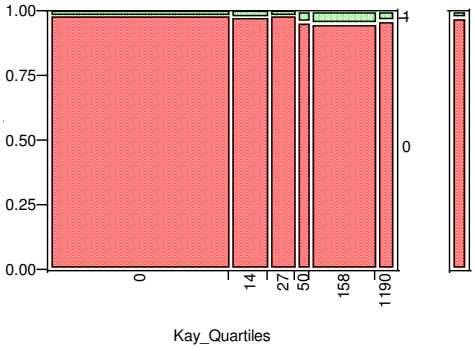
IE_Kay_Quantiles By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
20	286	11	297	3.7	0.2925		
40	82	9	91	9.9	0.0648		
60	67	8	75	10.7	0.0486	*	s
80	89	8	97	8.3	0.2123		
158	171	19	190	10.0	0.0116	*	
1190	79	8	87	9.2	0.1210		s
	1647	111	1758				



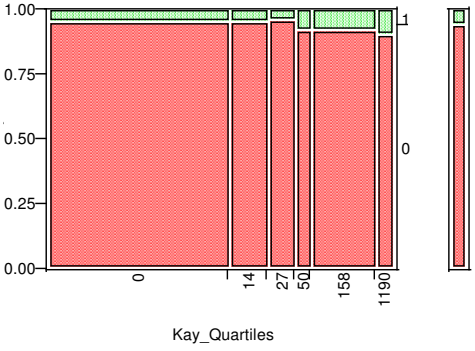
Contingency Table
IE_Kay_Quartiles By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
14	192	4	196	2.0	0.5147		s
27	136	2	138	1.4	0.9732		s
50	74	3	77	3.9	0.0958		s
158	315	16	331	4.8	0.0004	*	
1190	83	3	86	3.5	0.1413		s
	1707	41	1748				



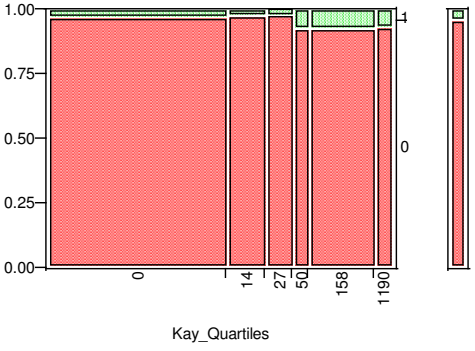
Contingency Table
IE_Kay_Quartiles By GE_UK_3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
14	184	9	193	4.7	0.9508		
27	132	6	138	4.3	0.8286		
50	70	6	76	7.9	0.2300		s
158	295	25	320	7.8	0.0412	*	
1190	75	8	83	9.6	0.0553		s
	1615	97	1712				



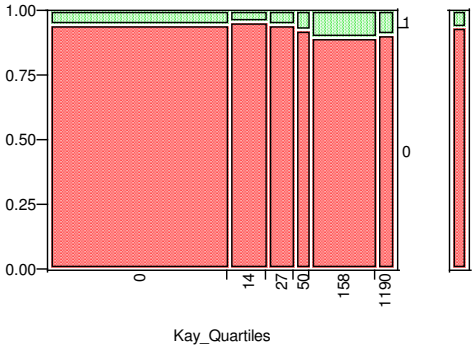
Contingency Table
IE_Kay_Quartiles By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
14	192	5	197	2.5	0.8250		
27	136	3	139	2.2	0.6543		s
50	72	6	78	7.7	0.0190	*	s
158	311	26	337	7.7	0.0001	*	
1190	81	6	87	6.9	0.0383	*	s
	1687	72	1759				



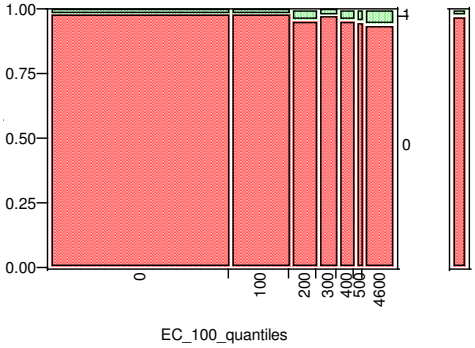
Contingency Table
IE_Kay_Quartiles By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
14	189	8	197	4.1	0.5015		
27	131	7	138	5.1	0.9452		
50	72	6	78	7.7	0.3522		s
158	303	34	337	10.1	0.0019	*	
1190	79	8	87	9.2	0.1210		s
	1647	111	1758				



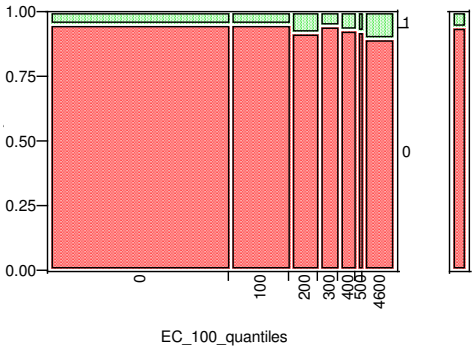
EC_100_quantiles By GE_UK

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
100	301	5	306	1.6	0.7807		s
200	133	6	139	4.3	0.0162	*	s
300	99	2	101	2.0	0.6529		s
400	87	4	91	4.4	0.0348	*	s
500	39	2	41	4.9	0.0799		s
4600	141	9	150	6.0	0.0002	*	s
	1707	41	1748				



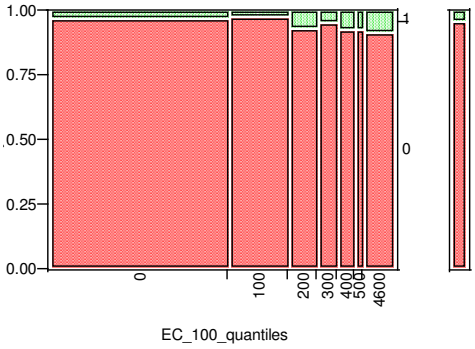
EC_100_quantiles By GE_UK_3-weeks

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	859	43	902	4.8			
100	289	14	303	4.6	0.9171		
200	128	11	139	7.9	0.1195		
300	92	5	97	5.2	0.8654		s
400	81	6	87	6.9	0.3821		s
500	37	3	40	7.5	0.4326		s
4600	129	15	144	10.4	0.0059	*	
	1615	97	1712				



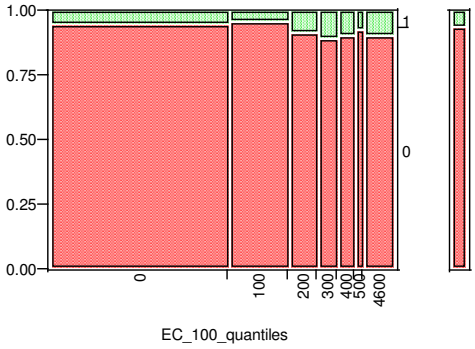
EC_100_quantiles By GE_UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
100	301	8	309	2.6	0.8281		
200	131	10	141	7.1	0.0091	*	s
300	99	5	104	4.8	0.2626		s
400	85	7	92	7.6	0.0137	*	s
500	38	3	41	7.3	0.0996		s
4600	138	13	151	8.6	0.0004	*	
	1687	72	1759				



EC_100_quantiles By GE_NL-2

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
100	295	13	308	4.2	0.4882		
200	129	12	141	8.5	0.1141		
300	93	11	104	10.6	0.0260	*	
400	83	9	92	9.8	0.0697		
500	38	3	41	7.3	0.5561		s
4600	136	15	151	9.9	0.0222	*	
	1647	111	1758				



Annex 29. Incidence rates of skin infections/cutireactions in quartile and quintile categories

In the tables and figures in this annex the incidence rates skin infections/cutireactions among non-bathers were compared to the incidence rates among bathers exposed in quartile and quintile categories of microbiological exposure concentrations. Pearson's Chi square tests were performed separately for each of the categories. Significant differences between bathers and non-bathers ($p<0.05$) were marked by an asterisk.

Legend

Disease definitions:

SKIN	Skin infections, cutireactions

Indicator parameter:

AE	Aeromonads (concentration per 100 ml)

Exposure definition:

Exposure Def. 1	"10 minutes bathing, ≥ 3 head immersions"

Exposure:

0	Non-bathers
xy	quartile or quintile cut points

Disease:

0	Participants without gastroenteritis according to specified definition
1	Participants with gastroenteritis according to specified definition

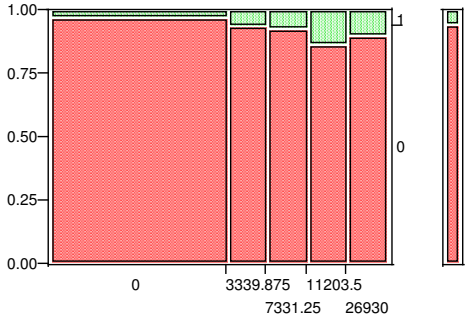
Miscellaneous:

n	Total number of participants in this analysis
p Chi^2	Probability of error in Pearson's Chi Square test
*	p $Chi^2 < 0.05$
s	"suspect"; if an expected cell value was less than 5

Contingency Table

Quartiles_Exposure Def. 1_AE By SKIN

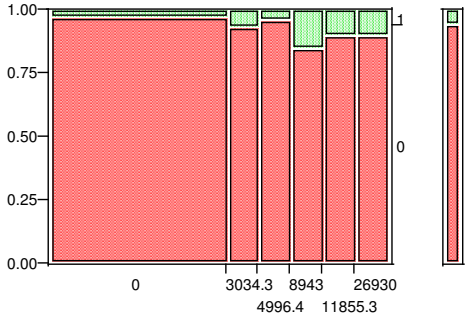
	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	930	27	957	2.8			
3339.875	202	14	216	6.5	0.0082	*	
7331.25	201	16	217	7.4	0.0013	*	
11203.5	187	29	216	13.4	0.0000	*	
26930	194	22	216	10.2	0.0000	*	
	1714	108	1822				



Contingency Table

Quintiles_Exposure Def. 1_AE By SKIN

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	930	27	957	2.8			
3034.3	161	12	173	6.9	0.0064	*	
4996.4	167	7	174	4.0	0.3932		
8943	146	26	172	15.1	0.0000	*	
11855.3	155	18	173	10.4	0.0000	*	
26930	155	18	173	10.4	0.0000	*	
	1714	108	1822				



Annex 30. Disease incidences by quartiles, quintiles and arithmetic concentration classes: Comparison with the predicted step models for dose-response relationships

The figures in this annex display the dose-response models (step-models) consisting of :

1. the incidence rate among non-bathers with 95% confidence intervals,
2. the threshold concentrations which are defined as the exposure concentrations with the most significant difference between the incidence rates among bathers exposed below and above that concentration (concentration with minimal Chi square p), and
3. the incidence rates among bathers exposed above threshold concentrations with 95% confidence intervals.

The incidence rates among bathers exposed in quartile and quintile categories of micro-biological exposure concentrations are displayed as horizontal lines indicating the quantile ranges. The geometric means of the quantile ranges are marked by a dot. Incidence rates which are significantly different from the non-bathers rate (Pearson's Chi square test, $p < 0.05$) are marked by an asterisk.

To enable a direct comparison between the results of previous studies performed in sea water and the results obtained in fresh water, gastroenteritis data were also classified using the quantile borders of the sea water studies published by Kay et al., 1994. In addition, data were classified by splitting them into 20 unit intervals of intestinal enterococci exposure and into 100 unit intervals of E. coli exposure. For enterococci split into 20 unit intervals the two upper classes are "80 to 158" and "158 to maximum". The class "80 to 158" was calculated for direct comparison with the sea water results again, as 158 was the maximum concentration found in the sea water studies.

From the comparison of the step models with the results of classification of the gastroenteritis data into quantile categories and into intervals with fixed arithmetic

ranges it was intuitively concluded that the following concentrations would be reasonable estimates for thresholds of effect (NOAEL's; no observed adverse effect levels) for the condition “bathing duration of 10 minutes including three or more head immersions”:

ca. 100 EC/100 ml
ca. 25 IE/100 ml
ca. 10 CP/100 ml (1 CP/10 ml)
ca. 10 SOMCP/100 ml

The relation of 100 EC/100 ml to 25 IE/100 ml also corresponds very well with the result of the orthogonal regression analysis for these two parameters in the original water samples, which revealed a regression function of log10(IE) = -0.270 + 0.836 * log10(EC). From this equation it can be calculated that 100 EC/100 ml would correspond to 25.23 IE/100 ml.

For skin infections or cutireactions concentrations of aeromonads above ca. 6000/100ml can explain part of the effect while a remaining part has to be attributed to other water-related factors which did not correspond with any of the microorganisms monitored in this study (two-step model).

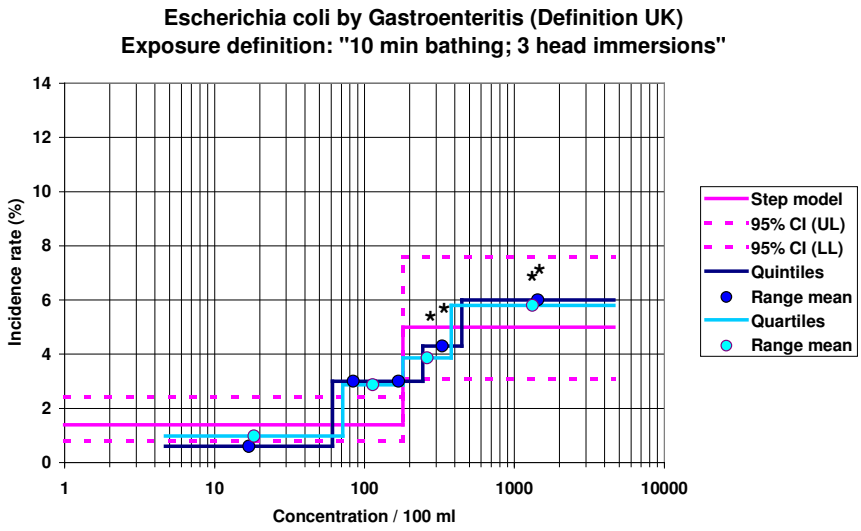


Fig. 1

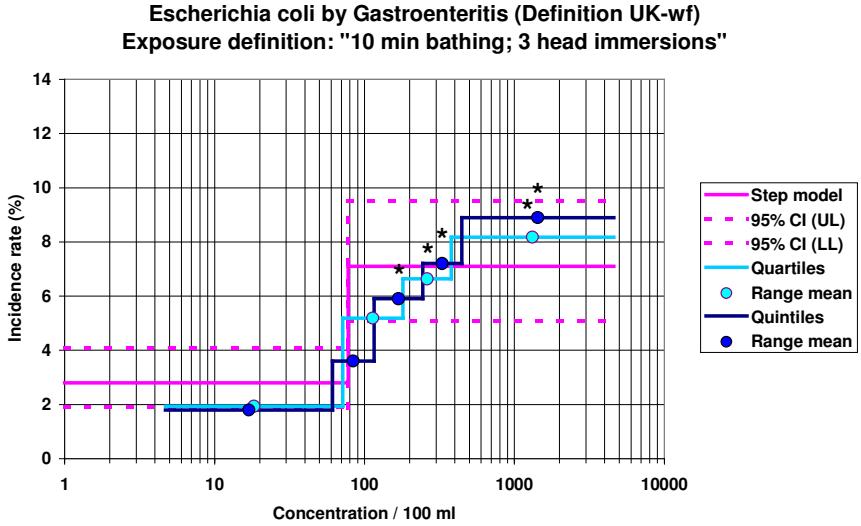


Fig. 2

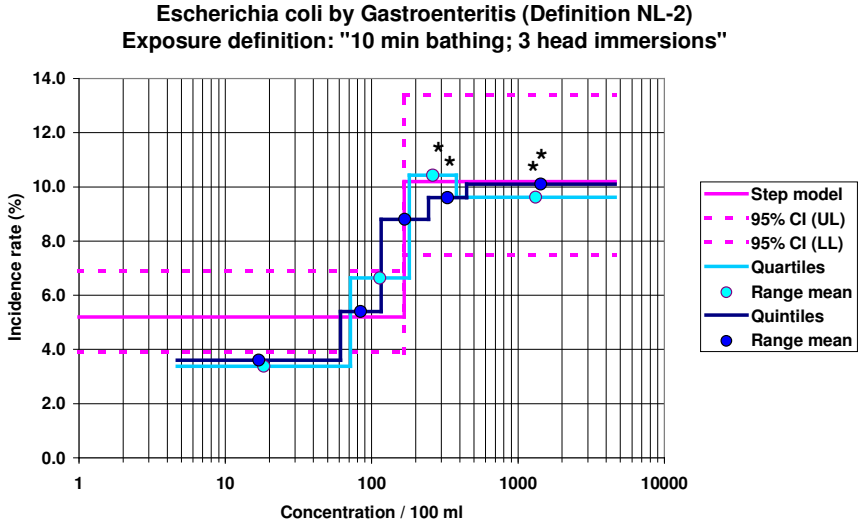


Fig. 3

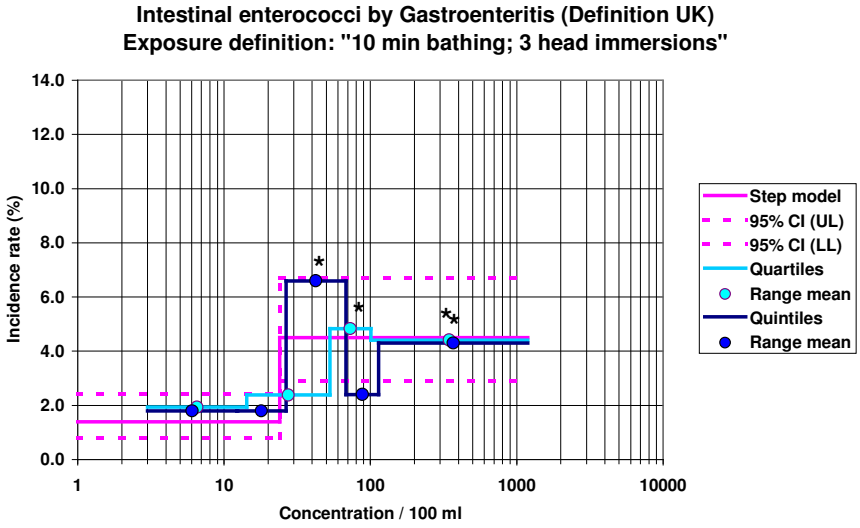


Fig. 4

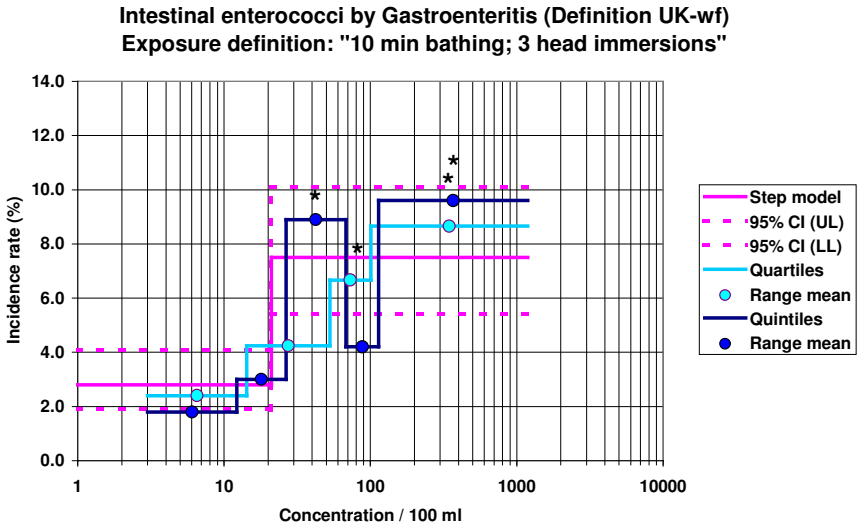


Fig. 5

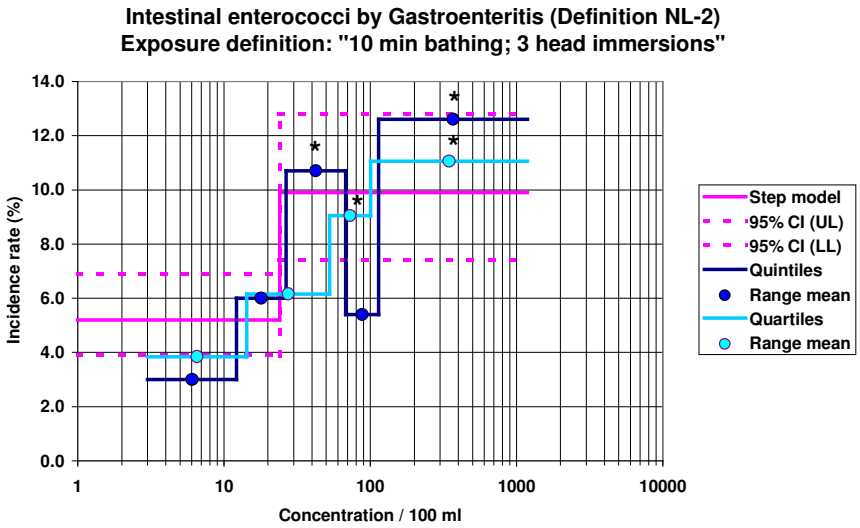


Fig. 6

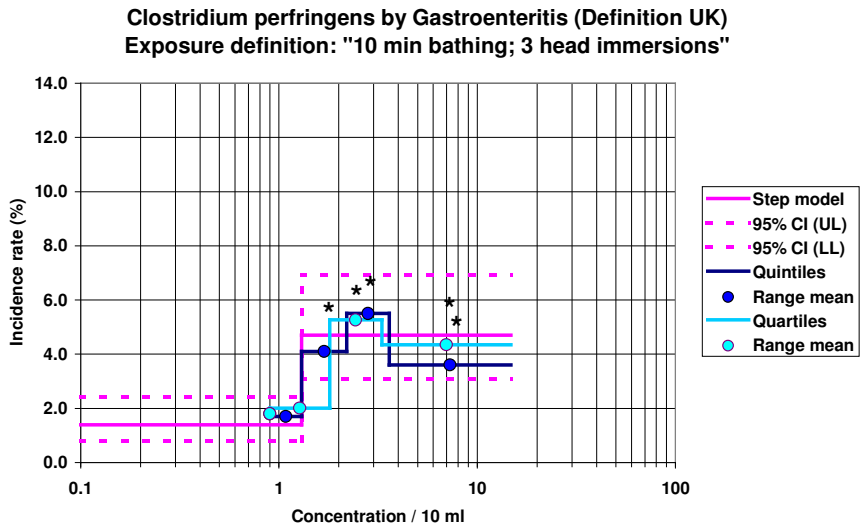


Fig. 7

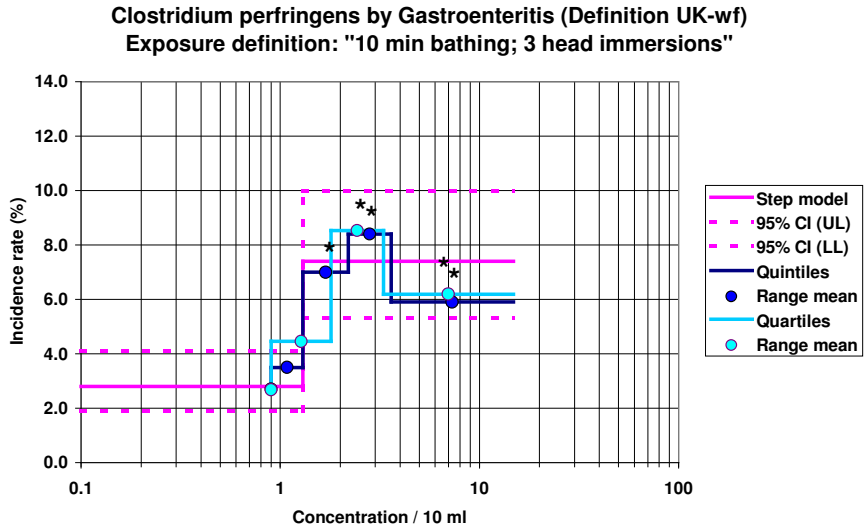


Fig. 8

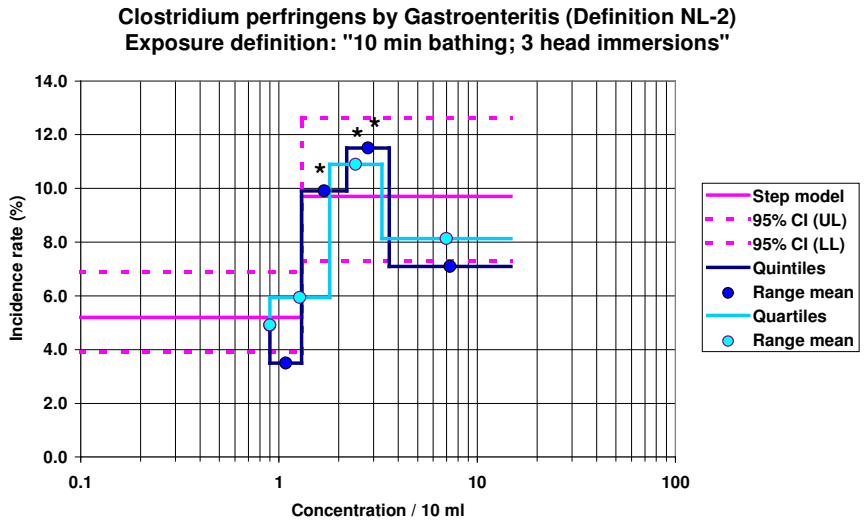


Fig. 9

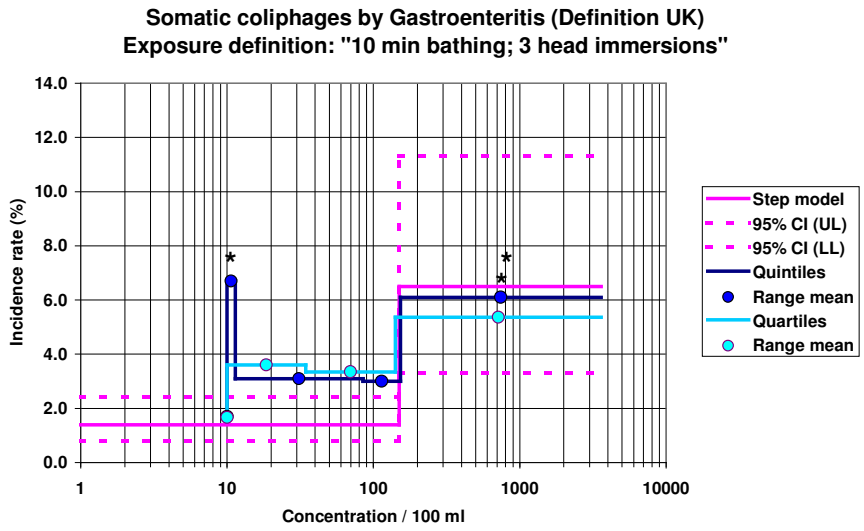


Fig. 10

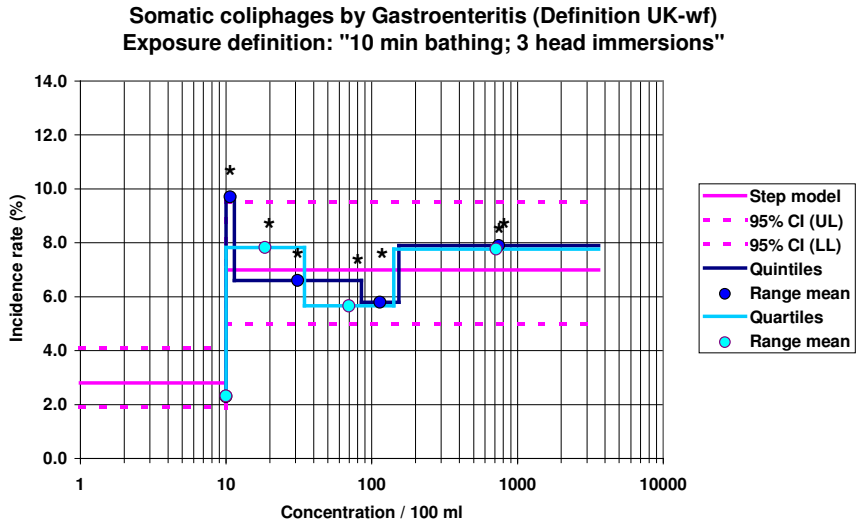


Fig. 11

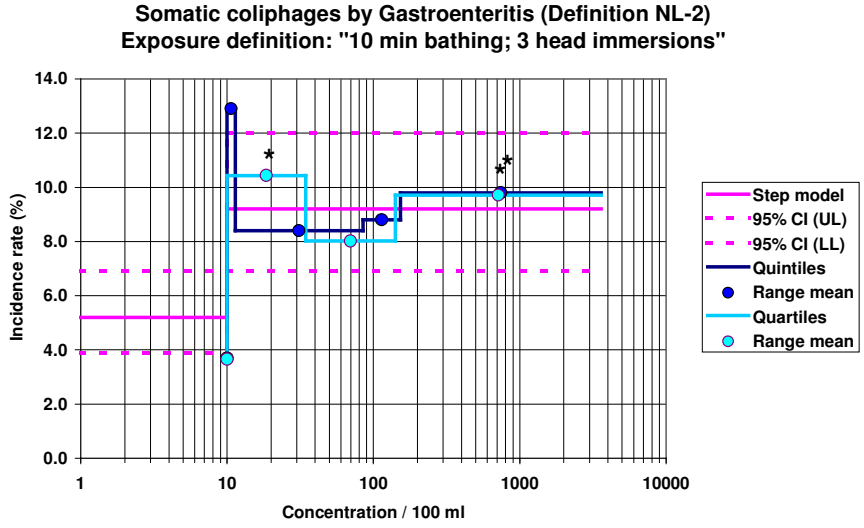


Fig. 12

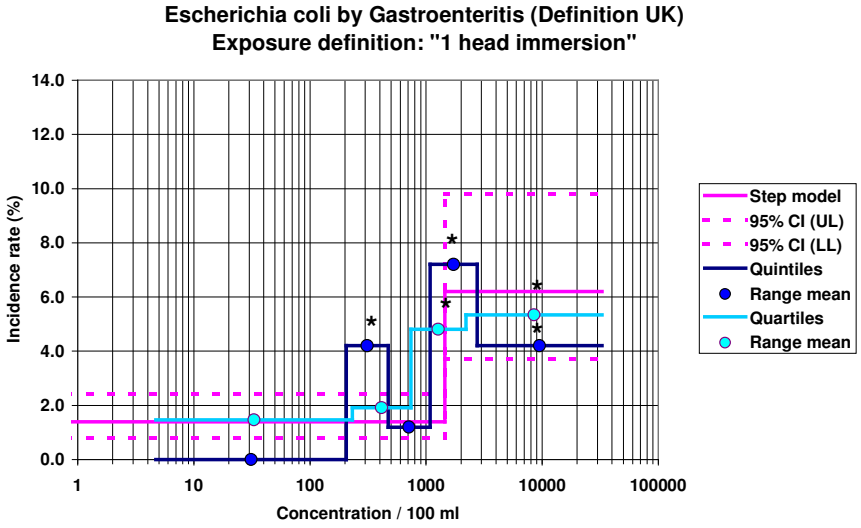


Fig. 13

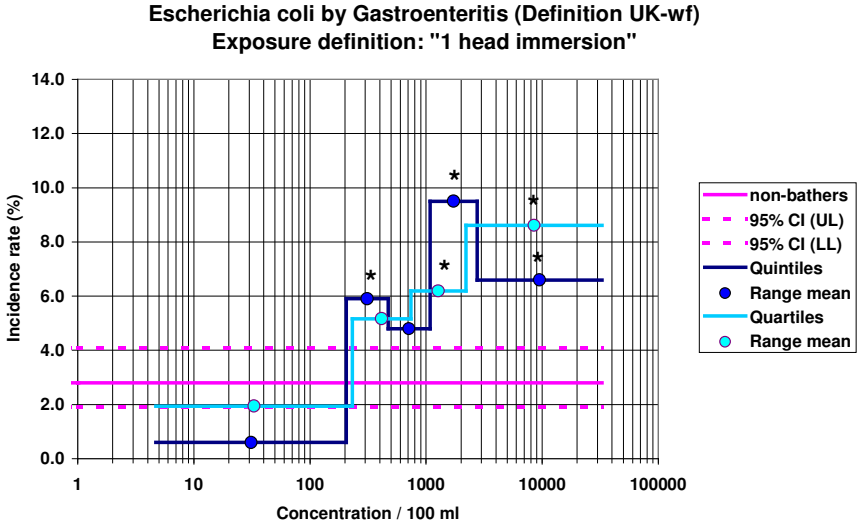


Fig. 14

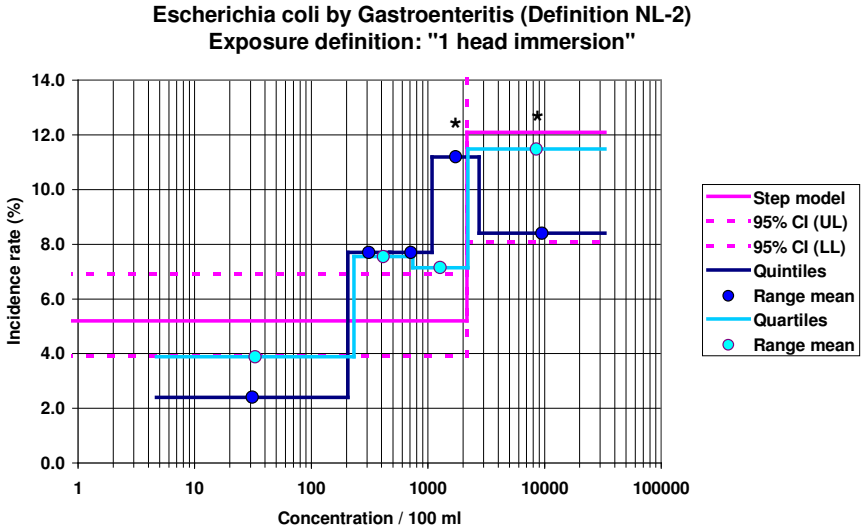


Fig. 15

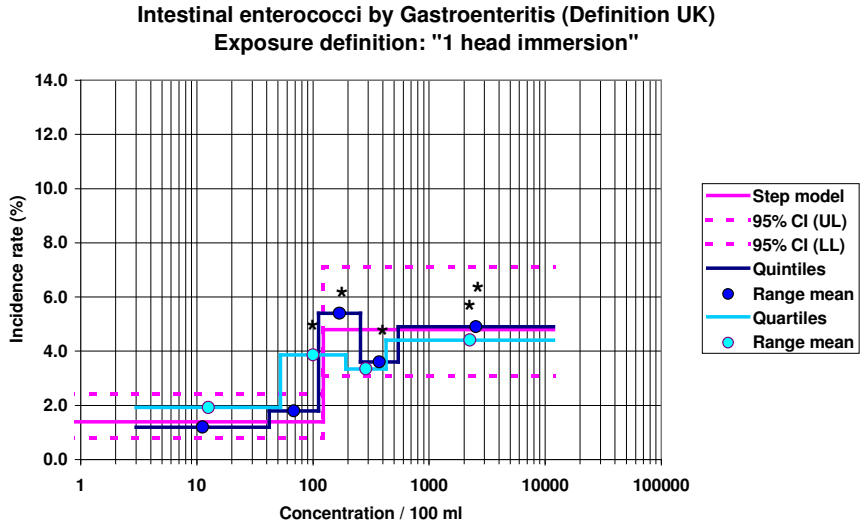


Fig. 16

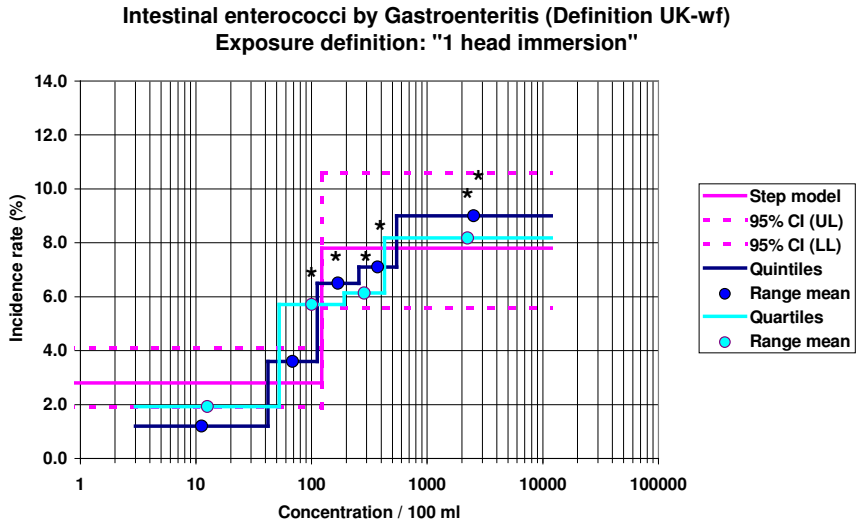


Fig. 17

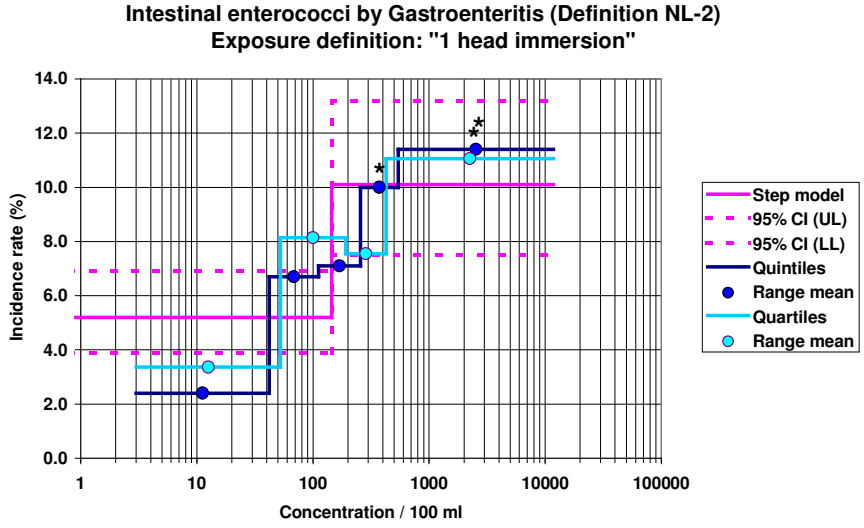


Fig. 18

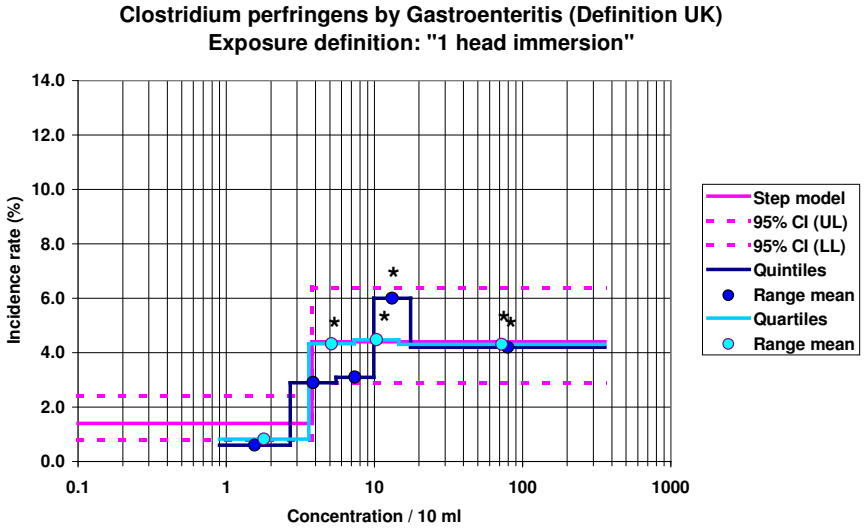
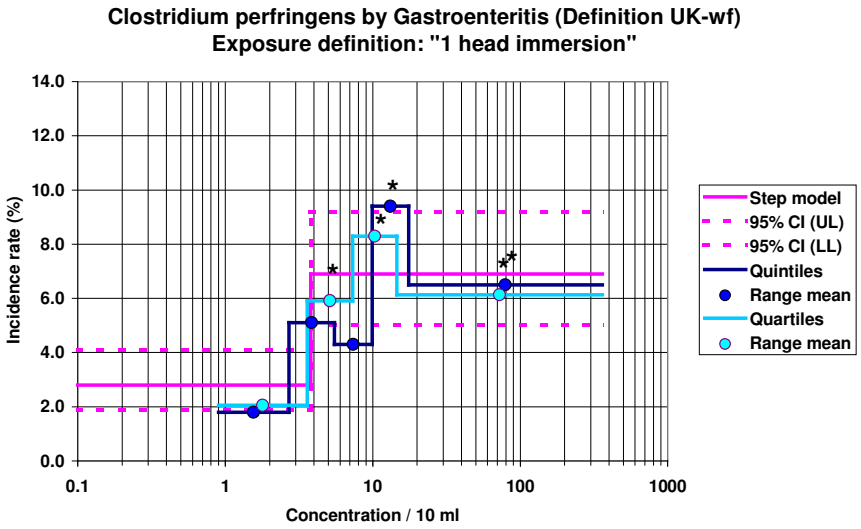


Fig. 19



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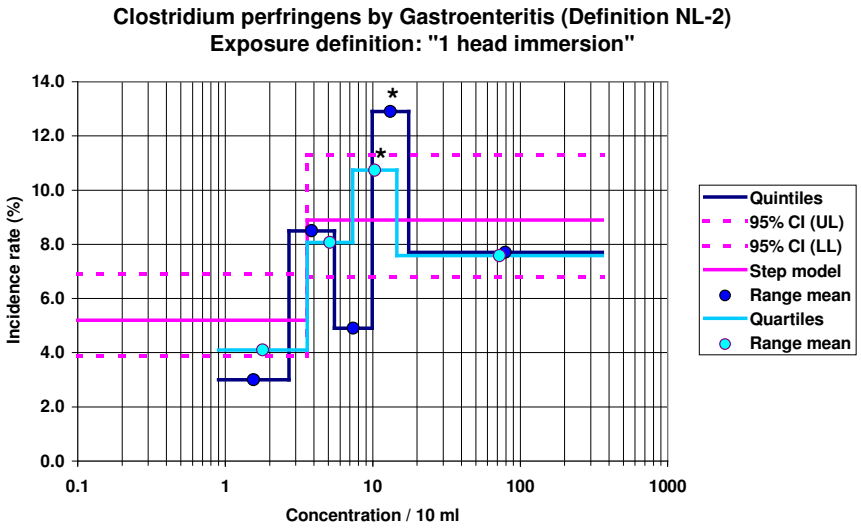


Fig. 21

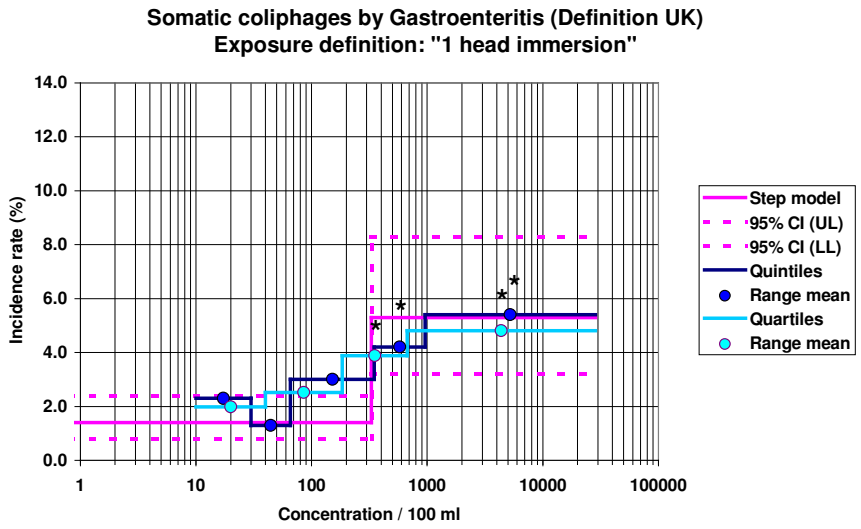


Fig. 22

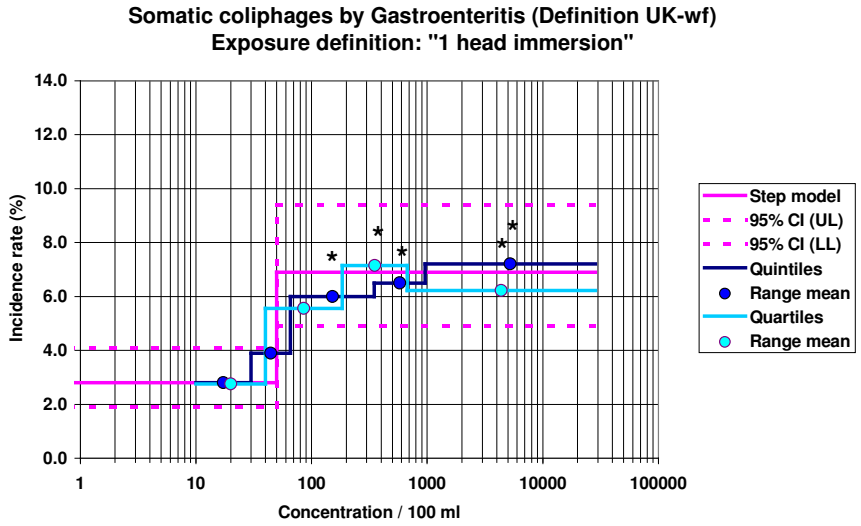


Fig. 23

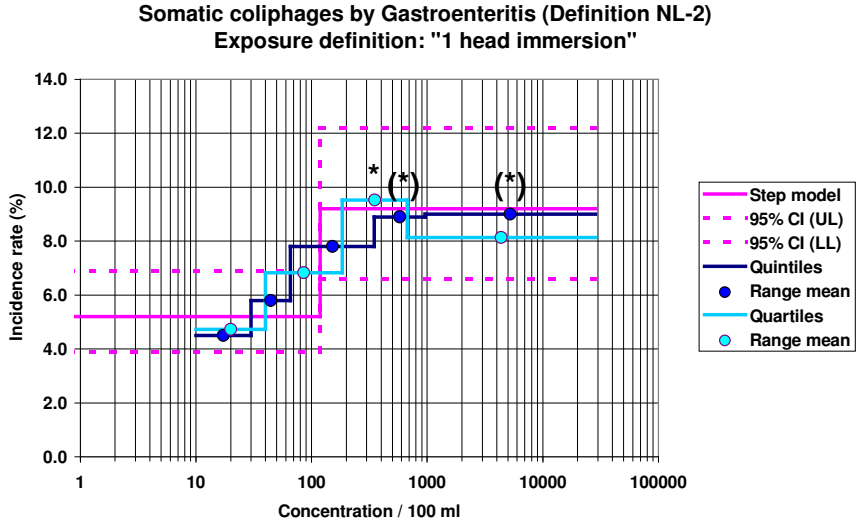


Fig. 24

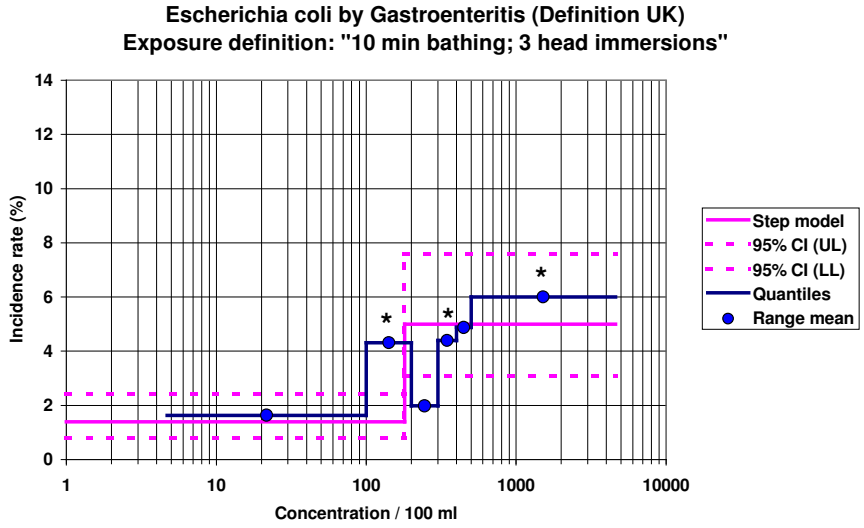


Fig. 25

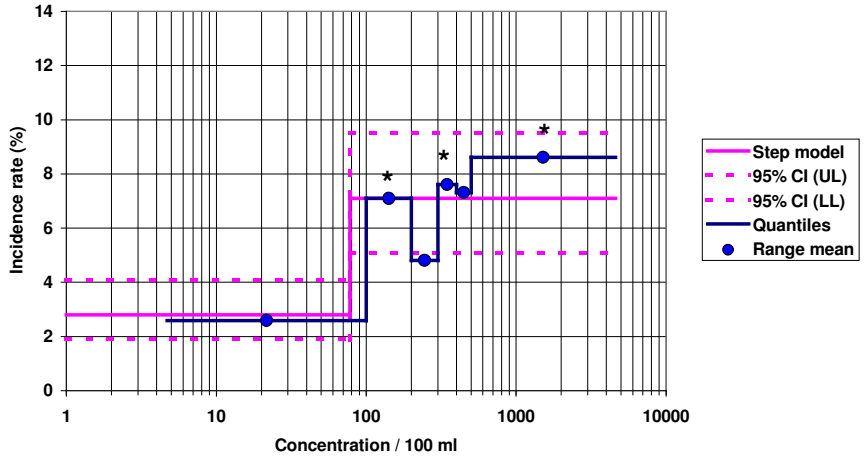


Fig. 26

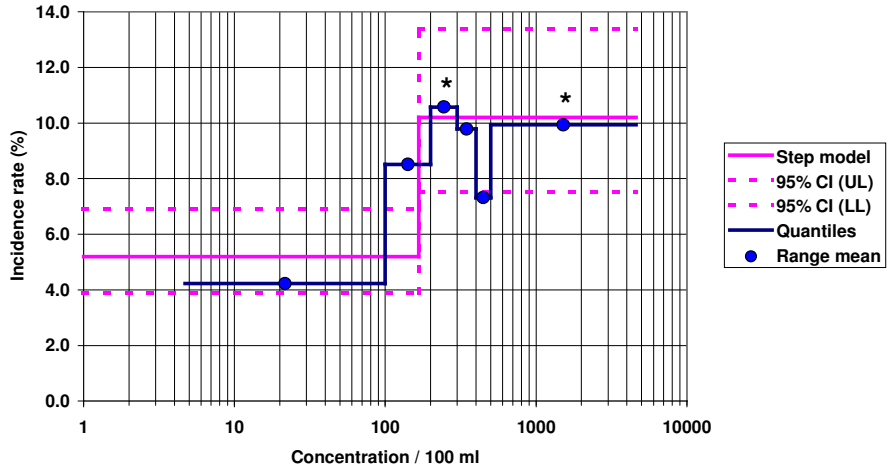


Fig. 27

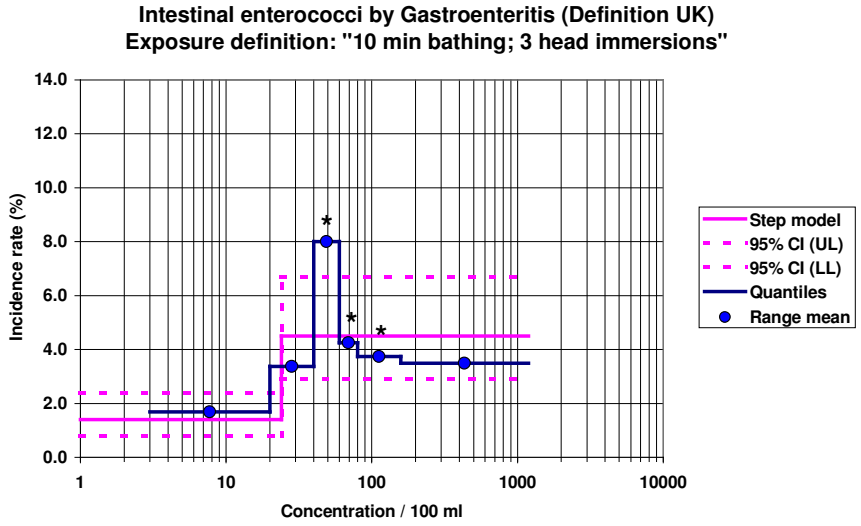


Fig. 28

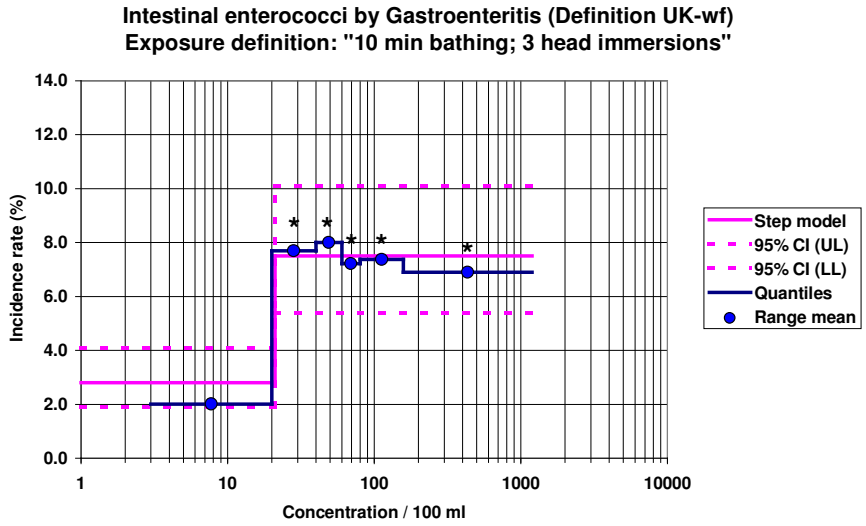


Fig. 29

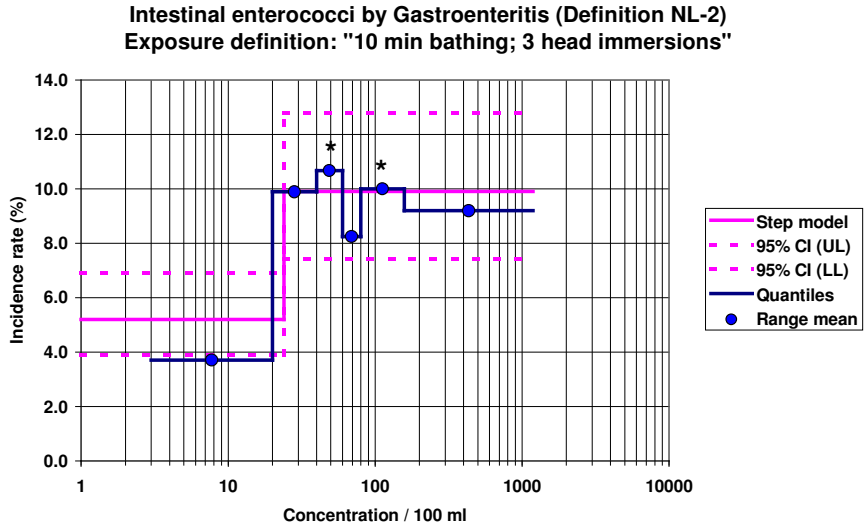


Fig. 30

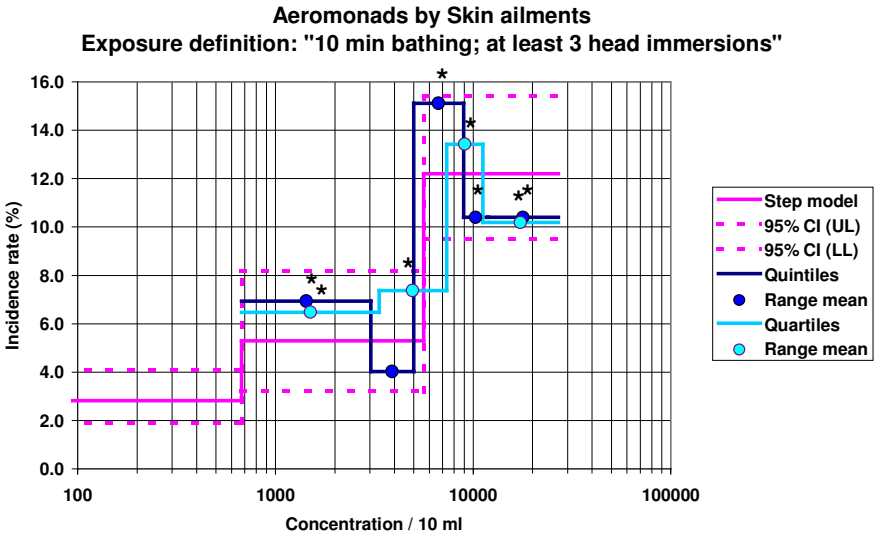

Aeromonads by Skin ailments
Exposure definition: "10 min bathing; at least 3 head immersions"
Incidence rate (%)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
100
1000
10000
100000
Concentration / 10 ml
Step model
95% CI (UL)
95% CI (LL)
Quintiles
Range mean
Quartiles
Range mean

Fig. 31

Annex 31. Effect of water ingestion: Incidence rates of gastroenteritis in non-bathers vs. bathers reporting to have swallowed water or not to have swallowed water below and above threshold concentrations

After 10 minutes of bathing participants were asked whether they had swallowed water. Incidence rates of gastroenteritis were calculated for bathers exposed below or above threshold concentrations of four faecal indicator parameters (EC, IE, CP, SOMCP) using three different definitions of gastroenteritis (GE_UK, GE_UK-wf, GE_NL-2) and two exposure definitions ("10 minutes bathing, ≥ 3 head immersions", "1 head immersion"). Bathers exposed below and above threshold concentrations were additionally sub-classified according to their response to the question "Did you swallow water?" Bathers who were not sure whether they had swallowed water were excluded from this analysis. Pearson's Chi Square tests were performed to compare the incidence rates of gastroenteritis in the four resulting categories of bathers to the incidence rate of gastroenteritis in the group of non-bathers. The tables and figures in this annex demonstrate the results of these analyses.

Legend

Disease definitions:

GE_UK	Gastroenteritis, UK definition
GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis, NL-2 definition

Faecal indicator parameters:

CP	*Clostridium perfringens*
EC	*Escherichia coli*
IE	Intestinal enterococci
SOMCP	Somatic coliphages

Exposure definitions:

Exposure Def. 1	"10 minutes bathing, ≥ 3 head immersions"
Exposure Def. 2	"1 head immersion"

Exposure:

0	Non-bathers
1	Bathers below threshold concentration who did not swallow water
2	Bathers below threshold concentration who swallowed water
3	Bathers above threshold concentration who did not swallow water
4	Bathers above threshold concentration who swallowed water

Disease:

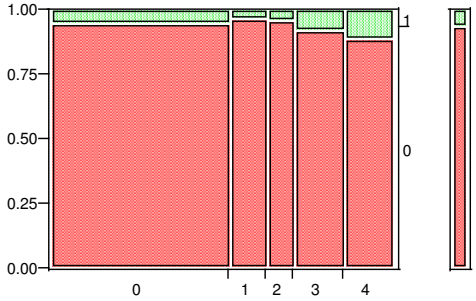
0	Participants without gastroenteritis according to specified definition
1	Participants with gastroenteritis according to specified definition

Miscellaneous:

n	Total number of participants in this analysis
p Chi^2	Probability of error in Pearson's Chi Square test
*	p $Chi^2 < 0.05$
s	"suspect"; if an expected cell value was less than 5

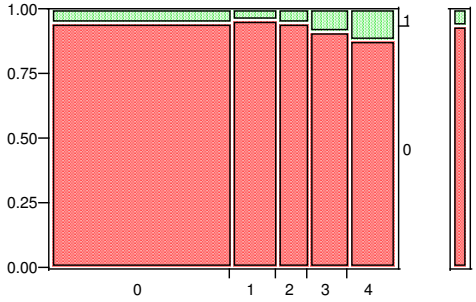
Exposure Def. 1_ CP By GE-NL-2

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
1	186	7	193	3.6	0.3555		
2	130	6	136	4.4	0.6925		
3	240	21	261	8.0	0.0847		
4	223	29	252	11.5	0.0003	*	
	1652	111	1763				



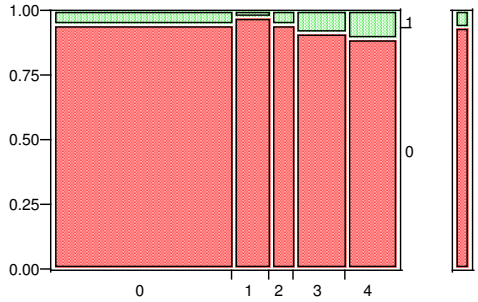
Exposure Def. 1_ EC By GE-NL-2

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
1	231	10	241	4.1	0.5002		
2	147	8	155	5.2	0.9791		
3	192	18	210	8.6	0.0609		
4	200	27	227	11.9	0.0003	*	
	1643	111	1754				



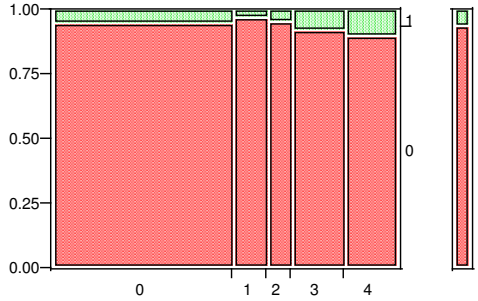
Exposure Def. 1_ IE By GE-NL-2

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
1	185	5	190	2.6	0.1287		
2	122	7	129	5.4	0.9184		
3	238	23	261	8.8	0.0307	*	
4	225	28	253	11.1	0.0008	*	
	1643	111	1754				



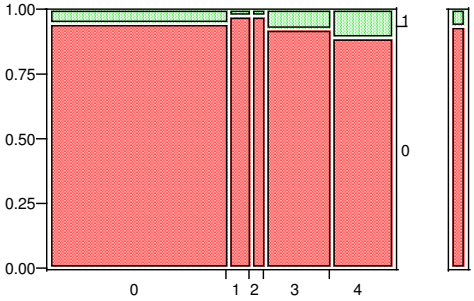
Exposure Def. 1_ SOMCP By GE-NL-2

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
1	175	5	180	2.8	0.1629		
2	116	6	122	4.9	0.8906		
3	245	22	267	8.2	0.0643		
4	234	27	261	10.3	0.0027	*	
	1643	108	1751				



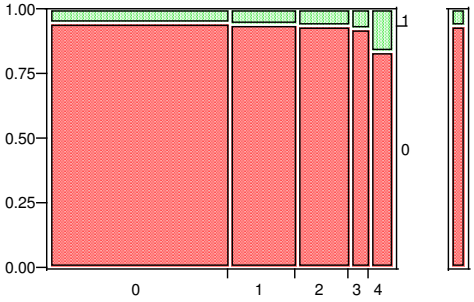
Exposure Def. 2_ CP By GE-NL-2

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
1	114	3	117	2.6	0.2120		
2	75	2	77	2.6	0.3124		s
3	312	25	337	7.4	0.1382		
4	278	33	311	10.6	0.0009	*	
	1652	111	1763				



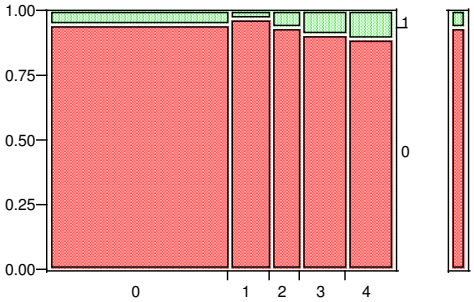
Exposure Def. 2_ EC By GE-NL-2

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
1	328	20	348	5.8	0.7056		
2	255	17	272	6.3	0.5074		
3	95	8	103	7.8	0.2794		
4	92	18	110	16.4	6E-06	*	
	1643	111	1754				



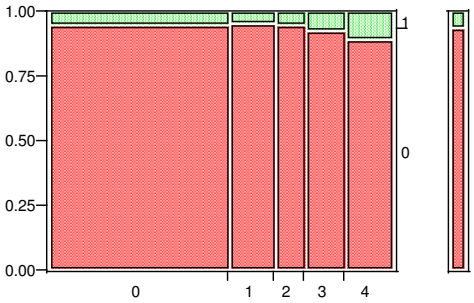
Exposure Def. 2_ IE By GE-NL-2

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
1	210	6	216	2.8	0.1301		
2	143	10	153	6.5	0.5022		
3	213	22	235	9.4	0.0173	*	
4	204	25	229	10.9	0.0015	*	
	1643	111	1754				



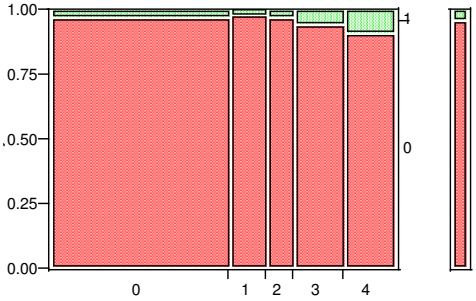
Exposure Def. 2_ SOMCP By GE-NL-2

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	873	48	921	5.2			
1	225	11	236	4.7	0.7315		
2	141	8	149	5.4	0.9362		
3	195	16	211	7.6	0.1786		
4	209	25	234	10.7	0.0021	*	
	1643	108	1751				



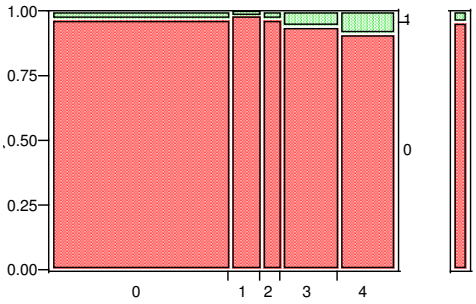
Exposure Def. 1_ CP By GE-UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
1	189	4	193	2.1	0.5581		
2	132	4	136	2.9	0.9383		s
3	246	15	261	5.8	0.0227	*	
4	230	23	253	9.1	1E-05	*	
	1692	72	1764				



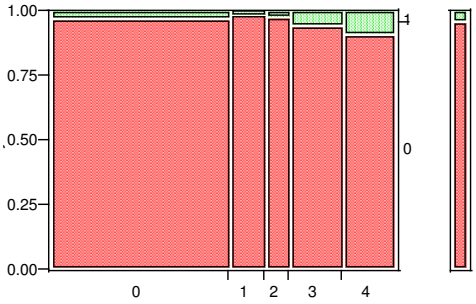
Exposure Def. 1_ EC By GE-UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
1	153	2	155	1.3	0.2675		s
2	101	3	104	2.9	0.9714		s
3	279	17	296	5.7	0.0179	*	
4	255	24	279	8.6	2E-05	*	
	1683	72	1755				



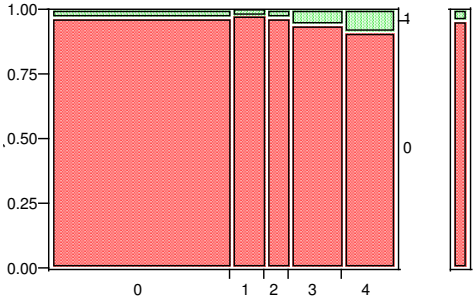
Exposure Def. 1_ IE By GE-UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
1	178	3	181	1.7	0.3705		s
2	123	3	126	2.4	0.7767		s
3	254	16	270	5.9	0.0151	*	
4	233	24	257	9.3	5E-06	*	
	1683	72	1755				



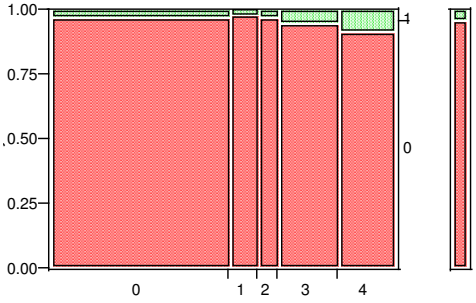
Exposure Def. 1_ SOMCP By GE-UK-wf

	Disease						
Exposure	0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
1	177	3	180	1.7	0.3756		s
2	119	4	123	3.3	0.7891		s
3	252	15	267	5.6	0.0276	*	
4	239	22	261	8.4	5E-05	*	
	1682	70	1752				



Exposure Def. 2_ CP By GE-UK-wf

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
1	143	3	146	2.1	0.5959		s
2	102	3	105	2.9	0.9841		s
3	292	16	308	5.2	0.0473	*	
4	260	24	284	8.5	3E-05	*	
	1692	72	1764				

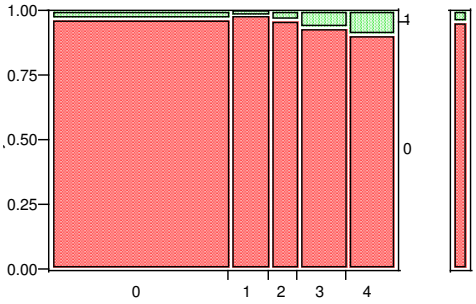


Exposure Def. 2_ EC By GE-UK-wf

Threshold not valid

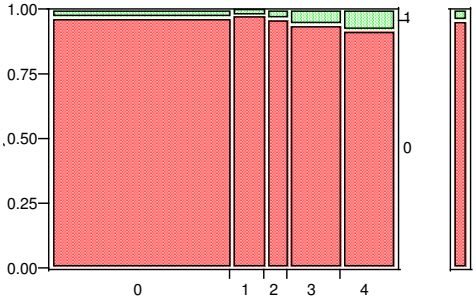
Exposure Def. 2_ IE By GE-UK-wf

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
1	201	3	204	1.5	0.2701		
2	142	5	147	3.4	0.6981		s
3	231	16	247	6.5	0.0062	*	
4	214	22	236	9.3	0.0000	*	
	1683	72	1755				



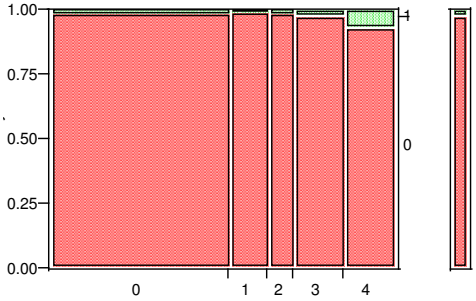
Exposure Def. 2_ SOMCP By GE-UK-wf

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	895	26	921	2.8			
1	176	3	179	1.7	0.3808		s
2	114	4	118	3.4	0.7292		s
3	253	15	268	5.6	0.0285	*	
4	244	22	266	8.3	0.0001	*	
	1682	70	1752				



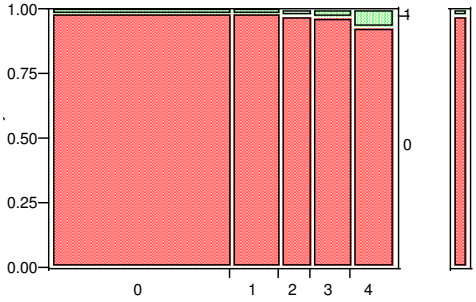
Exposure Def. 1_ CP By GE-UK

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
1	190	2	192	1.0	0.6849		s
2	133	2	135	1.5	0.9500		s
3	249	7	256	2.7	0.1481		s
4	233	17	250	6.8	0.0000	*	
	1712	41	1753				



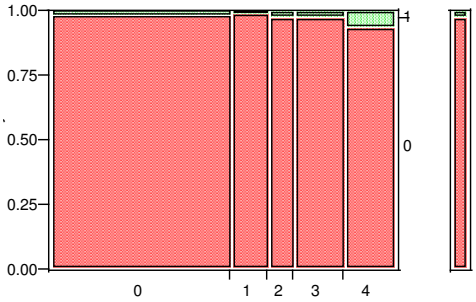
Exposure Def. 1_ EC By GE-UK

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
1	244	3	247	1.2	0.8118		s
2	158	4	162	2.5	0.3189		s
3	192	6	198	3.0	0.1102		s
4	202	15	217	6.9	0.0000	*	
	1703	41	1744				



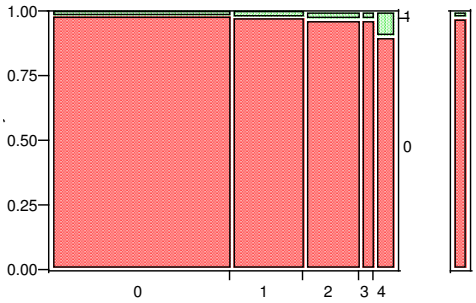
Exposure Def. 1_ IE By GE-UK

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
1	187	2	189	1.1	0.7005		s
2	126	3	129	2.3	0.4284		s
3	249	7	256	2.7	0.1481		s
4	234	16	250	6.4	0.0000	*	
	1703	41	1744				



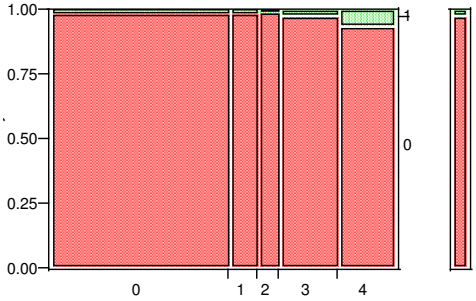
Exposure Def. 1_ SOMCP By GE-UK

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
1	362	7	369	1.9	0.5251		
2	276	9	285	3.2	0.0545		
3	70	2	72	2.8	0.3608		s
4	86	9	95	9.5	0.0000	*	s
	1701	40	1741				



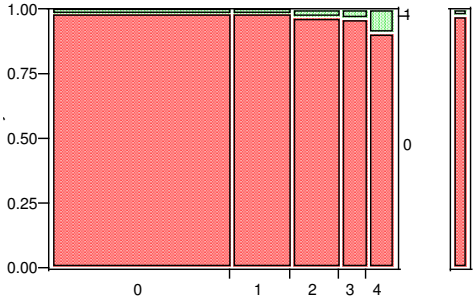
Exposure Def. 2_ CP By GE-UK

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
1	143	2	145	1.4	0.9744		s
2	103	1	104	1.0	0.7070		s
3	296	7	303	2.3	0.2855		s
4	263	18	281	6.4	4E-06	*	
	1712	41	1753				



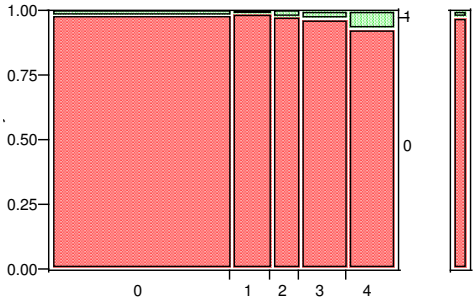
Exposure Def. 2_ EC By GE-UK

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
1	304	4	308	1.3	0.8818		s
2	238	7	245	2.9	0.1220		s
3	132	5	137	3.7	0.0591		s
4	122	12	134	9.0	8E-08	*	s
	1703	41	1744				



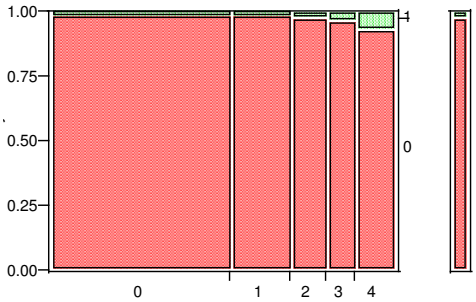
Exposure Def. 2_ IE By GE-UK

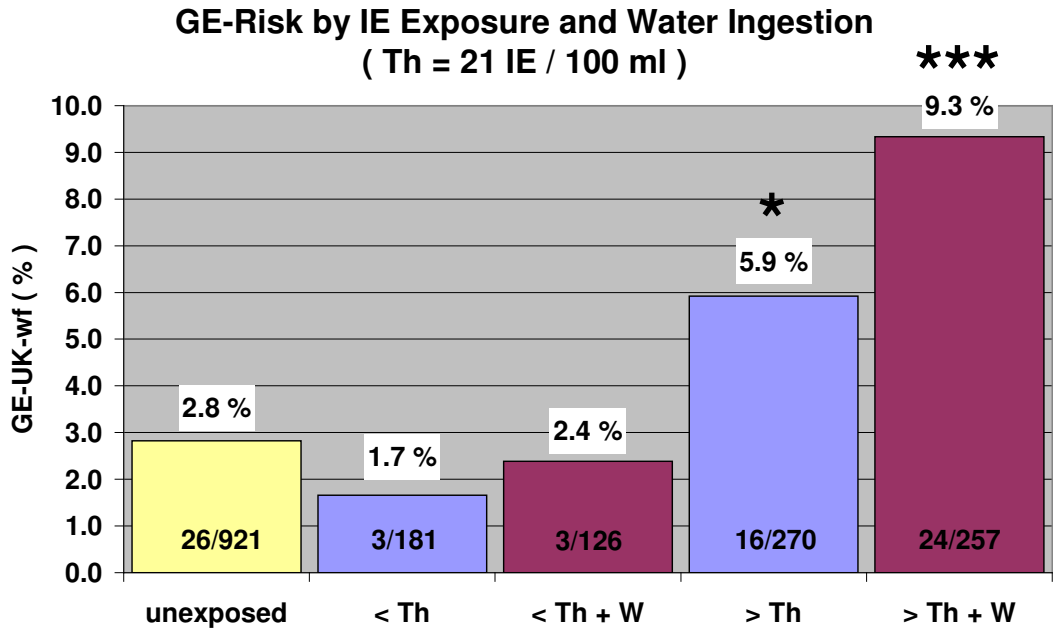
Exposure	Disease 0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
1	201	2	203	1.0	0.6308		s
2	143	3	146	2.1	0.5535		s
3	235	7	242	2.9	0.1153		s
4	217	16	233	6.9	2E-06	*	
	1703	41	1744				



Exposure Def. 2_ SOMCP By GE-UK

Exposure	Disease 0	1	n	%	p Chi²	*	s
0	907	13	920	1.4			
1	295	4	299	1.3	0.9232		s
2	181	5	186	2.7	0.21		s
3	137	5	142	3.5	0.0701		s
4	181	13	194	6.7	9E-06	*	s
	1701	40	1741				





Legend

Disease definition:

GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency

Faecal indicator parameter:

IE	Intestinal enterococci

Exposure definition:

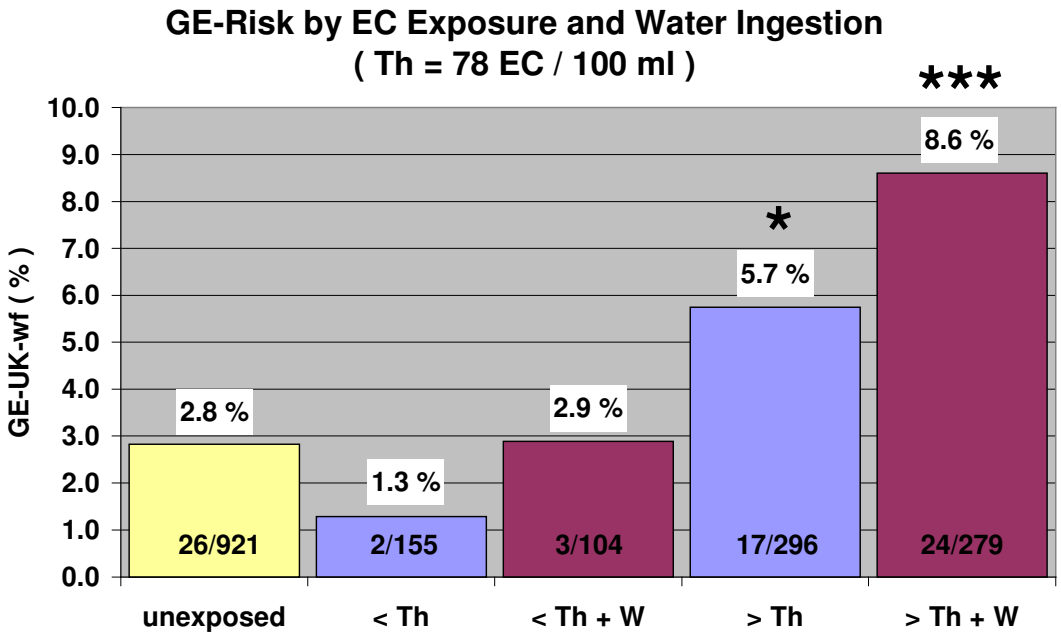
Exposure Def. 1	"10 minutes bathing, ≥ 3 head immersions"

Exposure:

unexposed	Non-bathers
< Th	Bathers below threshold concentration who did not swallow water
< Th + W	Bathers below threshold concentration who swallowed water
> Th	Bathers above threshold concentration who did not swallow water
> Th + W	Bathers above threshold concentration who swallowed water

Significance:

*	Pearson's Chi Square p exposed vs. unexposed < 0.05
***	Pearson's Chi Square p exposed vs. unexposed < 0.001



Legend

Disease definition:

GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency

Faecal indicator parameter:

EC	*Escherichia coli*

Exposure definition:

Exposure Def. 1	"10 minutes bathing, ≥ 3 head immersions"

Exposure:

unexposed	Non-bathers
< Th	Bathers below threshold concentration who did not swallow water
< Th + W	Bathers below threshold concentration who swallowed water
> Th	Bathers above threshold concentration who did not swallow water
> Th + W	Bathers above threshold concentration who swallowed water

Significance:

*	Pearson's Chi Square p exposed vs. unexposed < 0.05
***	Pearson's Chi Square p exposed vs. unexposed < 0.001

Annex 32. Effect of water ingestion: Risk of gastroenteritis attributable to swallowing water below and above threshold concentrations

Swallowing water above threshold concentrations for faecal indicator organisms (higher degree of faecal contamination) resulted in higher attributable risks than swallowing water below threshold concentrations (lower degree of faecal contamination). This effect was independent of the definition of exposure, the choice of the indicator organism and the definition of gastroenteritis.

Legend

Disease definition:

GE_UK	Gastroenteritis, UK definition
GE_UK-wf	Gastroenteritis, UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis, NL-2 definition

Faecal indicator parameter:

CP	*Clostridium perfringens*
EC	*Escherichia coli*
IE	Intestinal enterococci
SOMCP	Somatic coliphages

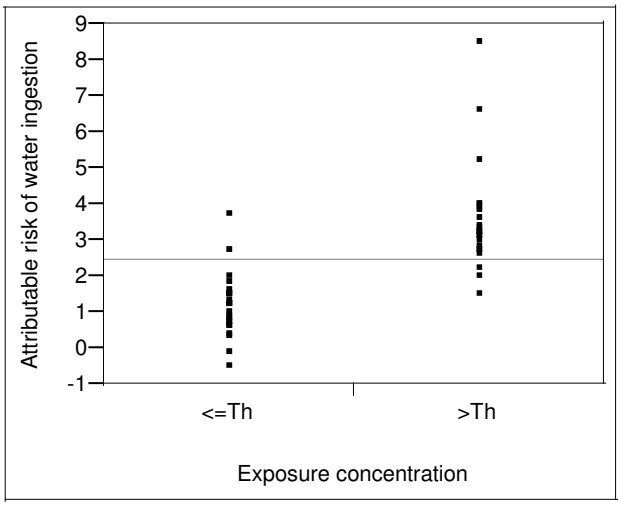
Exposure definition:

Exposure Def. 1	"10 minutes bathing, ≥ 3 head immersions"
Exposure Def. 2	"1 head immersion"

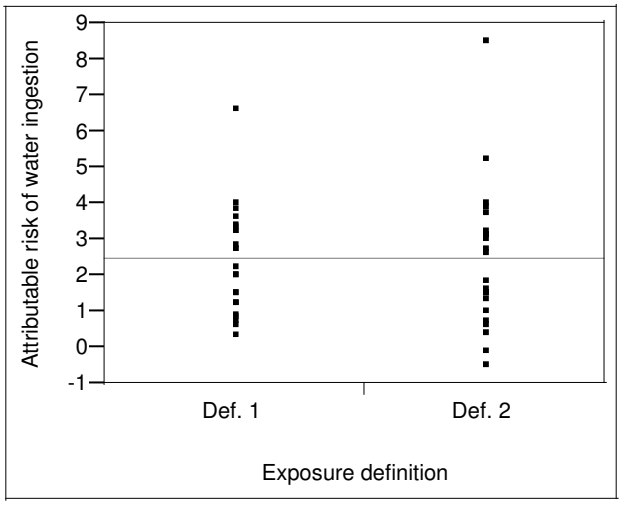
Miscellaneous:

aR	risk attributable to swallowing water

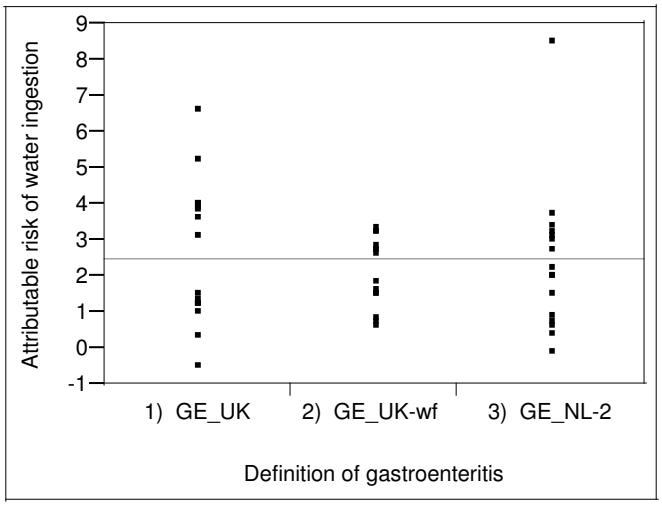
Exposure Definition	Indicator Parameter	Definition of Gastroenteritis	aR ≤ Threshold (%)	aR > Threshold (%)
Def. 1	CP	GE_UK	0.4	4.1
Def. 1	EC	GE_UK	1.3	3.9
Def. 1	EN	GE_UK	1.3	3.7
Def. 1	SOMCP	GE_UK	1.3	6.7
Def. 2	CP	GE_UK	-0.4	4.1
Def. 2	EC	GE_UK	1.6	5.3
Def. 2	EN	GE_UK	1.1	4.0
Def. 2	SOMCP	GE_UK	1.4	3.2
Def. 1	CP	GE_UK-wf	0.9	3.3
Def. 1	EC	GE_UK-wf	1.6	2.9
Def. 1	EN	GE_UK-wf	0.7	3.4
Def. 1	SOMCP	GE_UK-wf	1.6	2.8
Def. 2	CP	GE_UK-wf	0.8	3.3
Def. 2	EC	GE_UK-wf	not defined	not defined
Def. 2	EN	GE_UK-wf	1.9	2.8
Def. 2	SOMCP	GE_UK-wf	1.7	2.7
Def. 1	CP	GE_NL-2	0.8	3.5
Def. 1	EC	GE_NL-2	1.0	3.3
Def. 1	EN	GE_NL-2	2.8	2.3
Def. 1	SOMCP	GE_NL-2	2.1	2.1
Def. 2	CP	GE_NL-2	0.0	3.2
Def. 2	EC	GE_NL-2	0.5	8.6
Def. 2	EN	GE_NL-2	3.8	1.6
Def. 2	SOMCP	GE_NL-2	0.7	3.1
Mean			**1.3**	**3.6**



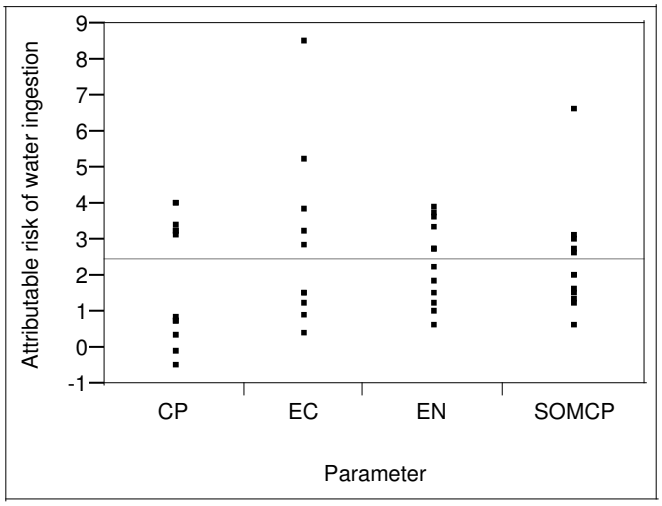
ANOVA: $p < 0.0001$



ANOVA: p = 0.86



ANOVA: p = 0.72



ANOVA: p = 0.62

Annex 33. Excess risk of gastroenteritis by 95th percentiles of *Escherichia coli* and intestinal enterococci concentrations

Estimates of the disease burden from bathing in recreational waters with lognormal distributed concentrations of microbiological indicator organisms have been made by WHO (1998, 2003) and by the European Commission in order to derive health standards. The dose-response relationships of intestinal enterococci with gastroenteritis (UK-definition) and acute febrile respiratory infections which were found in randomised controlled trials performed at coastal bathing sites in the UK (Kay et al, 1994) were used to calculate bathers' excess rates of disease at the 95th percentiles of lognormal distributions of faecal indicator densities (Commission, 2002). The figures in this annex display the estimated excess rates of gastroenteritis on the y-axis by 95th percentile concentrations of lognormal distributed concentrations of Escherichia coli and intestinal enterococci on the x-axis. The standard deviation of the distributions was uniformly set to 0.75. The dose response relationships used in these calculations were the step models for the gastroenteritis definitions GE_UK; GE_UK-wf and GE_NL-2. The maximum risk levels for these three definitions were harmonised for the two indicator organisms by calculating the arithmetic mean of the estimated maximum risk levels of both indicators for each of the three definitions. The threshold concentrations were harmonised as well: 25 IE/100 ml and 100 EC/100ml were used as threshold concentrations for all three definitions of GE. A step model was also adapted to the incidence rates of gastroenteritis in quartile categories of faecal streptococci published by Kay et al. 1994. The cut-point between the second and third quartile was modelled as threshold concentration. The model parameters for the three definitions of gastroenteritis are listed in the table below:

GE definition	Indicator	Original models				Harmonised models			
		Th (MPN)	BLR (%)	MRL (%)	aR (%)	Th (MPN)	BLR (%)	MRL (%)	aR (%)
GE_UK	EC /100 ml	180	1.4	5.0	3.6	100	1.4	4.75	3.35
GE_UK-wf	EC /100 ml	78	2.8	7.1	4.3	100	2.8	7.3	4.5
GE_NL-2	EC /100 ml	167	5.2	10.2	5.0	100	5.2	10.05	4.85
GE_UK	IE /100 ml	24	1.4	4.5	3.1	25	1.4	4.75	3.35
GE_UK-wf	IE /100 ml	21	2.8	7.5	4.7	25	2.8	7.3	4.5
GE_NL-2	IE /100 ml	24	5.2	9.9	4.7	25	5.2	10.05	4.85
GE_UK (*)	FS/100 ml	26	9.7	19.2	9.5	25	9.7	19.2	9.5

Legend

Th	Threshold of effect (NOAEL)
BLR	Base line risk (incidence rate among non-bathers)
MRL	Maximum risk level; incidence rate above threshold concentration
aR	attributable risk (excess risk); = MRL-BLR
MPN	Most probable number
GE_UK	Gastroenteritis; UK definition according to Kay et al., 1994
GE_UK-wf	Gastroenteritis; UK definition without consideration of stool frequency
GE_NL-2	Gastroenteritis; NL-2 definition according to van Asperen et al.; 1998
EC	Escherichia coli
IE; FS	Intestinal enterococci; faecal streptococci
(*)	results from sea water exposure

The curves in the figures displayed in this annex were calculated in MS Excel™ using the following formulae:

GE_UK (%) =(1-NORMDIST(LOG10(25);LOG10(IE);0.75;TRUE))*100*0.0335
=(1-NORMDIST(LOG10(100);LOG10(EC);0.75;TRUE))*100*0.0335

GE_UK-wf (%) =(1-NORMDIST(LOG10(25); LOG10(IE);0.75;TRUE))*100*0.045
=(1-NORMDIST(LOG10(100); LOG10(EC);0.75;TRUE))*100*0.045

GE_NL-2 (%) =(1-NORMDIST(LOG10(25); LOG10(IE);0.75;TRUE))*100*0.0485
=(1-NORMDIST(LOG10(100); LOG10(EC);0.75;TRUE))*100*0.0485

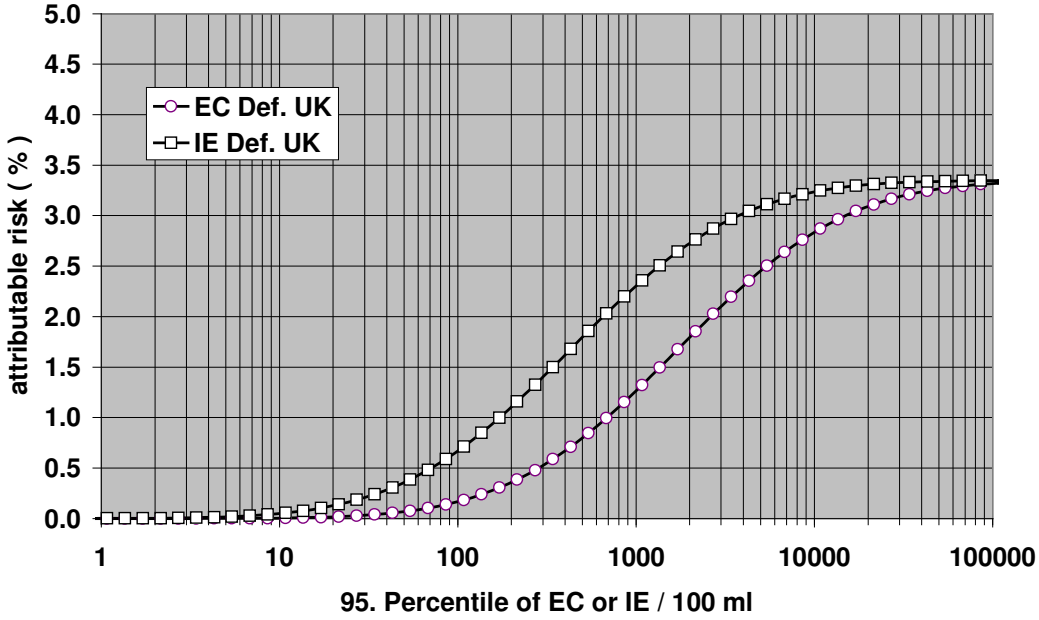
GE_UK (*) (%) =(1-NORMDIST(LOG10(25); LOG10(IE);0.75;TRUE))*100*0.095

The incidence rates of gastroenteritis among bathers and non-bathers exposed in quartile categories of faecal streptococci at sea water bathing sites in the UK were copied from Table 4, p. 907 in Kay et al., 1994. The step model parameters calculated from these figures (Th=26; BLR=9.7%; MRL=19.2%) are within the 95% confidence intervals of all quartiles.

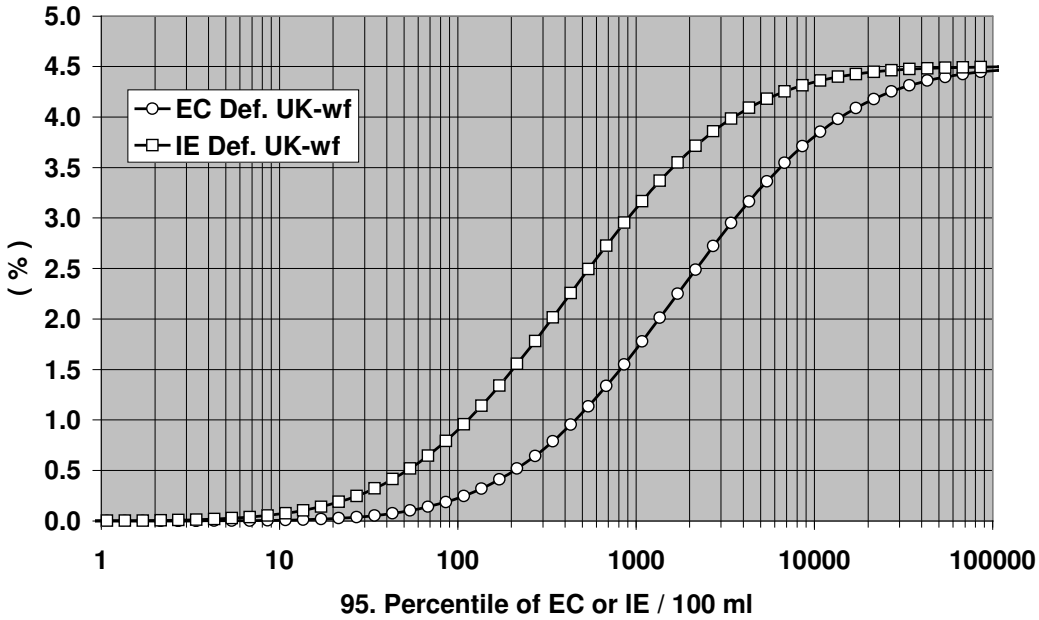
Faecal steptococci exposure class (/100 ml)	No.	Rate of gastroenteritis (%)	(95% CI)
Unexposed	605	9.7	(7-12)
Exposed 0-13	159	10.7	(6-15)
Exposed 14-26	109	11.0	(5-17)
Exposed 27-49	121	14.0	(8-20)
Exposed 50-158	118	24.6	(17-32)

**Attributable risk (excess risk) of gastroenteritis
by 95th percentiles of EC or IE exposure
at German fresh water bathing sites
(SD of log10 transformed concentration data = 0.75)**

Gastroenteritis definition: UK

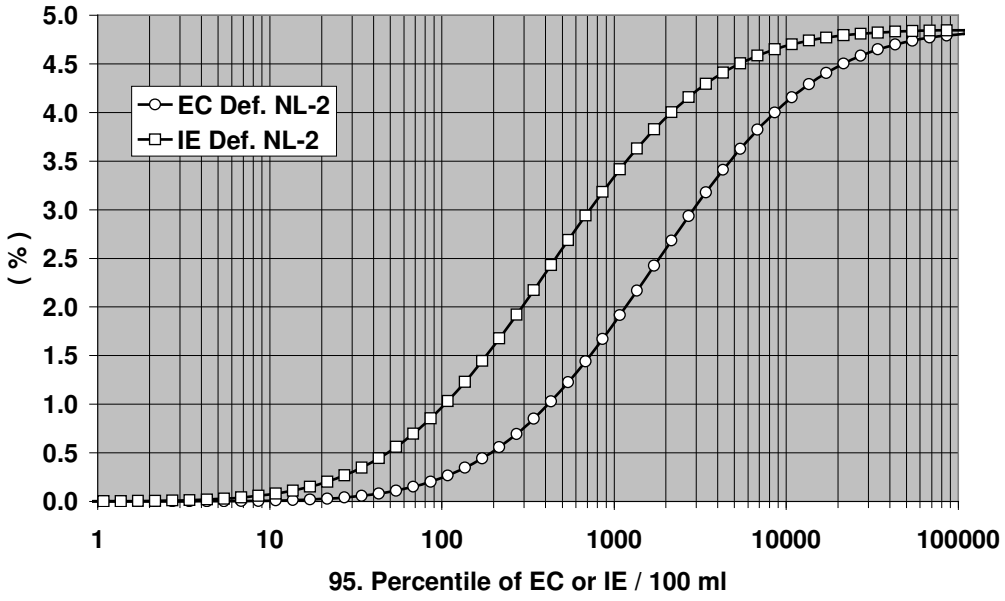


Gastroenteritis definition: UK-wf

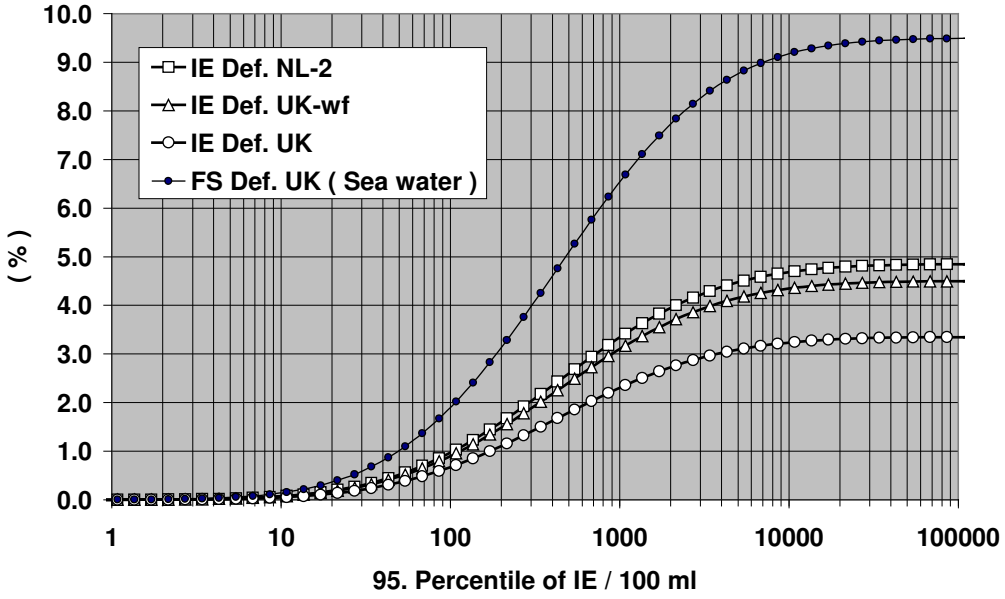


**Attributable risk (excess risk) of gastroenteritis
by 95th percentiles of EC or IE exposure
at German fresh water bathing sites
(SD of log10 transformed concentration data = 0.75)**

Gastroenteritis definition: NL-2



All definitions: Comparison with results from sea water exposure



Annex 34. Bases for a risk-related interpretation of monitoring results: Area-integrated sampling according to ISO 5667-4

Assuming that microbiological concentrations in a water body of a certain bathing site are lognormal distributed on the day of sampling, it is essential to increase the probability that the sample result is close to the true median concentration if the health risk from bathing at this site on the sampling day is to be estimated. The better the estimate for the mean water quality is, the more reliable the estimate for the potential health risk will be.

The best way to estimate the mean water quality would be to take multiple samples, analyse them separately and calculate the geometric mean. However, this would also multiply the costs of the analysis. ISO 5667-4 “Guidance on sampling from lakes, natural and man-made” defines in chapter 3.4.2. an area-integrated sample as a "Water sample obtained after combining a series of samples taken at various locations of a body of water from a particular depth". This technique would only increase the time for sampling but would not increase the costs of laboratory analysis.

The graphs in this annex illustrate the gain in precision when two-fold, three-fold or four-fold area-integrated samples are collected from a water matrix with lognormal distributed concentrations and a standard deviation of 0.4. The dot plots were generated by combining 1000 random numbers from lognormal distributions with a median of 250 indicator organisms per 100 ml or a median concentration with 10,000 indicator organisms per 100 ml on the x-axis with another set of 1000 random numbers from the same distribution on the y-axis. The present mandatory value for *E. coli* in the EU (2000 EC/100 ml) is marked as a red line. The graphs demonstrate that the area-integrated sampling technique would not only decrease the risk of false high estimates when the water quality is good; it would also decrease the risk of misinterpreting the true situation when the water quality is bad.

Legend:

left column: geometric mean = 250/100 ml
right column: geometric mean = 10,000/100 ml

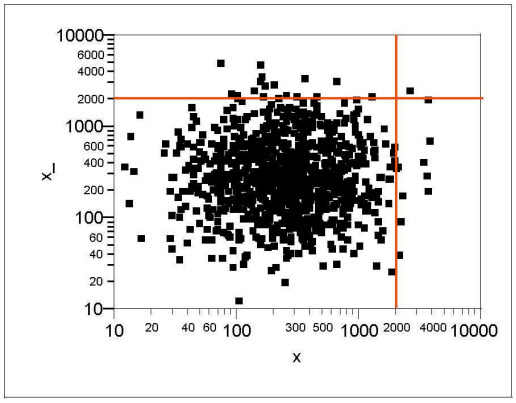
x random number from lognormal distribution (x-value)
x_ random number from lognormal distribution (y-value)

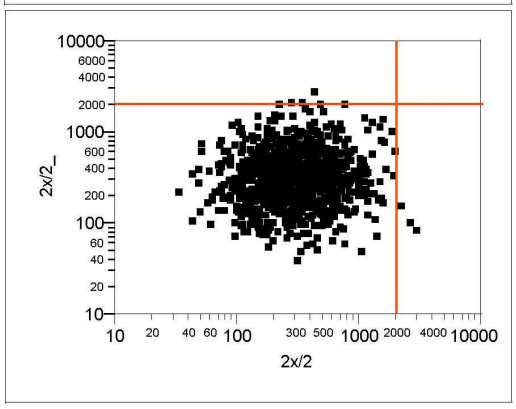
2x/2 sum of 2 random numbers from lognormal distribution divided by 2 (x-value)
2x/2_ sum of 2 random numbers from lognormal distribution divided by 2 (y-value)

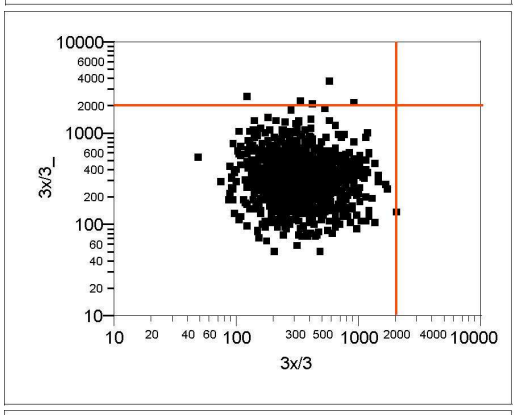
3x/3 sum of 3 random numbers from lognormal distribution divided by 3 (x-value)
3x/3_ sum of 3 random numbers from lognormal distribution divided by 3 (y-value)

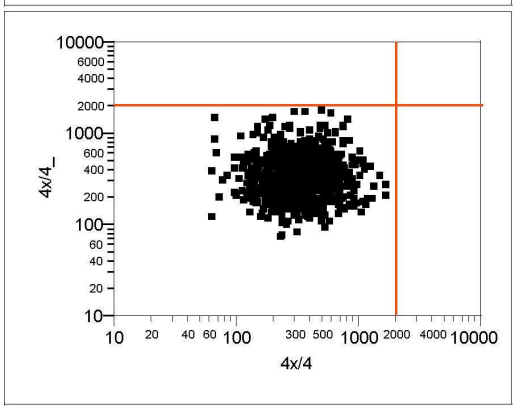
4x/4 sum of 4 random numbers from lognormal distribution divided by 4 (x-value)
4x/4_ sum of 4 random numbers from lognormal distribution divided by 4 (y-value)

geometric mean = 250

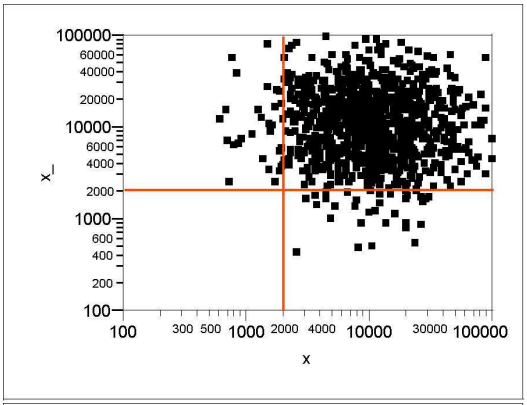


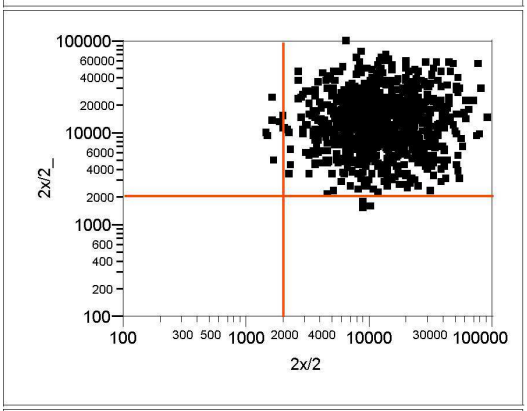


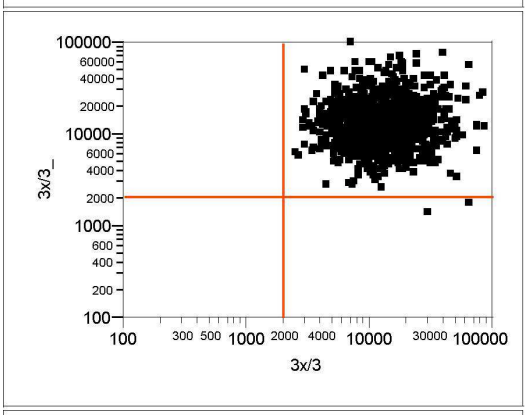


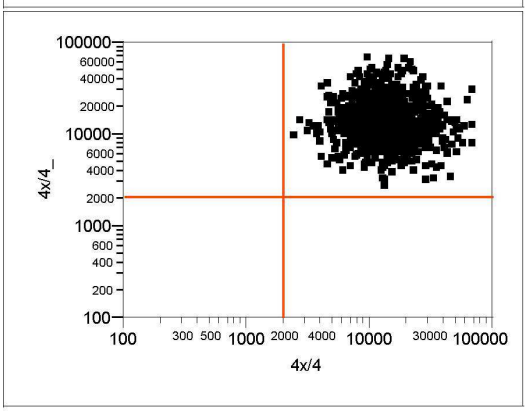


geometric mean = 10,000









Annex 35. Bases for a risk-related interpretation of monitoring results: Calculation of the percentage of concentrations exceeding the threshold of effect (NOAEL)

If a monitoring result is considered to be the most probable estimate for the mean water quality on the sampling day, the percentage of concentrations probably exceeding the threshold of effect (%>Th) can be calculated according to the following equation:

$$\%>Th = (1 - p, normal (x = log10(Th);\mu = log10(monitoring\ result);\ \sigma = 0.4))*100$$

When the bathers are randomly distributed over the beach it can be assumed that this is also the percentage of bathers who are exposed above the threshold concentration, i. e. the percentage of bathers who are at risk of acquiring e.g. gastroenteritis from 10 minutes of bathing and at least three head immersions. The percentage of bathers who become ill (disease burden) can then be estimated by combining this figure with a dose response model. When a step model is used the percentage of bathers who will probably suffer from bathing-associated disease is simply the product of the percentage of bathers exposed above threshold (%>Th) and the expected percentage of susceptible people in the cohort (maximum incidence rate - base line risk).

The figures in this annex illustrate how estimates for different mean water qualities (monitoring results) correspond with %>Th for lognormal distributed densities of *Escherichia coli* with a standard deviation of 0.4. If other standard deviations are found for certain bathing sites, these may of course replace the 0.4.

The formula for calculating %>Th values can easily be programmed in any standard spread sheet calculation program. In MS Excel, for example, the formula for *E. coli* concentrations (EC) and a threshold of 100 EC/100 ml would be:

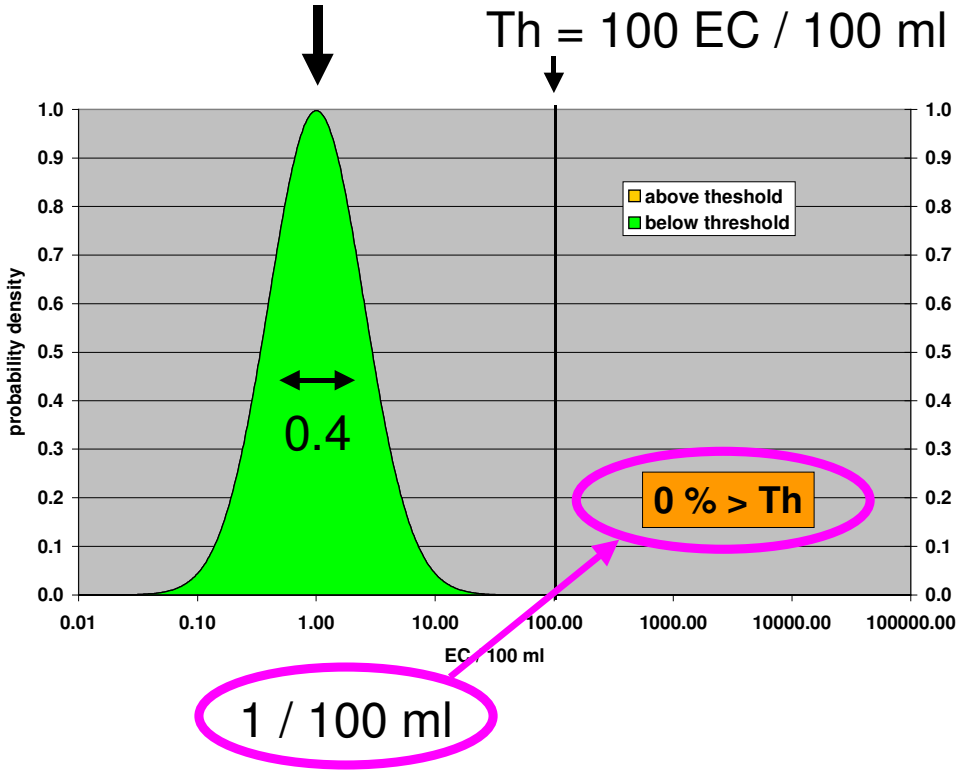
$$\%>Th = (1-NORMDIST(LOG10(100);LOG10(EC);0.4;TRUE))*100$$

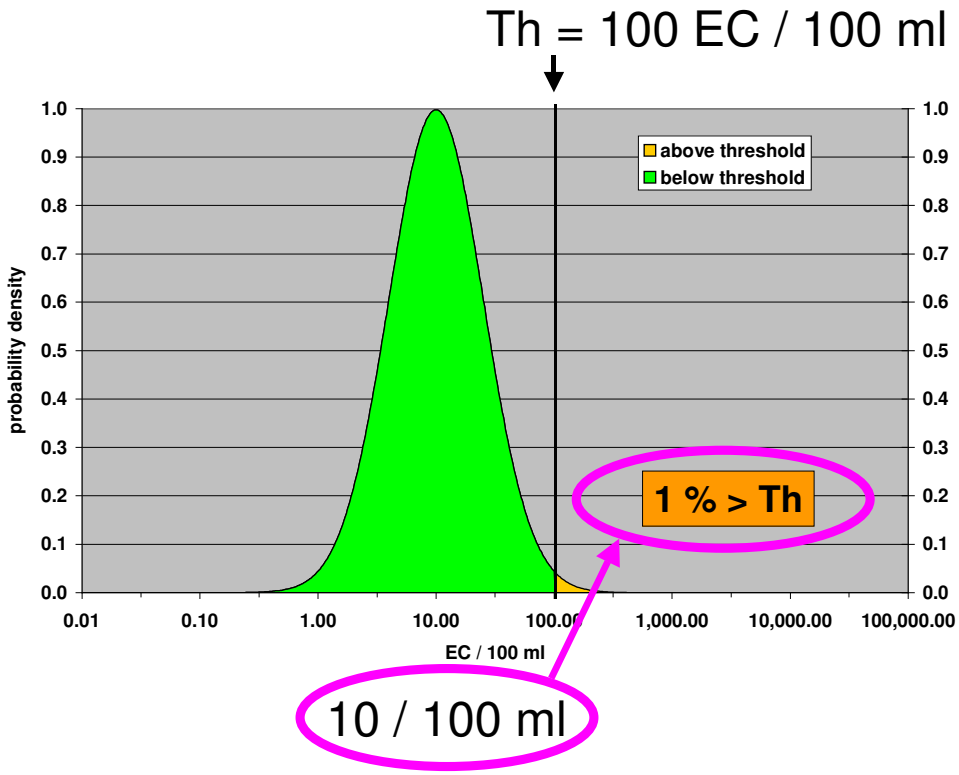
However, the %>Th values for all possible concentrations of *E. coli* and intestinal enterococci can also be listed in tables. Examples with standard deviations of 0.4 and threshold levels for gastroenteritis of 100 EC/100 ml and 25 IE/100 ml are also provided in this annex.

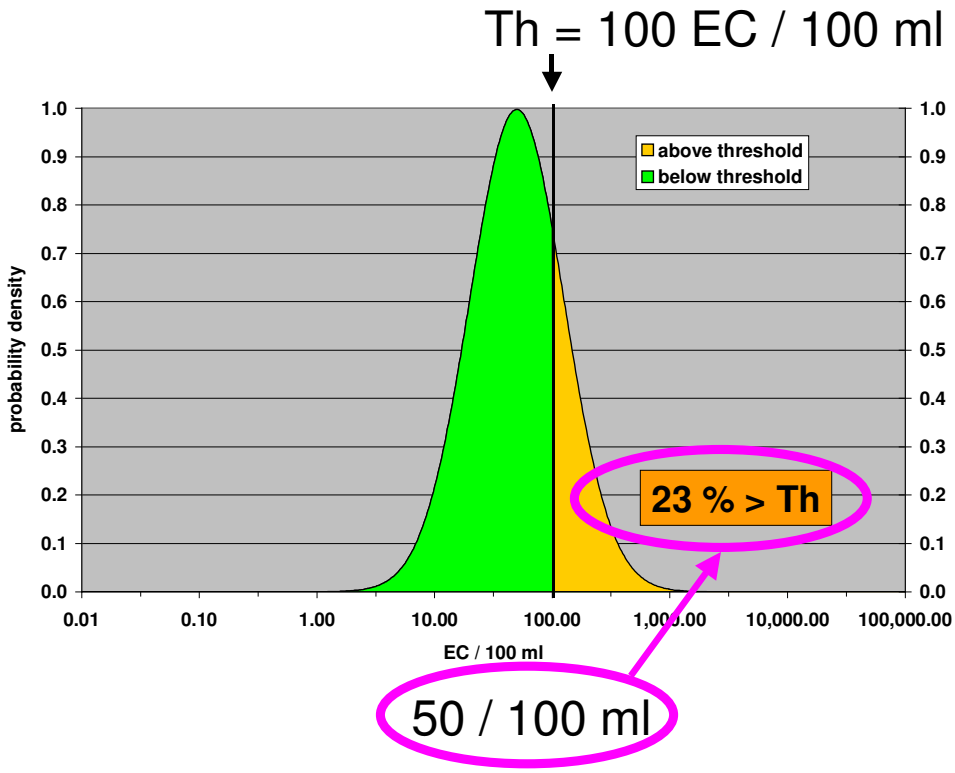
In this way concentrations of indicator organisms, which in and of themselves cannot be interpreted, can be transformed into meaningful figures.

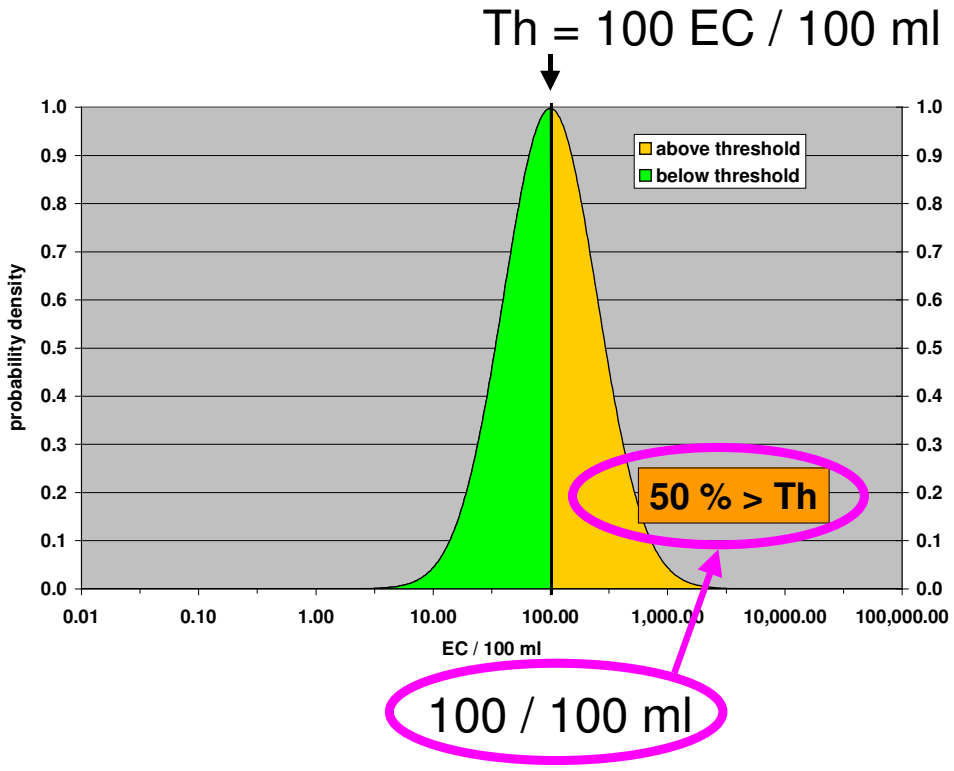
Legend:

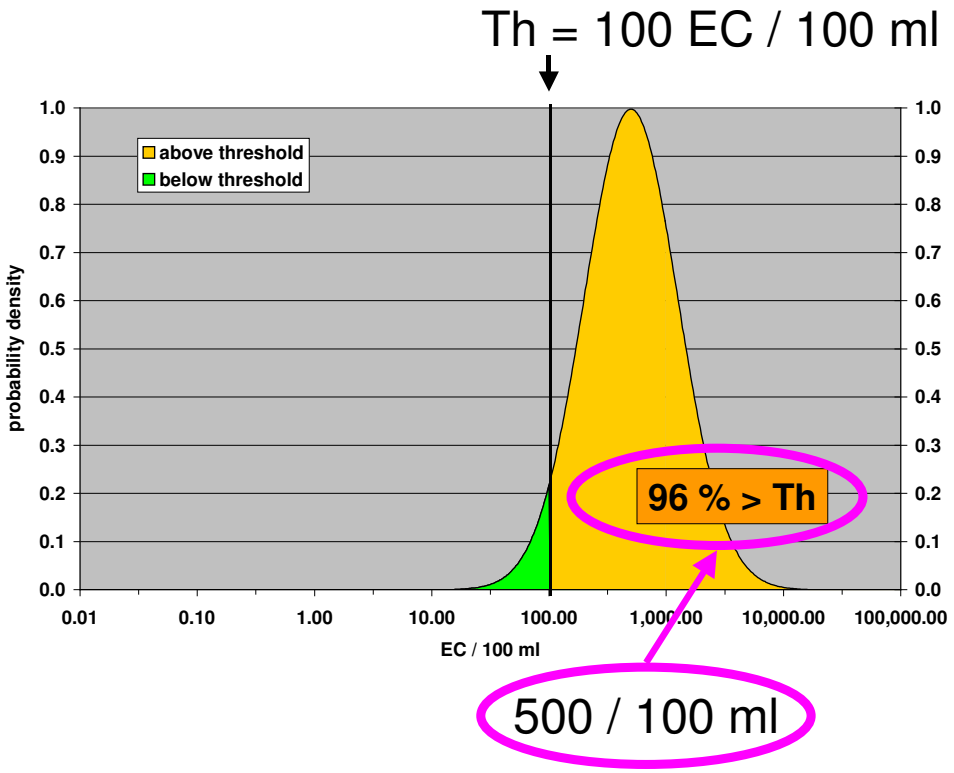
Th	Threshold of effect (NOAEL)
%>Th	percent concentrations exceeding the threshold
EC	*Escherichia coli*
IE	Intestinal enterococci


Th = 100 EC / 100 ml
above theshold
below threshold
probability density
0.4
0 % > Th
1 / 100 ml
EC / 100 ml
0.01
0.10
1.00
10.00
100.00
1000.00
10000.00
100000.00


Th = 100 EC / 100 ml
above threshold
below threshold
probability density
1 % > Th
10 / 100 ml
EC / 100 ml
0.01
0.10
1.00
10.00
100.00
1,000.00
10,000.00
100,000.00


Th = 100 EC / 100 ml
above threshold
below threshold
probability density
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.01
0.10
1.00
10.00
100.00
1,000.00
10,000.00
100,000.00
EC / 100 ml
23 % > Th
50 / 100 ml


Th = 100 EC / 100 ml
above threshold
below threshold
probability density
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.01
0.10
1.00
10.00
100.00
1,000.00
10,000.00
100,000.00
EC / 100 ml
50 % > Th
100 / 100 ml


Th = 100 EC / 100 ml
above threshold
below threshold
probability density
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.01
0.10
1.00
10.00
100.00
1,000.00
10,000.00
100,000.00
EC / 100 ml
96 % > Th
500 / 100 ml

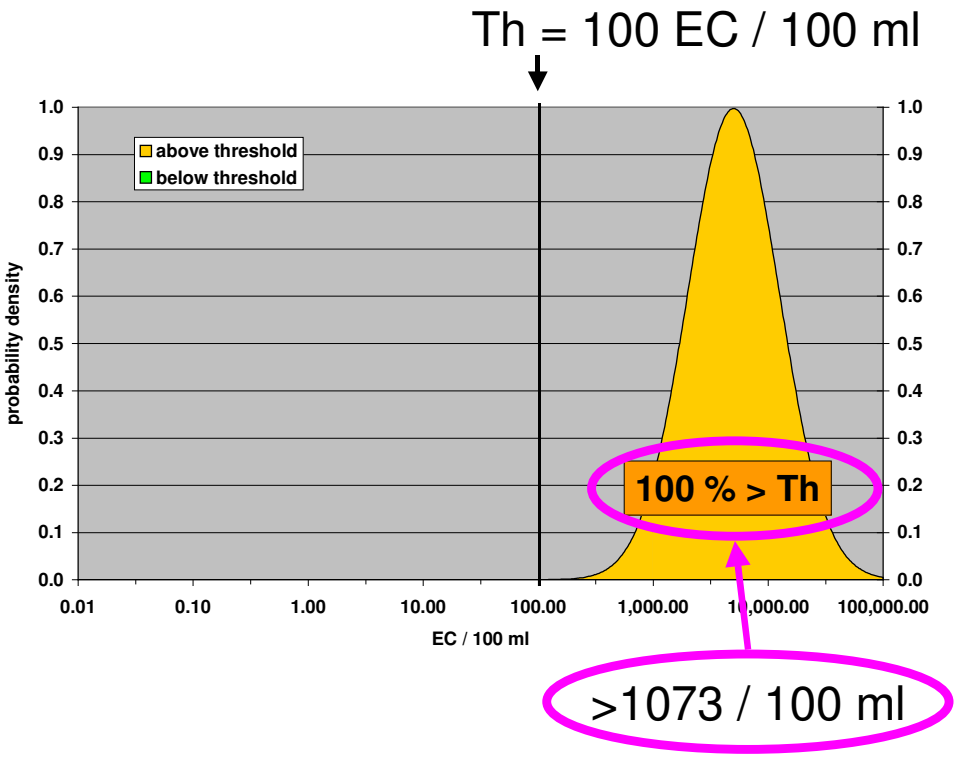

Th = 100 EC / 100 ml
above threshold
below threshold
probability density
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.01
0.10
1.00
10.00
100.00
1,000.00
10,000.00
100,000.00
EC / 100 ml
100 % > Th
>1073 / 100 ml

Table: % >Th for Escherichia coli Concentrations SD=0.4; Th = 100 EC/100 ml

EC / 100 ml				>Th %
0	-	9	=	0 %
10	-	13	=	1 %
14	-	16	=	2 %
17	-	18	=	3 %
19	-	20	=	4 %
21	-	22	=	5 %
23	-	24	=	6 %
25	-	26	=	7 %
27	-	28	=	8 %
29			=	9 %
30	-	31	=	10 %
32	-	33	=	11 %
34			=	12 %
35	-	36	=	13 %
37			=	14 %
38	-	39	=	15 %
40			=	16 %
41	-	42	=	17 %
43			=	18 %
44	-	45	=	19 %
46			=	20 %
47	-	48	=	21 %
49			=	22 %
50	-	51	=	23 %
52			=	24 %
53	-	54	=	25 %
55	-	56	=	26 %
57			=	27 %
58	-	59	=	28 %
60			=	29 %
61	-	62	=	30 %
63	-	64	=	31 %
65			=	32 %
66	-	67	=	33 %
68	-	69	=	34 %
70			=	35 %
71	-	72	=	36 %
73	-	74	=	37 %
75	-	76	=	38 %
77	-	78	=	39 %
79	-	80	=	40 %
81	-	82	=	41 %
83	-	84	=	42 %
85	-	86	=	43 %
87	-	88	=	44 %
89	-	90	=	45 %
91	-	92	=	46 %
93	-	94	=	47 %
95	-	96	=	48 %
97	-	98	=	49 %
99	-	101	=	50 %
102	-	103	=	51 %
104	-	105	=	52 %
106	-	108	=	53 %
109	-	110	=	54 %
111	-	113	=	55 %
114	-	117	=	56 %
118	-	119	=	57 %

EC / 100 ml				>Th %
120	-	121	=	58 %
122	-	124	=	59 %
125	-	127	=	60 %
128	-	130	=	61 %
131	-	134	=	62 %
135	-	137	=	63 %
138	-	140	=	64 %
141	-	144	=	65 %
145	-	148	=	66 %
149	-	151	=	67 %
152	-	155	=	68 %
156	-	159	=	69 %
160	-	164	=	70 %
165	-	168	=	71 %
169	-	173	=	72 %
174	-	178	=	73 %
179	-	183	=	74 %
184	-	188	=	75 %
189	-	194	=	76 %
195	-	200	=	77 %
201	-	206	=	78 %
207	-	213	=	79 %
214	-	220	=	80 %
221	-	228	=	81 %
229	-	236	=	82 %
237	-	245	=	83 %
246	-	254	=	84 %
255	-	265	=	85 %
266	-	276	=	86 %
277	-	288	=	87 %
289	-	302	=	88 %
303	-	317	=	89 %
318	-	334	=	90 %
335	-	353	=	91 %
354	-	376	=	92 %
377	-	403	=	93 %
404	-	435	=	94 %
436	-	476	=	95 %
477	-	530	=	96 %
531	-	608	=	97 %
609	-	737	=	98 %
738	-	1072	=	99 %
1073	-	> 1073	=	100 %

Table: %>Th for Intestinal Enterococci Concentrations SD=0.4; Th = 25 IE/100 ml

IE / 100 ml				> Th %
0	-	2	=	0 %
3			=	1 %
4			=	2 %
5			=	4 %
6			=	6 %
7			=	8 %
8			=	11 %
9			=	13 %
10			=	16 %
11			=	19 %
12			=	21 %
13			=	24 %
14			=	26 %
15			=	29 %
16			=	31 %
17			=	34 %
18			=	36 %
19			=	38 %
20			=	40 %
21			=	42 %
22			=	44 %
23			=	46 %
24			=	48 %
25			=	50 %
26			=	52 %
27			=	53 %
28			=	55 %
29			=	56 %
30			=	58 %
31			=	59 %
32			=	61 %
33			=	62 %
34			=	63 %
35			=	64 %
36			=	65 %
37			=	66 %
38			=	68 %
39			=	69 %
40	-	41	=	70 %
42			=	71 %
43			=	72 %
44			=	73 %
45			=	74 %
46	-	47	=	75 %
48			=	76 %
49	-	50	=	77 %
51			=	78 %
52	-	53	=	79 %

IE / 100 ml				> Th %
54	-	55	=	80 %
56	-	57	=	81 %
58	-	59	=	82 %
60	-	61	=	83 %
62	-	63	=	84 %
64	-	66	=	85 %
67	-	69	=	86 %
70	-	72	=	87 %
73	-	75	=	88 %
76	-	79	=	89 %
80	-	83	=	90 %
84	-	88	=	91 %
89	-	94	=	92 %
95	-	100	=	93 %
101	-	108	=	94 %
109	-	119	=	95 %
120	-	132	=	96 %
133	-	152	=	97 %
153	-	184	=	98 %
185	-	268	=	99 %
269	-	> 269	=	100 %

Annex 36. Bases for a risk-related interpretation of monitoring results: Calculation of time-integrated quality scores

The principle aim of monitoring recreational waters for faecal indicator organisms is to protect the health of bathers from infectious agents which can be spread with human or animal faeces. If at the end of a monitoring period beaches are classified into different quality categories according to the results of monitoring for faecal indicator organisms, the quality categories should reflect the mean health risk at the various sites in the most objective way possible. It is a nuisance when bathing sites with lower health risks are classified into lower quality categories than bathing sites with high health risks, as happens with the current classification system in the EU. Misclassifications detract from the confidence in a monitoring and classification system, and encourage counter-productive strategies to "outsmart" the system. An example: with the current monitoring system in the EU it is beneficial to collect additional samples in between the routine monitoring intervals when the water quality can be expected to be good, while it is disadvantageous to collect additional samples when the water quality is bad. This runs contrary to what a good HACCP concept should result in. Health authorities would often like to take additional samples when the water quality is bad, and they would also like to take multiple samples at various sampling points to look for spot contamination. However, if additional sampling results during short periods of low water quality have an over-proportional influence on the final classification result, health authorities can hardly justify their activities, especially when superior authorities also protect the touristic and financial interests of the municipality which is managing a beach. This problem would not be solved with any of the other monitoring and classification systems which have been proposed either by the WHO (1998, 2003) or by the Commission of the European Union (2002).

Therefore it has been proposed that bathing sites be classified according to a time-integrated quality score (Wiedenmann et al., 2003). The score is based on estimates for the percentage of indicator concentrations which exceed the thresholds of effect

(NOAEL's) for gastroenteritis ("%>Th") as explained in the corresponding Annex ("Bases for a risk-related interpretation of monitoring results: Calculation of the percentage of concentrations exceeding the threshold of effect (NOAEL)").

The score is expressed in a figure between 0 and 100%, and can be calculated according to a simple equation:

Time-integrated Quality Score = sum((%>Th)*days))/total days

“%>Th” is the estimated percentage of concentrations exceeding the threshold of effect (NOAEL) on the day of sampling, “days” is the number of days until the next sampling date, and “total days” is the total number of days of the bathing season. If two or more indicator organisms with different thresholds of effect are monitored simultaneously, the higher "%>Th"-value is used for the calculation in each case.

If it is assumed that bathing activities are randomly distributed over the bathing season, the score can be interpreted as an estimate for the percentage of bathers who were exposed above threshold of effect concentrations during that season. Thus, the score is easy to interpret, and its meaning can be easily explained to the public. It would then be a political decision to define score ranges which are considered to be "excellent", "good", "acceptable" or "poor."

Classification of bathing sites according to the time-integrated quality score would have the following advantages:

1. The classification would be strictly risk-related, i. e. based on estimates for the mean health risk from faecal water pollution at the various bathing sites.

2. These risk estimates are essentially based on epidemiological evidence from studies with a randomised, controlled trial design.

3. The quality score is independent of the variable susceptibility of different cohorts to infectious agents (e. g. tourists vs. local residents; UK cohort vs. German cohort, etc.).

4. The score accounts for the fact that the risk of infectious disease from bathing in faecally contaminated water does not increase with increasing indicator densities after having reached a maximum risk level. It also accounts for the fact that the risk from bathing cannot be less than zero. It is therefore less affected by statistical outliers (low or high) than other methods.

5. The score does not depend on any distribution assumptions for the changes in the mean microbiological water quality which occur during the bathing season; i. e. it doesn't matter whether these changes are e. g. lognormal distributed or whether they have a "messy" multiple-peak or chaotic distribution.

6. The score is free of any of the possible paradox reactions which can be encountered with classification according to the percentile approach, where, for example, a 95th percentile can increase when the water quality tends to become better or when a low value is added to a group of higher values. It is also impossible that the score has a higher value than any of the values which are used to calculate the score.

7. It would be advantageous to control a high value as soon as possible, to keep the time period short for which it counts; i. e. health authorities would not come into conflict with other interests (touristic, financial) for intensifying their control measures when the water quality has deteriorated.

8. If more than one sample is collected at a certain beach on a single day, e. g. due to complaints of bathers, the geometric mean of these sample results could be used in the score calculation.

9. The system would be open for supplementary monitoring schemes which may be performed by licensed laboratories e. g. on behalf of environmental organisations, tourist associations, etc.

10. If the number of bathers at a beach is recorded during the bathing season, it is very easy to compute e. g. the expected number of gastroenteritis cases that have probably occurred during that season ("disease burden"). This calculation can be done for different susceptibilities of cohorts. Thus, health authorities could calculate where infra-structural measures to improve the water quality would be most efficient.

One potential argument against this concept could be that a microbiological water quality detected on a certain day will almost never stay the same for the number of days until the next sample is collected. However, to carry a value forward until the next result is available is the most reasonable thing to do. All other assumptions would be even more speculative, and in fact health authorities as well as bathers "behave" accordingly during the time interval between two consecutive measurements. They assume that the latest result is the one which is valid for the moment, because no other information is available.

Another concern was that the final result could be skewed, if only the bad results are controlled by additional measurements but the good results are accepted as is. However,

classification results with conventional methods (e. g. calculation of 95% compliance rates with mandatory values or the calculation of 95th percentiles) are already skewed towards the "worse" side by the random error which is due to the limited number of samples which can be used in these calculations (Wiedenmann, 2003). In addition, it is a common experience of health authorities that unexpectedly high monitoring results often cannot be reproduced by control measurements nor can they be explained by any systematic contamination e. g. from agricultural run-off or sewage discharge. They may be due to faecal accidents of human or animal origin, especially water fowl, and have little significance for the classification of a bathing site as no water quality directive whatsoever can prevent such accidents. A mechanism that partially compensates for these sources of error is therefore reasonable as well.

The table and the figures in this annex illustrate how a time-integrated quality score is calculated using sampling dates and monitoring results for *Escherichia coli*, how the results can be transformed into estimates for disease burden of a bathing site, how the results can be plotted in quality control charts for the bathing season, and how the score correlates with other methods of classification of recreational water quality.

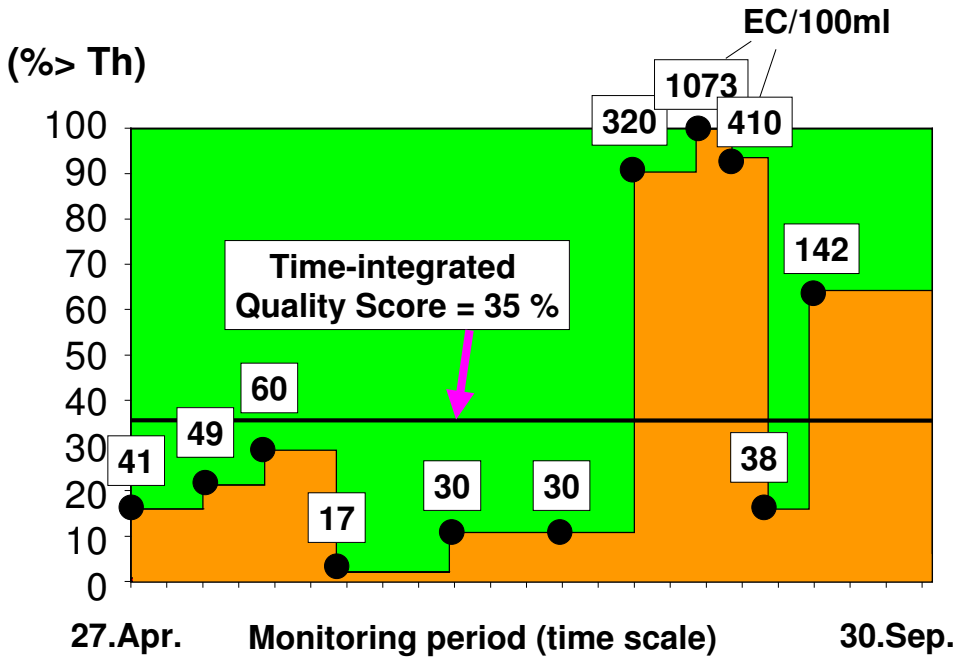
Sample No.	Sampling Date	No. of Days	EC/100ml	%>Th	(%>Th)*days	% GE	(%GE)*days
1	27.04.00	14	41	17	233	0.8	11.0
2	11.05.00	12	49	22	263	1.0	12.4
3	23.05.00	14	60	29	405	1.4	19.1
4	06.06.00	22	17	3	60	0.1	2.8
5	28.06.00	17	30	10	162	0.4	7.6
6	15.07.00	19	30	10	182	0.4	8.5
7	03.08.00	12	320	90	1076	4.2	50.6
8	15.08.00	7	1500	100	699	4.7	32.8
9	22.08.00	7	410	94	656	4.4	30.8
10	29.08.00	8	38	15	117	0.7	5.5
11	06.09.00	24	142	65	1556	3.0	73.1
End	30.09.00						
	Total days	156		**Sum**	5410	**Sum**	254.3
				TIQS	35 %	**DB**	1.6 %

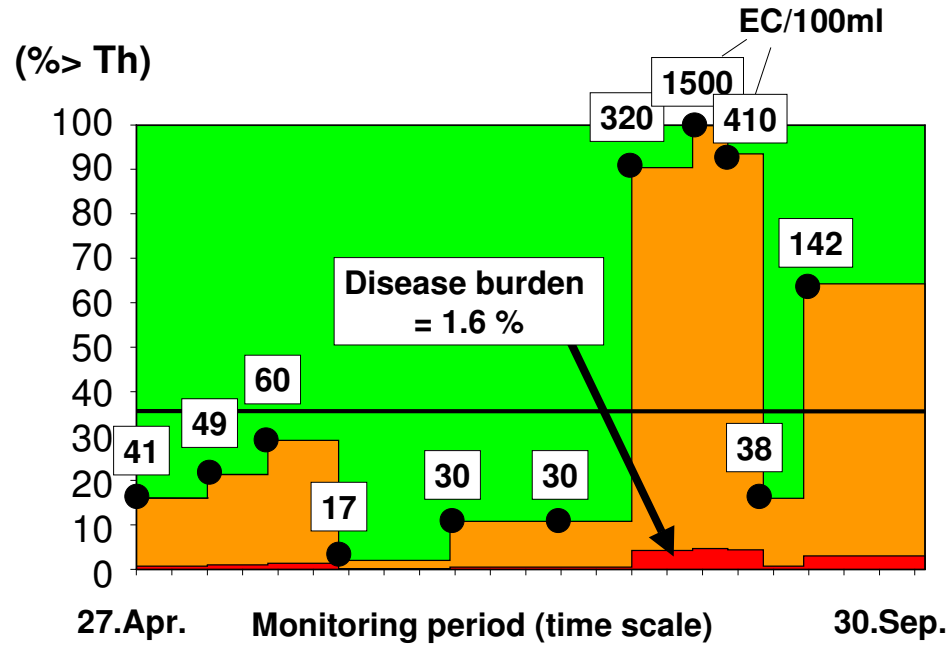
Table 1: Example for the calculation of a Time-integrated Quality Score

Legend

Sample No.	Sample number
Sampling Date	Date when the sample was collected
No. of Days	Number of days between two consecutive sampling days
EC/100ml	Concentration of *Escherichia coli* / 100ml
%>Th	Estimated percentage of concentrations above threshold concentration (NOAEL) on the sampling day. It is assumed that EC/100ml is the median of lognormal distributed concentrations with a standard deviation of 0.4 on the sampling day.
(%>Th)*days	Product of (%>Th) and (No. of Days)
Total days	Total number of days between first sampling day and end of the bathing season
TIQS	Time-integrated Quality Score (Estimated percentage of bathers exposed above threshold of effect during the monitoring period) Calculation: Sum((%>Th)*days)/total days
%GE	Estimated percentage of bathers who would acquire gastroenteritis (GE-Definition: UK-wf) from 10 minutes bathing and at least three head immersions at a German fresh water bathing site in a cohort of local residents with 4.7% susceptible individuals. Calculation: (%>Th)*0.047. Step model parameters of dose response relationship: baseline incidence rate = 2.8%; Threshold of effect = 100EC/100ml; maximum incidence rate = 7.5%
(%GE)*days	Product of (%GE) and (No. of Days)
DB	Disease burden (estimated percentage of bathers acquiring gastroenteritis from 10 minitues bathing and at least three head immersions during the monitoring period). Calculation: sum((%GE)*days)/total days

Quality Control Chart
based on the monitoring data listed in Table 1 of this annex.

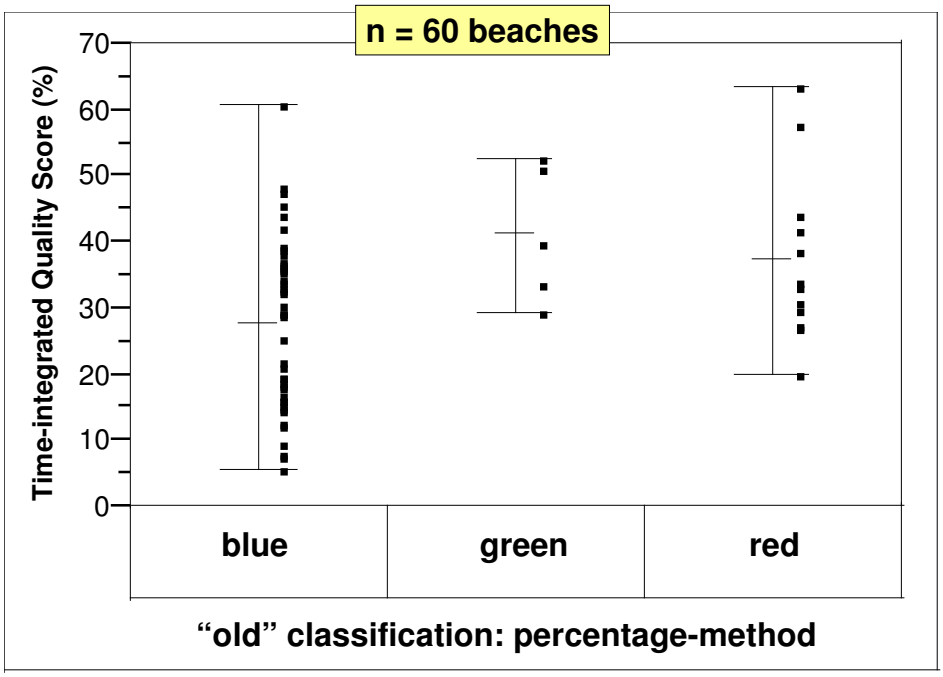




Comparison of the Time-integrated Quality Score with the results of classification of 60 German fresh water bathing sites according to current EU legislation and according to the 95th percentile approach in the revised EU draft directive.

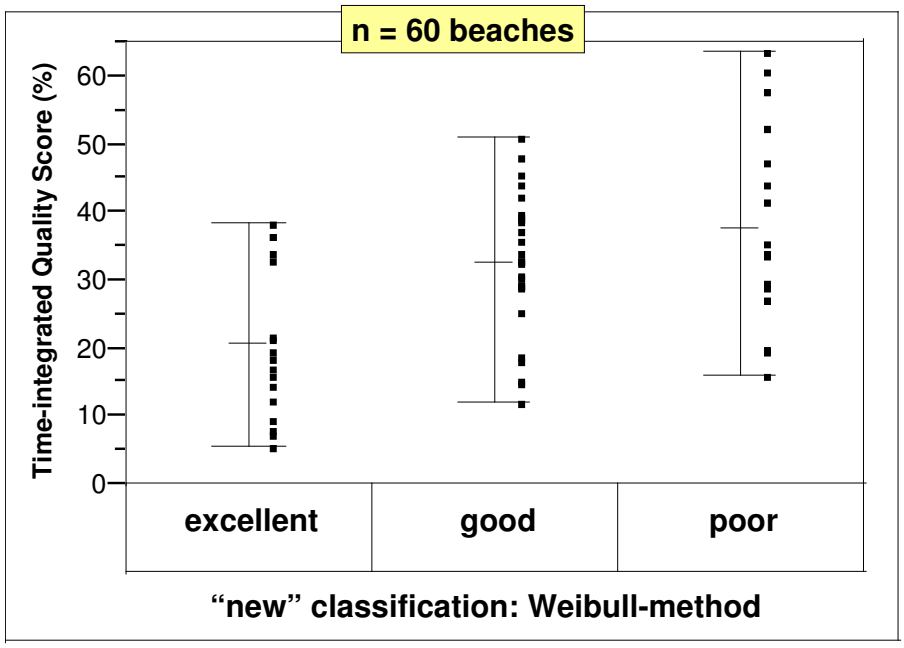
The monitoring data used in this comparison were donated by the Baden-Württemberg State Health Office. Between 26 and 35 sample results from three consecutive monitoring seasons were available for each of the 60 sites.

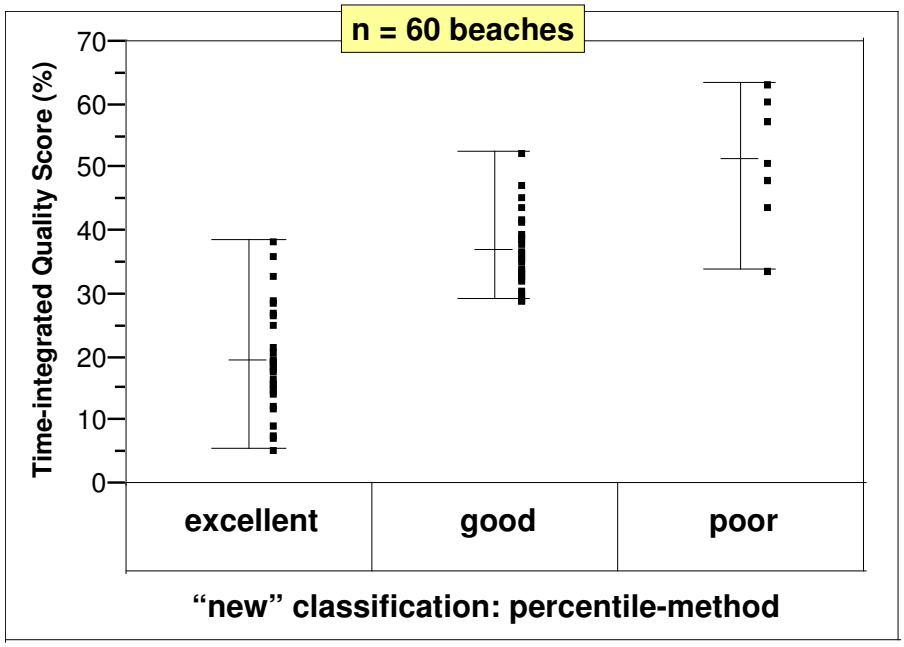
The comparison demonstrates that the current classification system does not correlate well with the health risk at the bathing sites. Sites with low risk may happen to be classified into lower quality categories than sites with high health risks. Classification according to the 95th percentile is not better, when the percentiles are calculated according to the Weibull method (WHO, 2003). The best correlation between the classification result and the time-integrated quality score could be observed when the 95th percentiles were calculated according to the formula antilog($\mu+1.65\sigma$) as proposed by the European Commission and by WHO. Therefore, if the time-integrated quality score itself is not used for classification, the latter method seems to be the best choice.



Legend

blue	site complied with guide values for total coliforms and faecal coliforms in at least 80%, and with mandatory values in at least 95% of the samples in each of the three years
green	site complied with mandatory values for total coliforms and faecal coliforms in at least 95% of the samples in each of the three years
red	site did not comply with mandatory values for total coliforms or faecal coliforms in more than 5% of the samples of at least one of the three years





Legend:

excellent	95th percentile for *Escherichia coli* ≤ 250/100ml, and for faecal streptococci ≤ 100/100ml
good	95th percentile for *Escherichia coli* ≤ 500/100ml, and for faecal streptococci ≤ 200/100ml
poor	95th percentile for *Escherichia coli* >250/100ml, and for faecal streptococci >200/100ml

Annex 37. Materials used for study organisation: Press release

To inform public media a press release was launched by the University of Tübingen three to four weeks ahead of each of the trials. The release explained the scientific background and aims of the study and included information on the location of the study site, the time-table, and phone numbers of the project managment team, an e-mail address and the URL of the study web site for more detailed information. An example in German language is given in this annex.

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

Pressemitteilung

Tübingen, 21. Mai 2000

"Planschen für die Wissenschaft"

Teil 4 der umweltmedizinischen Studie findet am 16. Juni im Strandbad Marli in Lübeck statt – 500 Badefans gesucht – Teilnehmer können sich ab sofort anmelden.

"Planschen für die Wissenschaft" - Unter diesem Motto findet am **Samstag, 16. Juni**, im Strandbad Marli in Lübeck in Zusammenarbeit mit dem Bereich Umweltschutz der Hansestadt Lübeck, der Lübecker Schwimmbäder GmbH und dem Institut für Hygiene und Umweltmedizin der Universität Kiel ein **Forschungsprojekt des Umweltbundesamts** und des Instituts für Allgemeine Hygiene und Umwelthygiene der Universität Tübingen statt. Dabei sollen die 1976 von der Europäischen Union festgelegten Richtlinien und Grenzwerte zur mikrobiologischen Qualität von Süßwasser-Badestellen unter der Leitung des Facharztes für Hygiene und Umweltmedizin Dr. Albrecht Wiedenmann überprüft werden. Für die groß angelegte Studie **werden 500 Versuchsteilnehmer aus Lübeck gesucht**, die unter dem Motto "Planschen für die Wissenschaft" für zehn Minuten Baden gehen.

- **Hintergrund:**

Das Umweltbundesamt lässt dieses Forschungsprojekt in der Badesaison 2000 und 2001 stellvertretend für die deutschen Süßwasser-Badestellen an insgesamt fünf verschiedenen Binnengewässern in Deutschland durchführen, die nach der

Badegewässerrichtlinie der Europäischen Union laufend kontrolliert werden und im Hinblick auf die Wasserqualität den Bestimmungen entsprechen: die ersten drei Studien fanden bereits im vergangenen Jahr am Baggersee Kirchentellinsfurt bei Tübingen, in Unteruhldingen am Bodensee und im Strandbad Plötzensee in Berlin statt. das fünfte Projekt ist im August in Bayern geplant. Obwohl die Badegewässerrichtlinie bereits **seit 1976** besteht, wurden **in Deutschland bisher noch nie Gesundheitsdaten im Zusammenhang mit dem Baden in Naturgewässern auf diese systematische Weise erhoben**.

Mit den fünf Studien soll festgestellt werden, ob durch die Badegewässerrichtlinie sichergestellt wird, dass es beim Baden in unseren heimischen Gewässern nicht zu gesundheitlichen Beeinträchtigungen durch Mikroorganismen kommt. Dabei sollen hauptsächlich leichtere Beschwerden erfasst werden, mit denen die Menschen normalerweise nicht zum Arzt gehen und die deshalb in der Regel unbekannt bleiben: z. B. leichte Augen-, Ohren- und Rachenentzündungen oder leichte Magen-Darm-Infekte. Da solche Beschwerden auch völlig unabhängig vom Baden auftreten können, werden die 500 Badewilligen erst im Strandbad nach dem Zufallsprinzip entweder der "Badegruppe" oder einer gleich großen "Nichtbadegruppe" zugeteilt, die dann als Vergleichsgruppe dient. Parallel zu der Badeaktion werden in kurzen Abständen Wasserproben gezogen und in einem mobilen Laboratorium gleich vor Ort mikrobiologisch untersucht. So können die Wissenschaftler überprüfen, ob evtl. auftretende gesundheitliche Beschwerden auch unterhalb der Grenzwerte der Badegewässerrichtlinie in einem Zusammenhang mit der im Wasser gemessenen Konzentration an Mikroorganismen stehen.

Mit der Untersuchung sollen keinerlei Aussagen zur Wasserqualität eines einzelnen Gewässers gemacht werden. **Die Ergebnisse sollen möglichst allgemein gültig und auf andere Binnengewässer übertragbar sein.** Daher werden die Daten aller fünf Erhebungen erst Ende des Jahres 2001 in anonymisierter Form zusammen gerechnet und gemeinsam ausgewertet.

Was genau erwartet die Teilnehmer aus Lübeck? Sie erhalten bei der Anmeldung zur Studie ein Informationsblatt und unterschreiben eine Teilnahmeerklärung. Ein erstes Interview zum allgemeinen Gesundheitszustand, zu den Ernährungsgewohnheiten etc., sowie eine kurze ärztliche Untersuchung (Augen-, Ohren- und Rachen-Inspektion) findet nach Terminabsprache am Mittwoch, dem 13. Juni, oder Donnerstag, dem 14. Juni, im Rathaus in Lübeck statt. Dabei wird der erste von insgesamt vier Fragebögen ausgefüllt. Der zweite folgt am Badetag selbst, der dritte eine Woche später (wieder im Rahmen eines Interviews mit kurzer Untersuchung) und der vierte wird drei Wochen nach dem Badetag zuhause ausgefüllt. Für den Badetag, Samstag, den 16. Juni, erhalten die Teilnehmer einen Termin für den Strandbadbesuch zwischen 11:30 Uhr und 14:30 Uhr. Eine Woche vor und eine Woche nach diesem Badetermin sollten die Studienteilnehmer nicht in Naturgewässern baden.

Am Badetag werden die "Bade-Gruppe" und "Nichtbade-Gruppe" gebildet. Wer baden geht, sollte zehn Minuten im Wasser bleiben und in dieser Zeit drei Mal den Kopf kurz unter Wasser tauchen. Sog. Badebetreuer protokollieren dabei genau, wann wer wo im Wasser war, damit die einzelnen Teilnehmer später den Messwerten der Wasserqualität exakt zugeordnet werden können. Wegen des großen Organisationsaufwandes läuft die Studie auch bei schlechtem Wetter. Allerdings kann dann am Strand in den ca. 30 Zeltpavillons Zuflucht gesucht werden. Für die Teilnehmer ist der Besuch des Strandbads an diesem Tag übrigens kostenlos. Damit alle Teilnehmer auch hinsichtlich der Ernährung ähnlichen Bedingungen ausgesetzt sind, wird außerdem für ein kostenloses Lunchpaket gesorgt.

- **Wer kann teilnehmen ?**

Teilnehmen kann, wer gesund und volljährig ist. Jugendliche zwischen 14 und 18 Jahren können mit einer Einverständniserklärung beider Eltern oder Erziehungsberechtigten selbständig teilnehmen. Kinder zwischen 4 und 14 Jahren benötigen ebenfalls die Einverständniserklärung beider Eltern oder Erziehungsberechtigten und können in Begleitung mindestens eines Elternteils oder eines Erziehungsberechtigten teilnehmen.

- **Wie kann man sich anmelden ?**

Anmelden kann man sich telefonisch unter unter 0177-300-1432 bzw. -1434 oder per e-mail unter webmaster@badegewaesserstudie.de. Man bekommt dann eine Teilnahmeerklärung zugeschickt, die zum 1. Interview mitgebracht werden muss. Nähere Informationen gibt's auch unter http://www.badegewaesserstudie.de im Internet. Hier kann auch das Merkblatt und die Teilnahmeerklärung abgerufen und ausgedruckt werden.

- **Was bekommt man dafür ?**

Wer die Studie bis zum letzten Fragebogen am 7. Juli mitmacht, erhält eine Aufwandsentschädigung von **50 Mark**. Da 500 Personen, die genügend Zeit und Begeisterung für ein solches Forschungsprojekt mitbringen, nicht leicht zu finden sind, werden die Teilnehmer gebeten, auch Familienangehörige, Freunde, Bekannte, Kollegen etc. auf das Projekt aufmerksam zu machen. Nach dem Prinzip "Teilnehmer werben Teilnehmer" erhält man für jeden weiteren angeworbenen Teilnehmer, der bis zum letzten Fragebogen mit dabei bleibt, **zusätzlich 10,- DM**.

Nähere Informationen für die Medien:

Dr. Albrecht Wiedenmann
Eberhard-Karls-Universität Tübingen
Institut für Allgemeine Hygiene und Umwelthygiene
Wilhelmstraße 31; D-72074 Tübingen
Tel. 0177-300-1431 / Fax. 0177-99-300-1431
Internet: http://www.badegewaesserstudie.de,
E-mail: webmaster@badegewaesserstudie.de

Annex 38. Examples for press reports before and after the bathing trials at the five study sites

Reutlinger_Generalanzeiger_2000-05-23:

Sie waren schon mal Probebaden für ihre Studie: Albrecht Wiedenmann und seine Mitarbeiterinnen vom Tübinger Hygiene-Institut testen den Kirchentellinsfurter Baggersee unter besonderer Berücksichtigung seiner Tauglichkeit als Badegewässer. Am Samstag sollen das im Dienst der Wissenschaft gleich 400 Testpersonen tun.
GEA-Foto: Meyer

Nichts wie rein in den Baggersee

Tübinger Wissenschaftler suchen für Badegewässerstudie noch freiwillige Planscher

Von Heiner Keller

Kirchentellinsfurt/Tübingen. (GEA) Dr. Albrecht Wiedenmann und sein Team vom Hygiene-Institut der Uni Tübingen zögerten nicht lange und sprangen in den Kirchentellinsfurter Baggersee. Mit dem Sprung ins frische Nass wollen sie dazu animieren, bei ihrer Badegewässerstudie mitzumachen. 400 Badelustige sollten möglichst mit von der Partie sein, knapp die Hälfte hat sich allerdings erst angemeldet.

Mit der Badegewässerstudie, die die Tübinger Wissenschaftler im Auftrag des Bundesumweltministeriums organisieren, geht es darum, in Deutschland fünf Gewässer darauf zu untersuchen, ob die von der EU erlassene Badegewässer-Richtlinie tatsächlich gewährleistet, dass es bei Badenden zu keinen gesundheitlichen Beeinträchtigungen kommt.

Der wissenschaftliche Ansatz liegt darin zu untersuchen, ob nicht selbst unterhalb der gültigen Grenzwerte aufgrund von Mikroorganismen Beschwerden wie Augen-, Ohren- oder Rachenentzündungen, Unwohlsein oder leichte Durchfallerkrankungen auftreten können.

Baden oder nicht baden

Mitmachen bei der Studie kann jedermann, er muss nur gesund und volljährig sein. Für die Untersuchung des Kirchentellinsfurter Baggersees suchen die Wissenschaftler noch für Samstag, 27. Mai, Testpersonen, die bereit sind mitzumachen. Sei der Untersuchung wird es eine Gruppe der Badenden, und eine der Nicht-Badenden geben. Während die einen mindestens zehn Minuten ins Wasser müssen und dabei auch mal den Kopf unter Wasser nehmen sollen, dürfen sich die anderen am Baggerseeufer mit Sonnenbaden, Ballspielen und anderen Outdoor-Aktivitäten vergnügen.

Dr. Albrecht Wiedenmann verweist darauf, dass sich die Testpersonen keineswegs einer großen gesundheitlichen Gefahr aussetzten, denn schließlich sei der Kirchentellinsfurter Baggersee im EU-Badegewässer-Atlas mit einem blauen Dreieck versehen, was ihm attestiere, dass die Grenzwerte eingehalten, ja sogar unterschritten würden.

Bevor es am kommenden Samstag an den See geht, wird mit den Teilnehmern ein Interview geführt und sie werden von einem Arzt, der sich Mund, Nase und Rachen ansieht, kurz untersucht. Eine Woche nach dem Badetag ist ein weiteres Interview vorgesehen und ganz am Schluss gilt es noch, einen Fragebogen auszufüllen.

Vespern für die Wissenschaft

Ganz umsonst muss man sich nicht in den Dienst der Wissenschaft stellen: Wer mitmacht, der bekommt 50 Mark und ein Vesper.

Anmelden für diese umweltmedizinische Studie kann man sich unter den Rufnummern 0177-800-1431, -1432, -1433 oder -1434.

Weitere Informationen: www.badegewaesserstudie.de

Südkurier_2000-06-30:

Ins Wasser für die Wissenschaft

400 Personen nehmen in Unteruhldingen an einer Badegewässerstudie teil

Friedrichshafen (hk) Zehn Minuten im Bodensee planschen für einen wissenschaftlichen Zweck – möglich wird das für rund 200 Personen am Samstag, 8. Juli, im Strandbad Unteruhldingen. Es handelt sich dabei um ein Forschungsprojekt des Umweltbundesamtes und des Hygieneinstitutes der Universität Tübingen in Zusammenarbeit mit dem Landratsamt und der Gemeinde Uhldingen-Mühlhofen. Die Forscher wollen überprüfen, ob die bereits 1976 erlassene Badegewässerrichtlinie der EU sicherstellt, dass es beim Baden in heimischen Naturgewässern nicht zu Beeinträchtigungen der Gesundheit durch Mikroorganismen kommt.

Die Studie wird mit lokalen Kooperationspartnern in den Sommermonaten 2000 und 2001 an insgesamt fünf verschiedenen kontrollierten Badegewässern in Deutschland durchgeführt, erläuterte der Tübinger Hygienearzt Dr. Albrecht Wiedenmann im Pressegespräch. In erster Linie geht es um die Erfassung leichterer Beschwerden wie etwa leichte Augenentzündungen, Unwohlsein oder Durchfall, wegen derer man normalerweise nicht zum Arzt geht und die daher meist unbekannt bleiben.

Anhand des Projektes sollen die Fragen beantwortet werden, wie oft solche Beschwerden auch bei Einhaltung der in den Richtlinien festgelegten mikrobiologischen Grenzwerte auftreten und ob ein Zusammenhang mit der Konzentration der im Wasser gemessenen Mikroorganismen besteht. Die Auswertung erfolgt für alle untersuchten Gewässer gemeinsam. „Die Untersuchung dient nicht dazu, die Wasserqualität des Strandbades Unteruhldingen zu ermitteln", stellte Wiedenmann klar.

50 Mark Entschädigung

An dem Projekt in Unteruhldingen nehmen rund 400 Personen teil, die am Strandbad nach dem Zufallsprinzip in zwei gleich große Gruppen eingeteilt werden. 200 davon „dürfen" für etwa zehn Minuten ins Wasser. Die 200 Teilnehmer der Vergleichsgruppe nehmen an normalen Strandaktivitäten teil, ohne jedoch in Kontakt mit dem Wasser zu kommen. Das ist notwendig, weil die erwähnten Beschwerden auch unabhängig vom Baden auftreten können. Als (fakultative) Verpflegung gibt es für alle ein mikrobiologisch kontrolliertes Lunch-Paket. Parallel zu den Badeaktivitäten werden in kurzen Zeitintervallen Wasserproben entnommen und auf verschiedene Arten von Mikroorganismen untersucht.

Jeder Teilnehmer muss für die Studie zusätzlich insgesamt vier Fragebögen ausfüllen, in denen es insbesondere um den Gesundheitszustand geht. Augen, Ohren und Rachen werden kurz ärztlich. Teilnehmen kann jede gesunde Person ab vier Jahren; Jugendliche zwischen 14 und 18 Jahren mit einer Einverständniserklärung der Eltern, Kinder zwischen bis 13 Jahre in Begleitung eines Elternteils. Nach Rücksendung des Fragebogens erhält jeder Teilnehmer eine Aufwandsentschädigung von 50 Mark.

❑ Anmeldungen und Infos unter ☎ 0177-300-1432 oder -1434,

@ im Internet „www.badegewaesserstudie.de".

Sie freuen sich auf das wissenschaftliche Planschen im See (v.l.n.r.): Dr. Albrecht Wiedenmann (Facharzt für Hygiene und Umweltmedizin Universität Tübingen), Biologin Petra Krüger und Dr. Bernhard Kiß vom Gesundheitsamt Bodenseekreis. Gestern stellten sie im Gesundheitsamt eine Badegewässerstudie des Umweltbundesamtes vor. Bild: Kleinstück

BILD_Berlin-Brandenburg_2000-08-02:

Senat sucht: Wer geht für 50 Mark baden?

Zehn Minuten baden, dreimal kurz untertauchen - dafür kassieren 200 Berliner 50 Mark. Und auch die 200, die nur zugucken...

„Planschen für die Wissenschaft" ist ein Projekt von Umweltbundesamt und der Universität Tübingen. Umweltmediziner Dr. Albrecht Wiedenmann (39): „Wir überprüfen die EU-Richtlinien für Süßwasser-Badeseen. Treten Beschwerden auf, müssen eventuell die Grenzwerte für Mikroorganismen geändert werden."

Badetag ist Sonnabend, der 19. August. Ab 11.30 Uhr im Strandbad Plötzensee (Nordufer 24). Dr. Wiedenmann: „Die Teilnehmer müssen auch vier Fragebögen ausfüllen - zwei davon in der Technischen Universität (TU) vor und nach dem Baden. Außerdem gibt's eine kleine ärztliche Untersuchung, bei der Augen, Ohren und Rachen inspiziert werden."

Gesucht werden insgesamt 400 Berliner. Sie werden am Badetag nach Zufallsprinzip in eine Bade- und eine Nichtbadegruppe getrennt - zur Kontrolle, um die Ergebnisse zu vergleichen. Dr. Wiedenmann: „Da es nicht einfach ist, Leute mit genügend Begeisterung und Zeit für ein Forschungsprojekt zu finden, gibt's für jeden, der Freunde, Verwandte, Kollegen wirbt, zu den 50 Mark Aufwandsentschädigung noch zehn Mark extra."

Teilnehmen kann jeder, der gesund und volljährig ist, Kinder ab vier nur in Begleitung der Eltern, Jugendliche mit Einverständniserklärung der Eltern.

INFO Anmeldung: Ab sofort ☎ 89031500 oder -1401, sowie 0177 300-1432. Im Internet: **www.badegewaesserstudie.de**

Neues_Deutschland_2000-08-03:

Baden für die Wissenschaft

Freiwillige sollen den Weddinger Plötzensee testen

(ddp). Die Universität Tübingen sucht 400 Testpersonen, die sich im Dienste der Wissenschaft am Plötzensee in Wedding vergnügen. 200 der wissenschaftlichen Badegäste sollen am Sonnabend, dem 19. August, für genau zehn Minuten im See planschen und tauchen. Die andere Hälfte der Teilnehmer wird sich währenddessen ohne Wasserkontakt am Strand tummeln.

Der wissenschaftliche Badetag ist Teil einer Badegewässerstudie des Instituts für Allgemeine Hygiene und Umwelthygiene der Universität Tübingen und des Umweltbundesamts. Dabei werden im Sommer 2000 und 2001 insgesamt fünf deutsche Badeseen von jeweils 400 Personen getestet. Die Untersuchung soll feststellen, ob die 1976 erlassene Badegewässerrichtlinie der Europäischen Union ein gesundes Baden sicherstellt. Dabei geht es vor allem um die Erfassung leichterer Beschwerden.

Interessenten können sich unter den Telefonnummern 0177/ 300-1432/ -1434 oder 030/8903-1500/ -1401 anmelden. Das zehnminütige Probebaden lohnt sich: Für jede Testperson gibt es ein mikrobiologisch kontrolliertes Lunch-Paket und 50 Mark. Einen Haken hat die Sache allerdings: Wegen des großen Organisationsaufwandes kann die Durchführung der Studie nicht vom Wetter abhängig gemacht werden. Die Testplanscher müssen also auch bei Regen und Kälte baden gehen.

Weitere Informationen übers wissenschaftliche Plantschen unter: www.badegewaesserstudie.de

Lübecker_Nachrichten_2001-06-17:

Das Freibad der Forschung

Mehrere hundert Lübecker stiegen für Gewässerstudie in die kühle Wakenitz

Gerade aufgetaucht aus den Fluten der Wakenitz: Janneke Clasen (15, v. l.), Kathrine Clasen (12) und Heike Wichmann (39), die gestern am Forschungsprojekt im Strandbad Marli teilnahmen. 580 Probanden aus Lübeck und Umgebung hatten sich für das Massenplanschen zu wissenschaftlichen Zwecken gemeldet. Fotos: MAXWITAT

AZ_München_2001-08-06:

■ *Bundesumweltamt sucht „Versuchskaninchen": Es gibt 50 Mark*

Baden gehen und abkassieren

vc. **München**

Baden gehen für die Wissenschaft und dafür noch bares Geld kassieren! Klingt nach einer fiebrigen Idee nach zu langer Sonneneinstrahlung, ist aber der ganz ernst gemeinte Plan der Universität Tübingen und des Umweltbundesamtes: Erstmals wollen Forscher untersuchen, welche Auswirkungen das Baden in Naturgewässern auf die Gesundheit hat. Deshalb suchen sie nach Freiwilligen, die in den Lerchenauer See tauchen. Der Lohn: 50 Mark. Vorher und nachher müssen sie einen Gesundheitscheck durchlaufen. Samstag (18. August) ist Badetag.

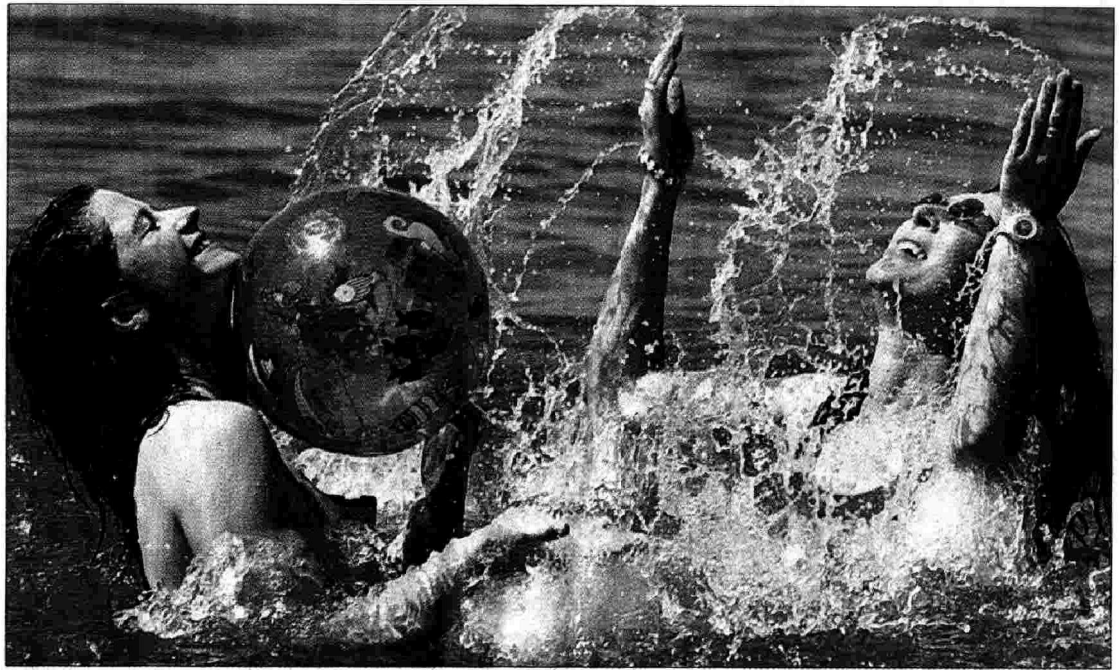
Bei der Studie kann jeder mitmachen, der gesund und älter als vier Jahre ist. Minderjährige brauchen eine Einverständniserklärung ihrer Eltern. Anmeldung unter 0177/3001432 oder 0177/3001434 Internet: www.badege-waesserstudie.de

Dann wird's am Ufer aussehen wie beim Triathlon: 250 Probanden müssen sich ins Wasser stürzen, die Vergleichsgruppe – 250 weitere – bleiben im Trockenen.

Die Münchner Seen werden alle 14 Tage untersucht um sicherzustellen, dass sie die Richtwerte von 1976 noch einhalten. Doch gewährleisten diese Richtwerte für Schadstoffe überhaupt das, was sie sollen? Schützen sie die Gesundheit der Badegäste? In dieser Studie werden also die Richtwerte selbst auf den Prüfstand gestellt. In Tübingen, Berlin, Lübeck und am Bodensee sind bereits Schwimmfans für die Wissenschaft baden gegangen – jetzt planschen die Münchner.

Die Versuchsteilnehmer werden vor allem auf Beschwerden untersucht, wegen derer man nicht sofort zum Arzt geht: zum Beispiel leichte Ohren- und Augenentzündungen oder Magenbeschwerden.

Und so geht's: Einfach unter der Telefonnummer anrufen und einen Termin zum Interview vereinbaren. Eine Woche nach dem Wassergang kommt eine zweite Gesundheitskontrolle und zum Schluß wird allen Teilnehmern noch ein Fragebogen nach Hause geschickt. Wer einen „Versuchsschwimmer" anwirbt, kann sich noch zehn Mark extra verdienen.



Erfrischende Badefreude und dafür auch Geld kriegen: Am 18. August wird das für hunderte Münchner am Lerchenauer See wahr Foto: dpa

Annex 39. Information flyer used for recruitment of participants

Umwelt Bundes Amt
für Mensch und Umwelt

- Planschen für die Wissenschaft -

Badegewässerstudie des Umweltbundesamts
& des Hygiene-Instituts der Universität Tübingen
in Zusammenarbeit
mit dem Landratsamt Bodenseekreis / Gesundheitsamt
& der Gemeinde Uhldingen-Mühlhofen

Universität Tübingen

"Machen Sie mit !"

Samstag, 8. Juli

Strandbad Unteruhldingen

Es können 400 Personen teilnehmen !

Anmeldung /Info: ☎ Tel. 0177-300-1432 oder -1434
Internet: www.badegewaesserstudie.de
e-mail: webmaster@badegewaesserstudie.de

Am Samstag 8. Juli können Sie am Strandbad Unteruhldingen zwischen 11:30 und 15:00 an einem **Forschungsprojekt** teilnehmen, das **bundesweit an insgesamt fünf verschiedenen repräsentativen Binnengewässern** durchgeführt wird. Dabei sollen die Auswirkungen des Badens in Naturgewässern auf die **menschliche Gesundheit** untersucht werden. Es geht dabei hauptsächlich um die Erfassung leichterer Beschwerden, wegen derer man normalerweise nicht zum Arzt geht und die in Folge dessen in der Regel **unbekannt** bleiben. Die Untersuchungen erfolgen ausschließlich an Gewässern, die den Bestimmungen der **EU-Badegewässerrichtlinie** entsprechen. Teilnehmer erhalten eine **Aufwandsentschädigung von 50,- DM**.

Das müssen **Sie** tun:

BADEN (10 min planschen, schwimmen ....
& 3 x Kopf untertauchen)

oder NICHT BADEN (wird ausgelost !)
... ein mikrobiologisch kontrolliertes Lunch-Paket verzehren ...
...und insgesamt **4 Fragebögen** beantworten

- teilnehmen kann, wer **gesund und älter als 4 Jahre** ist -

---

Das müssen **wir** tun:

- die Teilnehmer nach dem Zufallsprinzip in **200 Badende** und **200 Nicht-Badende** einteilen (es gibt keine Wahl- oder Tauschmöglichkeit)
- alle 20 min **Wasserproben** entnehmen und im Labor analysieren
- 2-3 Tage vor und 1 Woche nach dem Baden eine kurze ärztliche Inspektion von Augen, Ohren und Rachen durchführen: **am 5./6. Juli von 15:00 - 21:00 und am 15. Juli von 10:00 - 18:00 (nach Terminvereinbarung)**

Annex 40. Information leaflet for participants and declaration of participation

An information leaflet with a declaration of participation was handed out or sent by mail or e-mail to people who took an interest in participation. The signed declaration of participation was collected on registration before the first interview, and was kept in the files together with the questionnaires.

Informationsblatt und Teilnahmeerklärung

Badegewässerstudie des Umweltbundesamts und des Instituts für Allgemeine Hygiene und Umwelthygiene der Universität Tübingen

Wissenschaftliche Leitung:	**Dr. med. Albrecht Wiedenmann** Institut für Allgemeine Hygiene und Umwelthygiene der Universität Tübingen Direktor: Prof. Dr. med. K. Botzenhart Wilhelmstr. 31, 72074 Tübingen
Telefonische Auskunft:	**Hygiene-Institut** Tel: 0177-300-1431; -1432; -1433 oder -1434
Internet:	http://www.badegewaesserstudie.de
E-mail:	webmaster@badegewaesserstudie.de
	Umweltbundesamt Dr. med. Juan López-Pila, Dr. rer. nat. Regine Szewzyk
Terminvereinbarung:	**Mo – Sa von 9:00-20:00 unter** **Tel.: 0177-300-1432 oder -1434** **Tel.: 030-8903-1500 oder -1401**

1. Zielsetzung

Ziel dieser Untersuchung ist es festzustellen, ob die im Jahr 1976 von der Europäischen Union (EU) erlassene Badegewässerrichtlinie sicherstellt, dass es beim Baden in unseren heimischen Gewässern nicht zu Beeinträchtigungen der Gesundheit durch Mikroorganismen kommt. Es geht dabei hauptsächlich um die Erfassung von Beschwerden, bei denen man normalerweise nicht zum Arzt geht und die infolgedessen

in der Regel unbekannt bleiben: z. B. leichte Augen-, Ohren- und Rachenentzündungen, Unwohlsein, Erbrechen, leichte Durchfallerkrankungen und Ähnliches.

Durch die Studie sollen folgende Fragen beantwortet werden:

1.) wie häufig treten solche Beschwerden auch bei Einhaltung der in der Badegewässerrichtlinie festgelegten mikrobiologischen Grenzwerte auf ?
2.) besteht hierbei ein Zusammenhang mit der Konzentration der im Wasser gemessenen Mikroorganismen ?

Hierzu werden insgesamt ca. 2000 Teilnehmer benötigt, die bereit sind, an einem Wochenende an ein ganz normales öffentliches Badegewässer zu kommen, und in einem mit Markierungsseilen abgegrenzten Bereich für 10 Minuten baden zu gehen. Gleichzeitig werden in diesem Bereich in kurzen Abständen Wasserproben entnommen und mikrobiologisch untersucht. Vor und einige Tage nach dem Baden werden die Teilnehmer über ihren Gesundheitszustand befragt.

Die o. g. Beschwerden können natürlich auch völlig unabhängig vom Baden aus ganz verschiedenen Ursachen auftreten. Es ist deshalb notwendig, die Häufigkeit dieser Beschwerden auch bei einer Gruppe von Personen zu bestimmen, die nicht gebadet hat. Die Gruppe der Nicht-Badenden sollte sich ansonsten aber möglichst wenig von der Gruppe der Badenden unterscheiden. Die Teilnehmer an dieser Studie werden daher nach dem Zufallsprinzip eingeteilt und erfahren erst am Strand, ob sie zur Gruppe der Badenden oder Nicht-Badenden gehören. Da die mikrobiologische Untersuchung der Wasserproben im Labor mindestens 1-2 Tage dauert bis die Ergebnisse vorliegen, können weder die Studienteilnehmer noch die Organisatoren schon am Badetag wissen, wie hoch oder niedrig die Konzentration von Mikroorganismen innerhalb der einzelnen Badebereiche ist. Sie wissen lediglich, dass die mikrobiologischen Grenzwerte in diesem Badegewässer eingehalten werden.

Da die gewonnenen Ergebnisse möglichst allgemein gültig sein sollen, wird die Untersuchung an fünf verschiedenen Badegewässern innerhalb Deutschlands durchgeführt. Die Auswertung erfolgt für alle untersuchten Gewässer gemeinsam. Es geht also nicht darum, die Qualität eines bestimmten Badesees oder Badestrands zu ermitteln. Es erfolgt aus diesem Grund auch keine Mitteilung von Messwerten an die einzelnen Teilnehmer. Die Studie beschäftigt sich auch nicht mit ökologischen Fragestellungen, z. B. mit Belangen des Naturschutzes oder Problemen wie Gewässerüberdüngung, Algenwachstum, Verlandung usw.. Solche Probleme können an manchen Badegewässern völlig unabhängig von der nach Badegewässerrichtlinie ermittelten mikrobiologischen Wasserqualität vorhanden sein.

2. Beschreibung des Studienablaufs

Die Studie wird ausschließlich an Badegewässern durchgeführt, die laufend nach EU-Richtlinie kontrolliert werden und von den Gesundheitsbehörden offiziell zum Baden zugelassenen sind. Die Vorgaben der Badegewässerrichtlinie im Hinblick auf die mikrobiologische Wasserqualität wurden an diesen Gewässern in den vergangenen Jahren eingehalten.

Für die Studie werden pro Badestelle 400 Teilnehmer benötigt. Die Teilnehmer sollten gesund und mindestens 4 Jahre alt sein. Jugendliche zwischen 14 und 18 Jahren können mit einer Einverständniserklärung beider Eltern oder Erziehungsberechtigten selbständig teilnehmen. Kinder zwischen 4 und 14 Jahren benötigen ebenfalls die Einverständniserklärung beider Eltern oder Erziehungsberechtigten und können in Begleitung mindestens eines Elternteils oder eines Erziehungsberechtigten teilnehmen. Die Teilnehmer werden gebeten, sich 2-3 Tage vor dem Badetag zu einem telefonisch vereinbarten Termin an einem zentral gelegenen Ort zu einem ersten Interview (Befragung) einzufinden. Nach Vorlage der unterschriebenen Teilnahme-Erklärung (nicht vergessen!) füllen Mitarbeiter des Projekts gemeinsam mit dem Teilnehmer einen Fragebogen aus, in dem hauptsächlich Angaben zum Gesundheitszustand, zu den Ernährungsgewohnheiten und den persönlichen Lebensverhältnissen gemacht werden

(Fragebogen Nr.1). Im Anschluß an das Interview prüft ein ebenfalls anwesender Arzt die Angaben im medizinischen Teil des Fragebogens und bespricht sie mit dem Teilnehmer. Der Arzt misst mit einem Infrarot-Thermometer im äußeren Gehörgang die Körpertemperatur und schaut sich Augen, Ohren und Rachen an, um sicherzustellen, dass keine Infektionskrankheit in diesem Bereich besteht. Er entscheidet dann, ob die Person aus seiner Sicht an der Studie teilnehmen kann. Anschließend erhält der Teilnehmer einen Termin für den Badetag (11:30; 12:00; 12:30; 13:00; 13:30 oder 14:00 Uhr) zu dem er sich am Badestrand einfinden soll.

Am Badetag melden sich die Teilnehmer zum vereinbarten Zeitpunkt am Informationsstand. Sie erfahren erst jetzt, ob sie in die Gruppe der Badenden oder Nicht-Badenden eingeteilt worden sind. Es besteht hierbei also keine Wahlmöglichkeit. Auch ein Tausch mit anderen Teilnehmern, z. B. Freunden oder Verwandten, ist nicht möglich. Wir empfehlen aus diesem Grund auch, dass jeder Teilnehmer seine eigenen Strandutensilien (eigenes Handtuch, eigene Bastmatte etc.) mitbringt. Kinder, die nicht selbständig baden können, werden zusammen mit mindestens einem Elternteil zum Baden oder Nicht-Baden eingeteilt.

Um für alle Teilnehmer während des Aufenthalts am Strand möglichst gleiche Ernährungsbedingungen zu schaffen, sollten am Badetag keine Lebensmittel am Strand eingekauft oder mitgebracht werden, auch kein Eis, keine belegten Brote, Currywurst etc.. Die Teilnehmer erhalten statt dessen ein einheitliches kostenloses Lunchpaket mit Getränk.

Sie erfahren jetzt auch die Nummer ihres Betreuers am Strand, bei dem sie sich anschließend melden sollen. Der Strand ist in vier jeweils 15-20 Meter breite Streifen eingeteilt, die mit A bis D gekennzeichnet sind. Dorthin wenden sich die Badenden. Die Nicht-Badenden sind in einem separaten Strandabschnitt untergebracht wo sie nicht in Kontakt mit dem Wasser kommen, ansonsten aber die üblichen Aktivitäten ausüben können (Sonnenbaden, Ballspielen etc.). Alle Projekthelfer tragen eine deutlich sichtbare Nummer auf dem T-Shirt. Die Betreuer der Badenden haben ein blaues T-

Shirt und blaue Schirmmütze, die Betreuer der Nicht-Badenden tragen rote T-Shirts. Andere Projekthelfer z. B. an den Info-Tischen sind an den grünen T-Shirts zu erkennen. Der Betreuer führt gemeinsam mit den Teilnehmern, die sich bei ihm gemeldet haben, das Badetag-Interview durch (Fragebogen Nr.2). Auch am Badetag sind wieder Ärzte vor Ort, die bei Gesundheitsproblemen, die seit dem ersten Interview neu aufgetreten sind, zu Rate gezogen werden. Nach dem Interview gehen die Teilnehmer, die zum Baden eingeteilt sind, für 10 Minuten ins Wasser. Der Betreuer protokolliert in diesen 10 Minuten alle Aktivitäten im Wasser (Schwimmen, Planschen etc.). Die Teilnehmer werden gebeten, sich während dieser 10 Minuten möglichst dicht bei einer der beiden Probenahmestellen im Wasser aufzuhalten. Diese sind mit einem weißen oder blauen Luftballon gekennzeichnet. Zusätzlich sollten die Teilnehmer mindestens 3-mal kurz den Kopf unter Wasser tauchen (Kinder natürlich nur, wenn sie selbst Lust dazu haben!). Es werden auch genügend Schwimmhilfen (Schwimmbretter, Wasserbälle etc.) zur Verfügung stehen, so dass man sich während der 10 Minuten Badezeit im Wasser auch ausruhen kann. Nach dem Baden können die Teilnehmer wieder nach Hause gehen oder noch weiter am Strand bleiben. Sie sollten jedoch nicht erneut ins Wasser gehen. Alle Teilnehmer, auch die Nicht-Badenden, sollten unbedingt versuchen, 1 Woche vor bis 1 Woche nach dem Badetag den Kontakt zu Naturgewässern zu meiden.

Wegen des großen Organisationsaufwandes kann die Durchführung der Studie nicht vom Wetter abhängig gemacht werden. Die Teilnehmer sollten daher bereit sein, auch bei relativ schlechtem Wetter an die Badestelle zu kommen. Es ist in jedem Fall für ausreichenden Regenschutz in Zelten gesorgt. Auch im umgekehrten Fall, bei zu starker Sonneneinstrahlung, besteht die Möglichkeit, sich im Schatten der Zelte aufzuhalten.

Wie alle Badegäste sind auch die Teilnehmer dieser Studie gehalten, am Strand ist jeweils gültige Strandordnung zu beachten.

1 Woche nach dem Badetag findet das 3. Interview statt, zu dem sich die Teilnehmer erneut einen Termin geben lassen. Das Interview verläuft wieder wie das 1. Interview,

d.h. es wird ein Fragebogen ausgefüllt (Fragebogen Nr. 3), und es findet wieder eine kurze ärztliche Untersuchung statt.

3 Wochen nach dem Badetag füllen die Teilnehmer den letzten Fragebogen aus (Fragebogen Nr.4). Sie bekommen diesen Fragebogen zwei Tage vorher zugeschickt und können ihn gleich nach dem Ausfüllen in einem mitgelieferten vorfrankierten Umschlag an das Hygiene-Institut der Universität Tübingen zurückschicken.

3. Gesundheitsrisiko

Da die Studie an einem nach EU-Richtlinie kontrollierten und offiziell zugelassenen Badegewässer durchgeführt wird, besteht für die Teilnehmer kein größeres Gesundheitsrisiko, als für viele Millionen Menschen, die ebenfalls jedes Jahr an den nach EU-Richtlinie kontrollierten Stränden Europas baden. Am Badetag werden zusätzliche Gesundheitsschutzmaßnahmen getroffen, z. B. durch Anwesenheit von Mitarbeitern des DRK, der DLRG o. Ä.. Jeder Badende wird außerdem von einem Projekthelfer ständig beobachtet. Das allgemeine Baderisiko ist für die Studienteilnehmer daher erheblich geringer als normalerweise.

Wie bei anderen alltäglichen Freizeitaktivitäten in der freien Natur, z. B. Joggen im Wald, bestehen auch beim Baden bestimmte Risiken, die nicht hundertprozentig ausgeschlossen werden können. Hierunter fallen Verletzungen, z. B. durch Glasscherben, oder durch Sturz nach Ausrutschen oder Abgleiten von der Uferböschung. Im Extremfall droht beim Baden das Risiko des Ertrinkens, z. B. beim Auftreten von Muskelkrämpfen oder Bewußtseinsverlust. In äußerst seltenen Fällen können Erreger, die über wildlebende Nagetiere ins Wasser oder in den Boden gelangen, durch die Haut oder durch Verschlucken von Wasser in den Körper eindringen und zu hochfieberhaften Erkrankungen oder schweren Magen-Darm-Infekten führen. Durch Ausscheidungen von Wasservögeln kann es zu Hautausschlägen mit heftigem Juckreiz kommen. Ähnliche Symptome können von kleinen Parasiten verursacht werden, die sich im Gras auf den Liegewiesen aufhalten. Auch nach

Zeckenstichen sind Hautausschläge oder schwere fieberhafte Erkrankungen möglich. Wir empfehlen daher allen Teilnehmern, den behandelnden Arzt/Ärztin in solchen Fällen auf den erfolgten Besuch eines Badesees oder einen erfolgten Zeckenstich hinzuweisen. Alle diese Erkrankungen sind nicht Gegenstand dieses Forschungsprojekts. Sie stehen in keinem Zusammenhang mit der nach Badegewässerrichtlinie ermittelten Wasserqualität oder sind so selten, dass keine Chance bestünde, sie im Rahmen einer Studie mit 2000 Teilnehmer überhaupt zu erfassen.

4. Versicherungsschutz

Für alle mit der Studie in Zusammenhang stehende Aktivitäten besteht ein zusätzlicher Unfallversicherungsschutz mit einer Deckungssumme von 50.000/100.000 DM (Ecclesia Versicherungsdienst GmbH). Für Schäden, die durch Projekthelfer oder Projektmitarbeiter verursacht werden, tritt die Haftpflichtversicherung des Universitätsklinikums Tübingen ein. Im Falle von Erkrankungen ist die normale Krankenversicherung oder der Sozialversichungsträger erstattungspflichtig.

5. Datenschutz

Die personenbezogenen Daten der Teilnehmer werden streng vertraulich behandelt. Eine Weitergabe an unbeteiligte Dritte erfolgt nicht. Zum Zwecke der wissenschaftlichen Auswertung werden die Daten anonymisiert. D. h. jeder Teilnehmer wird nur noch anhand einer Nummer identifiziert. Alle Helfer und Projektmitarbeiter stehen entweder selbst unter ärztlicher Schweigepflicht oder sie haben eine Schweigepflichtserklärung unterschrieben, wie sie von der Ärztekammer für Mitarbeiter eines Arztes vorgesehen ist.

6. Spesen / Auslagen

Als Aufwandsentschädigung für die Teilnahme an der Studie erhalten alle Teilnehmer am Ende 50,- DM. Voraussetzung für die Auszahlung der Aufwandsentschädigung ist die Teilnahme an allen Interviews und am Badetag und die Rücksendung des vollständig ausgefüllten 4. Fragebogens drei Wochen nach dem Badetag.

7. Zeitplan

Datum	Uhrzeit	Ort	Anlass
Mi 16.08.00 oder Do 17.08.00	15:00 bis 21:00	Technischen Universität, Mathematikgebäude, Raum 301, Straße des 17. Juni 135, gegenüber Hauptgebäude, U-Bahnhof Ernst Reuter Platz	1. Interview
Samstag 19.08.00	ab 11:30 bis 5:00	**Strandbad Plötzensee**	Badetag 2. Interview
Samstag 26.08.00	10:00 bis 18:00	Technischen Universität, Mathematikgebäude, Raum 301, Straße des 17. Juni 135, gegenüber Hauptgebäude, U-Bahnhof Ernst Reuter Platz	3. Interview
Samstag 09.09.00	-----	------	4. Fragebogen per Post

8. Zeitbedarf

- 1. Interview mit kurzer ärztlicher Untersuchung: ca. 30 min
- Badetag incl. 2. Interview: ca. 1-1,5 Std., nach Belieben auch länger
- 3. Interview mit kurzer ärztlicher Untersuchung: ca. 20 min
- 4. Fragebogen zu hause: ca. 20 min

9. <u>Terminvereinbarung für das 1. Interview:</u>

(Tel.: 0177-300-1432 bzw. -1434, Sprechzeiten: Mo - Sa 9:00-20:00

oder

(Tel.: 030-8903-1500 oder -1401, Sprechzeiten: Mo - Do 8:00-16:00

Termin vergessen / ändern / absagen? (Telefon wie oben

Bitte 1 Woche <u>vor</u> und 1 Woche <u>nach</u> dem Badetag
nicht mehr in einem Naturgewässer (Strandbad, See, Baggersee, Fluss, Meer)
baden !

<u>Zustimmung zur Badegewässerstudie des Umweltbundesamts und des Instituts für Allgemeine Hygiene und Umwelthygiene der Universität Tübingen</u>

- Ich habe Abschnitt 1-9 der Teilnehmerinformation gelesen und verstanden. Eventuelle zusätzliche Fragen wurden mir ausreichend beantwortet
- Ich werde am Badetag nur teilnehmen, wenn ich mich gesund fühle und keine Erkrankungen habe, bei denen Baden ein ernsthaftes Risiko für mich bedeuten würde (z. B. frische Operationswunden, Herzbeschwerden, Kollapsneigung, offene Hautausschläge, Immundefekt). Im Zweifelsfall werde ich ärztlichen Rat einholen. Ich werde auch nicht kommen, wenn ich unter akuten Infektionskrankheiten leide, die auf andere Teilnehmer übertragbar sind. z. B. Durchfall, Fieber, Erbrechen, ansteckende Hautausschläge)
- Ich weiß, dass ich, ggf. zusammen mit meinen Kindern, am Badetag nach dem Zufallsprinzip in die Gruppe der Badenden oder der Nicht-Badenden eingeteilt werde und keine Wahl- oder Tauschmöglichkeit habe.

- Ich habe zur Kenntnis genommen, dass am Badetag ein zusätzlicher Unfallversicherungsschutz besteht und dass ich Unfälle unverzüglich der Projektleitung melden muss, um den Versicherungsschutz nicht zu verlieren.
- Ich weiß, dass ich jederzeit und ohne jede Begründung die Teilnahme an dieser Studie beenden kann. Ich versichere aber, in diesem Fall das Projektteam umgehend hierüber zu informieren.
- Ich erkläre mich bereit, an dieser Studie und an den damit verbundenen Befragungen teilzunehmen.
- Es wurde mir zugesichert, dass alle Informationen über meine Person streng vertraulich behandelt und keine personenbezogenen Daten an unbeteiligte Dritte weitergegeben werden.
- Ich willige ein, dass für die wissenschaftliche Auswertung meine Angaben auf elektronischen Datenträgern gespeichert werden.

Teilnehmer:

Name, Vorname: _____

(bitte in Druckbuchstaben)

Alter : (zutreffendes ankreuzen)

□ 4-6 □ 7-13 □ 14-17 □ 18 oder älter

Teilnehmer ist

□ Schwimmer □ Nicht-Schwimmer

Datum: _____

_____

Unterschrift des Teilnehmers

Einverständniserklärung beider Eltern od. Erziehungsberechtigten (für alle Teilnehmer unter 18 Jahren):

Ich willige ein, dass mein o. g. Sohn / meine o. g. Tochter an der Badegewässerstudie teilnimmt. 4-13-Jährige können nur in Begleitung eines Elternteils/Erziehungsberechtigten teilnehmen.

_____

Unterschrift der Mutter / 1. Erzeihungsberechtigter

_____

Unterschrift des Vaters / 2. Erziehungsberechtigter

Bei nur einer Unterschrift: □ Ich bin der/die alleinige Erziehungsberechtigte

Anschrift des Teilnehmers:

Straße: _____

PLZ / Ort: _____

Telefon:_____ Fax: _____ e-mail: _____

Bankverbindung für die Überweisung der Aufwandsentschädigung:

Name des Kontoinhabers: _____

Name des Bankinstituts: _____

Bankleitzahl: _____ Kontonummer: _____

Meine Teilnahme wurde vermittelt durch::

_____

(Name, Vorname in Druckbuchstaben)

Annex 41. Memorandum with time-table for participants

Umweltbundesamt / Institut für Allgemeine Hygiene und Umwelthygiene der Uni Tübingen
Landratsamt Bodenseekreis - Gesundheitsamt / Gemeinde Uhldingen-Mühlhofen

Badegewässerstudie
am Strandbad Unteruhldingen

Merkblatt

1. Termin für's erste Interview vereinbaren

Sprechzeiten: Mo - Sa 9:00 -20:00
☎ Tel.: 0177-300-1432 oder -1434

1. Interview am Mittwoch, 05.07. _____ Uhr
oder am Donnerstag, 06.07. _____ Uhr

Wo? ☐ **Haus des Gastes**, Unteruhldingen, Schulstr. 12
☐ Landratsamt **Hauptgebäude**, Großer Sitzungssaal, 4.OG, Glärnischstr. 1-3, Friedrichshafen
☐ Landratsamt **Nebengebäude**, Bibliothek, Albrechtstr. 75, Friedrichshafen

2. Zum ersten Interview kommen

unterschriebene Teilnahmeerklärung mitbringen !
Merkblatt mitbringen !
Termin für den Badetag geben lassen, und bei Punkt "3." eintragen
Termin für's dritte Interview geben lassen, und bei "4." eintragen

3. Samstag, 8. Juli: "Badetag" um _____ Uhr

Zur vereinbarten Uhrzeit zum Strandbad kommen
BITTE Fahrgemeinschaften bilden!
am INFO-Stand anmelden
Sie erfahren, ob Sie baden oder nicht baden
Sie können nicht wählen oder tauschen! Daher sollte jeder seine eigenen Badeutensilien (Handtücher, Bastmatte usw.) mitbringen
Sie erhalten die Nummer Ihres Badetag-Betreuers
Sie erhalten ihr Lunch-Paket
Suchen Sie Ihren Betreuer und melden Sie sich bei ihm zum zweiten Interview und zum Baden / Nicht-Baden

4. Zum dritten Interview kommen

Samstag, 15. Juli _____ Uhr

Wo? ☐ **Haus des Gastes**, Unteruhldingen, Schulstr. 12
☐ Landratsamt **Hauptgebäude**, Großer Sitzungssaal, 4.OG, Glärnischstr. 1-3, Friedrichshafen

Termin vergessen / ändern / absagen? → ☎ Telefon wie oben

5. Samstag, 29. Juli:

letzten Fragebogen ausfüllen (wird zugeschickt)
und gleich zurückschicken

GESCHAFFT !!! ("50,- DM verdient")

Annex 42. Questionnaire No. 1: two or three days before exposure

Interview Nr.1

Badegewässerstudie des Umweltbundesamtes und des Hygiene-Instituts der Universität Tübingen

Datum: _____

Name, Vorname des Interviewers:_____

1. TEIL – PERSÖNLICHE DATEN

1. Name, Vorname des Teilnehmers:

_____

2. Geburtsdatum: ____/_____/_____

3. Geschlecht: männl. weibl.

4. Adresse unter der Sie die nächsten 3 Monate erreichbar sind:

Straße:_____

PLZ/Ort:_____

Telefon (privat):_____

5. Telefon an Arbeitsplatz:

_____

6. Welchen Abschluß haben Sie?

Hauptschule	Realschule	Fachhochschulreife
Hochschulreife	Fachhochschule	Universität
Anderer*	Keinen	Keine Angabe

***bitte genauere Angaben:**

_____

_____

_____

7. Welcher beruflichen Beschäftigung gehen Sie zur Zeit nach? Oder sind Sie noch Schüler?

_____

_____

8. Wieviel Personen leben in Ihrem Haushalt?
(Unter Haushalt ist gemeint, wenn Einrichtungen wie Küche, Bad und Toilette gemeinsam genutzt werden.)

Anzahl: _____

Keine Angabe

Wieviel sind davon unter 5 Jahre? _____

Keine Angabe

9. Hatte jemand in Ihren Haushalt innerhalb der letzten 2 Wochen eines der folgenden Symptome?
(es sind nur die Haushaltsmitglieder gemeint, nicht der Teilnehmer)

	Ja	Nein	Nicht sicher
(1) Durchfall			
(2) Übelkeit			
(3) Erbrechen			
(4) Erkältung (Husten, Schnupfen)			
(5) Fieber			
(6) Ohrinfektion			
(7) Lungeninfektion / Bronchitis			
(8) Halsschmerzen / Rachenentzündung			
(9) Augeninfektion			
(10) Akuter Hautausschlag			

10. Nehmen noch weitere Personen Ihres Haushalts an dieser Studie teil?

Ja Nein Nicht sicher

Falls ja, notieren Sie bitte die Namen:

1: _____

2: _____

3: _____

4: _____

2.TEIL – ALLGEMEINER GESUNDHEITSZUSTAND

11. Leiden Sie an einer der folgenden chronischen Krankheiten?

	Ja	Nein	Nicht sicher
(1) RÜCKENSCHMERZEN (z.B. Hexenschuß/Bandscheiben-Probleme)			
(2) Erhöhter BLUTDRUCK			
(3) ATEMWEGSBESCHWERDEN (z.B. Asthma/Bronchitis)			
(4) DIABETES (Zuckerkrankheit)			
(5) MAGENPROBLEME (z.B. Magengeschwür) genauere Beschr.:_____			
(6) DARM PROBLEME (z.B. Verstopfung, Durchfall, Reizdarm) genauere Beschr.:_____			
(7) OHREN PROBLEME / GEHÖRSCHÄDEN Genauere Beschr.:_____			
(8) HERZKRANKHEITEN (z.B. Angina pectoris) genauere Beschr.:_____			

Fortsetzung: ALLGEMEINER GESUNDHEITSZUSTAND

	Ja	Nein	Nicht sicher

(9) LEBERLEIDEN (z. B. Hepatitis)
Genauere Beschr.:_____
___

Falls Hepatitis, welcher Typ?

Hepatitis A

Hepatitis B

Andere Typen

(10) HABEN SIE EINE NICHT VERHEILTE VERLETZUNG?
genauere Beschr.:_____

(11) NIEREN- ODER BLASEN-PROBLEME

genauere Beschr.:_____

(12) NEUROLOGISCHE STÖRUNGEN
(z.B. Epilepsie/Migräne/Schlaganfall/Lähmung)

genauere Beschr.:_____

(13) HEUSCHNUPFEN

(14) HAUTKRANKHEITEN
(z.B. Ekzem, Schuppenflechten, Neurodermitis)

genauere Beschr.:_____

(15) AUGENLEIDEN
häufige Entzündungen (rote Augen)

genauere Beschr.:_____

(16) ANDERE GESUNDHEITLICHE PROBLEME
Bitte beschreiben Sie kurz: _____

_____

_____

12. Besuchen Sie regelmäßig wegen einem der oben genannten Probleme einen Arzt?

Ja Nein Nicht sicher

13. Wie häufig leiden Sie an Durchfall?

Häufig (ca. 1-2 mal pro Monat) Selten (ca. 1-2 mal pro Jahr) Nie Nicht sicher

14. Waren Sie in den letzten 6 Monaten so krank, daß Sie gezwungen waren Ihrer Arbeit fern zu bleiben, Ihren Gewohnheiten nicht nachgehen konnten oder das Krankenhaus aufsuchen mußten?

Ja Nein Keine Angabe

Wenn NEIN, fahren Sie bitte mit Frage 15 fort.
Wenn JA, vervollständigen Sie bitte den folgenden Teil.

Krankheits-Diagnose:_____

_____

Wurden Sie in ein Krankenhaus eingewiesen?

Ja Nein Keine Angabe

Wie lang waren Sie krank/arbeitsunfähig? WOCHEN ___ TAGE ___

Wenn Sie mehr als einmal krank waren geben Sie bitte die Details der schwersten Erkrankung an.

Krankheitsbeginn:

Monat_____ Jahr_____

Haben Sie aufgrund einer dieser Erkrankungen noch Beschwerden?

Ja Nein Keine Angabe

15. Hatten Sie innerhalb der letzten 3 Wochen eines der unten aufgeführten Symptome länger als 24 Stunden?
Bitte jede Frage mit JA, NEIN oder NICHT SICHER beantworten

Grippe / Erkältungssymptome (über 24 Std.)

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen
(1) Fieber (heiß u. kalt, Schüttelfrost)				____/____	_____
(2) Kopfschmerzen				____/____	_____
(3) Schmerzende Beine, Arme, Gelenke				____/____	_____
(4) Rachenentzündung / Halsschmerzen				____/____	_____

Atemwegssymptome (über 24 Std.)

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen
(5) Beschwerden im Brustkorb				____/____	_____
(6) Trockener Husten				____/____	_____
(7) Husten (Abhusten von Schleim)				____/____	_____
(8) Keuchen / Kurzatmigkeit				____/____	_____
(9) Schnupfen				____/____	_____

Ohren /Augensymptome (über 24 Std.)

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen
(10) Ohreninfektion (Entzündung, Vereiterung)				____/____	_____
(11) Augeninfektion (rote, entzündete Augen)				____/____	_____
(12) Sehstörungen				____/____	_____

Magen-, Bauch- und Darmsymptome (über 24 Std.)

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen
(13) Appetitlosigkeit				____/____	_____

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen
(14) Verdauungsstörung				____/____	_____
(15) Magenschmerzen (Koliken, Krämpfe, Bauchschmerzen)				____/____	_____
(16) Lockerer Stuhlgang (weicher als normal)				____/____	_____
(17) Durchfall				____/____	_____
Wenn ja, waren die Durchfälle					
breiartig				____/____	_____
flüssig braun - gelb				____/____	_____
wässerig klar				____/____	_____
blutig				____/____	_____
Wieviel Stuhlgänge traten pro Tag maximal auf?				_____ pro Tag	

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen
(18) Brechreiz (Übelkeit / Unwohlsein)				____/____	_____
(19) Erbrechen				____/____	_____
Wie häufig mußten Sie erbrechen?				_____ pro Tag	

<u>Hautsymptome (über 24 Std.)</u>

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen
(20) Hautausschläge				____/____	_____
(21) Hautgeschwüre /offene Stellen				____/____	_____
(22) Jucken (Hautreizung)				____/____	_____

Andere Symptome (über 24 Std.)

		Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen
(23)	Ausgeprägte Müdigkeit (unübliche Müdigkeit, Mattigkeit)				____/____	_____
(24)	Benommenheit /Schwindelgefühl				____/____	_____
(25)	Stechen / Kribbeln				____/____	_____
(26)	Muskelkrämpfe (in Armen und Beinen)				____/____	_____
(27)	Harnwegsinfektion / Blasenentzündung				____/____	_____

(28) Wenn Sie irgendein anderes Symptom über 24h hatten, das nicht auf dieser Liste steht, so nennen Sie es bitte mit Datum und Krankheitsdauer:

	Datum	Dauer in Tagen
1._____	____ /____	_____
2._____	____ /____	_____

16. Rauchen Sie, Pfeife bitte extra ankreuzen ?

Ja Pfeife Nein Keine Angabe

Wenn Sie nie geraucht haben fahren Sie bitte mit Frage 18 fort.

Wenn Sie rauchen, wie viele Zigaretten rauchen Sie am Tag _____
(Zigarren und „Selbstgedrehte“ zählen wie eine Zigarette)

17. Wenn Sie ein Ex-Raucher sind, wie lange rauchen Sie schon nicht mehr?

_____ JAHRE _____MONATE _____TAGE

18. Wie oft, falls überhaupt, trinken Sie Alkohol?

Niemals	Weniger als einmal pro Woche	Mind. einmal pro Woche	nicht sicher

Wenn die Antwort auf Frage 18 niemals lautet fahren Sie bitte mit Frage 21 fort.

19. Wie viele Einheiten Alkohol haben Sie ungefähr in den letzten 7 Tagen getrunken?

_____

Eine Einheit = eine halbe Bier, ¼ l Wein, 1/8l Portwein/Sherry/Martini, 3 x 0,2 cl Schnaps/Whisky/Cognac/Likör etc.

20. Würden Sie sagen, daß Sie in der letzten Woche ein übliches Trinkverhalten hatten?

JA	NEIN -Ich trinke normalerweise weniger Alkohol-	NEIN -Ich trinke normalerweise mehr Alkohol-	KEINE ANBGABE

21. Haben Sie in den letzten 4 Wochen irgendwelche Tabletten oder Medikamente genommen?

Ja Nein Keine Angabe

wenn ja, waren es:

	Ja	Nein	Nicht sicher	Name der Tabletten / Medizin
Antibiotika (z.B. Penicillin)				_____
Immunsuppressiva (z. B. Kortison)				_____
Abführmittel				_____
Magenmittel				_____
Andere				_____

3.TEIL – Aufenthalte im In- und Ausland

22. Haben Sie in den letzten 4 Wochen außerhalb Ihres Haushaltes übernachtet
(z.B. Ferien/Dienstreisen/Besuche bei Verwandte)

Ja Nein Nicht sicher

Wenn JA, war der Aufenthalt in Deutschland oder im Ausland?

DEUTSCHLAND AUSLAND BEIDES

Bitte geben Sie das Datum und die besuchten Orte -möglichst mit Postleitzahl- an, die Sie in den letzten 4 Wochen besucht haben

Orte	Datum	Dauer des Aufenthaltes

TEIL 4 – Aktivitäten in der Freizeit

23. Wie oft nehmen Sie durchschnittlich in einem Monat in dieser Jahreszeit an den unten aufgeführten Aktivitäten teil?

	öfters >3 mal	manchmal 1-3 mal	überhaupt nicht	Nicht sicher	wie oft im letzten Monat
(1) Kneipenbesuche					_____
(2) Party-/Disco-besuche					_____
(3) Sportverein					_____
(4) Kino/Theater					_____
(5) Andere*					_____

Genauere Angaben: _____

24. Wie oft üben Sie durchschnittlich in einem Sommermonat mit normalen Witterungsverhältnissen die folgenden Wassersportarten in Naturgewässern aus?

	oft >3mal	manchmal 1-3 mal	überhaupt nicht	nicht sicher	wie oft im letzten Monat
(1) Kanufahren, Schlauchboot, Rudern					_____
(2) Motor- oder Schnellbootfahren					_____
(3) Tauchen/ Schnorcheln					_____
(4) Surfen/ Wasserski/ Jetski/Segeln					_____
(5) Angeln					_____
(6) Planschen/ Wassertreten					_____
(7) Anderes*					_____

*Genauere Beschreibung:_____

_____

25. Wie oft gehen Sie in Naturgewässern schwimmen oder baden?
(Bitte geben Sie die durchschnittliche / typische Häufigkeit pro Monat -während des Sommers- an)

oft >3 mal	manchmal 1-3 mal	überhaupt nicht	nicht sicher	wie oft im letzten Monat
				_____

Wenn JA, wie weit und/oder wie lange schwimmen Sie normalerweise?

(Die Länge eines Pools = 25m) _____ Meter _____ Minuten

Bitte geben Sie genau an wo Sie in den letzten 3 Wochen gebadet haben -möglichst mit Postleitzahl-

Ort	Deutschland	Ausland	wie oft?
1.			
2.			
3.			
4.			
5.			

26. Wie oft gehen Sie an einen Strand (Meer, See oder Fluß) ohne in das Wasser zu gehen?

oft >3 mal	manchmal 1-3 mal	überhaupt nicht	nicht sicher	wie oft im letzten Monat
				_____

27. Wie oft benutzen Sie in einem Sommermonat mit normalen Witterungsverhältnissen ein Schwimmbecken (Freibad / Hallenbad)?

1: Öffentliches Schwimmbecken

oft >3 mal	manchmal 1-3 mal	überhaupt nicht	nicht sicher	wie oft im letzten Monat
				_____

2. Andere Schwimmbecken (z.B. priv. Pool)

oft >3 mal	manchmal 1-3 mal	überhaupt nicht	nicht sicher	wie oft im letzten Monat
				_____

28. Waren Sie in den letzten Monaten in einem Freizeitpark und haben dort irgendwelche Vergnügungsangebote im oder auf dem Wasser genutzt?
(z.B. Wasserrutschen / Rafting / Floßfahrten)

Ja Nein Nicht sicher

Wenn JA war der Park im Inland oder im Ausland?

Inland Ausland Nicht sicher

Bitte geben Sie den Namen des Parks an:

_____

29. Können Sie gut schwimmen?
(Können Sie mind. 2 Beckenlängen durchschwimmen)

Ja Nein Keine Angabe

30. Haben Sie noch irgendwelche ergänzende Hinweise oder Kommentare, die uns in diesem Zusammenhang helfen könnten?
(z.B. Informationen über allg. Gesundheit, Reisen / Arbeit im Ausland und Freizeitbeschäftigungen im Zusammenhang mit Wasser.)

Arztbogen - vom untersuchenden Arzt auszufüllen -

Name des Arztes:
_____

	Normal	Gerötet	Belegt
Rachen und Tonsillen			

	ja	Nein
Anhalt auf einen Ohreninfekt ?		
Anhalt auf einen Augeninfekt?		
Anhalt auf einen sonstigen Infekt?		
Temperatur >38°C		

Wenn Ja, bitte kurze schriftliche Angaben machen (Verdachtsdiagnose):

_____

_____

_____

Wurde der medizinische Teil des Fragebogens geprüft?	Ja	Nein
Empfohlen Hausarzt aufzusuchen?	Ja	Nein
Sind Sie für einen Ausschluß des Probanden?	Ja	Nein

Annex 43. Questionnaire No. 2: Exposure day interview

Badegewässer-Studie des Umweltbundesamtes und des Hygiene-Instituts der Universität Tübingen

Interview am Badetag **Datum: _____**

Name, Vorname des Interviewers:

_____

ABSCHNITT 1 KONSUMIERTE LEBENSMITTEL

1. Name, Vorname des Teilnehmers:

_____

2. Haben Sie innerhalb der letzten 3 Tage eines der folgenden Lebensmittel zu sich genommen? Wenn **Ja** vermerken Sie bitte ob es sich um selbst zubereitete oder fertig gekaufte Lebensmittel handelte.

	Ja	Nein	Nicht sicher	selbst zubereitet	gekauft
(1) Speiseeis					
(2) Belegte Brote / Brötchen					
(3) Hähnchen / Hühnerfleisch					
(4) Eier / Eierspeisen (Omelett, Rührei, Pfannkuchen, frische Waffeln)					
(5) Mousse au Chocolat, Tiramisu					
(6) Mayonnaise (selbst zubereitet)					
(7) Brat- oder Grillwürste (Hot dogs etc.)					
(8) Hamburger (Hacksteak etc.)					

	Ja	Nein	Nicht sicher	Selbst zubereitet	gekauft
(9) Salate					
(10) Rohmilch, Vorzugsmilch (nicht pasteurisiert)					
(11) Andere Rohmilchprodukte (z. B. Rohmilchkäse)					
(12) Rohes Fleisch (z.B. Tatar, Mettwurst)					
	Ja	Nein	Nicht sicher	Selbst zubereitet	gekauft
(13) Wurstwaren					
(14) Imbiß aus Straßenverkauf (Fast food)					
(15) Meeresfrüchte * (Muscheln, Schnecken etc.)					

*nähere Bezeichnung: _____

_____

3. Haben Sie in den letzten 3 Tagen an einem Grillfest oder Buffet teilgenommen?

Ja Nein Nicht sicher

ABSCHNITT 2 ALLGEMEINER GESUNDHEITSZUSTAND

4. Haben Sie heute oder in den letzten 3 Tagen irgendeines der folgenden Symptome gehabt? Antworten Sie bitte auf alle Fragen mit **Ja, Nein oder Nicht sicher**

Lesen Sie bitte die Abschnittsüberschriften vor und fragen Sie, ob der Teilnehmer irgendeines dieser Symptome hatte.

Grippe / Erkältungssymptome

	Ja	Nein	Nicht sicher
(1) Fieber (heiß- und kalt, Schüttelfrost)			
(2) Kopfschmerzen			
(3) Schmerzende Beine, Arme, Gelenke			
(4) Rachenentzündung / Halsschmerzen			

Atemwegssymptome

(5) Beschwerden im Brustkorb			
(6) Trockener Husten			
(7) Husten (Abhusten von Schleim)			
(8) Keuchen / Kurzatmigkeit			
(9) Schnupfen			

Ohren- / Augensymptome

	Ja	Nein	Nicht sicher
(10) Ohrinfektionen (Entzündung, Vereiterung)			
(11) Augeninfektionen (rote, entzündete Augen)			
(12) Sehstörungen			

Magen- und Darmsymptome

	Ja	Nein	Nicht sicher
(13) Appetitlosigkeit			
(14) Verdauungsstörungen			
(15) Magenschmerzen (Koliken ,Krämpfe, Bauchschmerzen)			
(16) Lockerer Stuhlgang (weicher als normal)			
(17) Durchfall			
Wenn ja, waren die Durchfälle			
breiartig			
flüssig braun - gelb			
wässerig klar			
blutig			
Wieviel Stuhlgänge traten pro Tag maximal auf?			_____ pro Tag
(18) Brechreiz (Übelkeit / Unwohlsein)			
(19) Erbrechen			
Wie häufig mußten Sie erbrechen?			_____ pro Tag

Hautsymptome

	Ja	Nein	Nicht sicher
(20) Hautausschläge			
(21) Hautgeschwüre / offene Stellen			
(22) Jucken (Hautreizung)			

Andere Symptome

	Ja	Nein	Nicht sicher
(23) Ausgeprägte Müdigkeit (unübliche Müdigkeit, Mattigkeit)			
(24) Benommenheit / Schwindelgefühl			
(25) Stechen / Kribbeln			
(26) Muskelkrämpfe (in Armen-u.Beinen)			
(27) Harnwegsinfektion / Blasenentzündung			

Hatten Sie irgendein anderes Krankheitssymptom?
wenn ja, welches

_____

_____

Keine Symptome in den letzten 3 Tagen

Wenn keine Krankheit vorliegt, so gehen Sie bitte zu Frage 8, sonst fahren Sie bitte mit Frage 5 fort

5. Wann begann die Krankheit, wann hörte sie auf und wie lange dauerte sie ? Bitte auf dem Kalender alle Tage einkreisen, an denen irgendeines dieser Symptome aufgetreten ist.

Monat:_____ Jahr:_____

Mo Di Mi Do Fr Sa

6. Welches war das erste Symptom ? _____
[Tragen Sie bitte die Symptom-Nummer aus Seite 2, 3 und 4 ein.]

7. Haben Sie Ihren Arzt wegen dieser Symptome aufgesucht ?

Ja Nein Nicht sicher

Wenn ja, wurde eine Krankheit diagnostiziert ?

Diagnose: _____

8. Waren Sie seit dem 1. Interview dieser Studie wieder schwimmen oder baden, bzw. haben Sie Wassersport betrieben?

Ja Nein Nicht sicher

wenn ja, war dies

im Schwimmbad (Beckenbad)

im Naturgewässer (Meer, Fluß, See, Baggersee etc.)

bitte genauere Angaben (z.B. Baden, Bootfahren,... Datum und Ort)

_____

9. Nehmen noch weitere Personen Ihres Haushalts an dieser Studie teil?

Wenn ja, notieren Sie bitte die Namen:

1: _____

2: _____

3: _____

10. Möchten Sie noch weitere Informationen anfügen?

_____

Arztbogen - vom untersuchenden Arzt auszufüllen -

Name des Arztes:
_____

	Normal	Gerötet	Belegt
Rachen und Tonsillen			

	ja	Nein
Anhalt auf einen Ohreninfekt ?		
Anhalt auf einen Augeninfekt?		
Anhalt auf einen sonstigen Infekt?		
Temperatur >38°C		

Wenn Ja, bitte kurze schriftliche Angaben machen (Verdachtsdiagnose):

_____

_____

_____

Wurde der medizinische Teil des Fragebogens geprüft?	Ja	Nein
Empfohlen Hausarzt aufzusuchen?	Ja	Nein

Sind Sie für einen Ausschluß des Probanden? Ja

Nein

Annex 44. Questionnaire No. 3:

Interview one week after exposure

Badegewässerstudie des Umweltbundesamtes und des Hygiene-Instituts der Universität Tübingen

anschließende Befragung **Datum:**_____
(1 Woche nach dem Baden)

Name, Vorname des Interviewers:

ABSCHNITT 1 KONSUMIERTE LEBENSMITTEL

1. Name, Vorname des Teilnehmers:

2. Haben Sie seit dem Badetag eines der folgenden Lebensmittel zu sich genommen?

	Ja	Nein	Nicht sicher
(1) Speiseeis			
(2) Belegte Brote / Brötchen (fertig gekauft)			
(3) Hähnchen / Hühnerfleisch			
(4) Eier / Eierspeisen (Omelett, Rührei, Pfannkuchen, frische Waffeln)			
(5) Mousse au Chocolat, Tiramisu			
(6) Mayonnaise (selbst zubereitet)			
(7) Brat- oder Grillwürste (Hot dogs etc.)			
(8) Hamburger (Hacksteak etc.)			
(9) Salate			
(10) Rohmilch / Vorzugsmilch (nicht pasteurisiert)			

	Ja	Nein	Nicht sicher
(11) Andere Rohmilchprodukte (z. B. Rohmilchkäse)			
(12) Rohes Fleisch (z.B. Tatar, Mettwurst)			
(13) Wurstwaren			
(12) Imbiß aus Straßenverkauf (Fast food)			
(13) Meeresfrüchte* (z.B. Muscheln, Schnecken etc..)			

*nähere Bezeichnung: _____

Haben Sie seit dem Badetag an einem Grillfest oder Buffet teilgenommen?

Ja Nein Nicht sicher

ABSCHNITT 2 ALLGEMEINER GESUNDHEITSZUSTAND

4. Überlegen Sie bitte, ob seit dem Badetag eines der folgenden Symptome bei Ihnen aufgetreten ist? Antworten Sie bitte auf alle Fragen bitte mit **JA, NEIN** oder **NICHT SICHER**

Lesen Sie bitte die Abschnittsüberschriften vor und fragen Sie, ob irgendeines dieser Symptome aufgetreten ist. Zeigen Sie bitte den mitgelieferten Kalender, um das Anfangsdatum und die Dauer jeder einzelnen Beschwerde festzustellen.

Grippe / Erkältungssymptome

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen
Fieber (heiß und kalt, Schüttelfrost)				___/___	_____
Kopfschmerzen				___/___	_____
Schmerzende Beine, Arme, Gelenke				___/___	_____
Rachenentzündung / Halsschmerzen				___/___	_____

Atemwegssymptome

Beschwerden im Brustkorb				___/___	_____
Trockener Husten				___/___	_____
Husten (Abhusten von Schleim)				___/___	_____
Keuchen /Kurzatmigkeit				___/___	_____
Schnupfen				___/___	_____

Ohren- / Augensymptome

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen
Ohrinfektionen (Entzündung, Vereiterung)				___/___	_____
Augeninfektionen rote, entzündete Augen				___/___	_____
Sehstörungen				___/___	_____

Darmsymptome

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen
Appetitlosigkeit				___/___	_____
Verdauungsstörungen				___/___	_____
Magenschmerzen (Koliken, Krämpfe, Bauchschmerzen)				___/___	_____
Lockerer Stuhlgang (weicher als normal)				___/___	_____
Durchfall				___/___	_____
Wenn ja, waren die Durchfälle					
breiartig				___/___	_____
flüssig braun - gelb				___/___	_____
wässerig klar				___/___	_____
blutig				___/___	_____
Wieviel Stuhlgänge traten pro Tag maximal auf?				_____ pro Tag	
Brechreiz (Übelkeit, Unwohlsein)				___/___	_____
Erbrechen				___/___	_____
Wie häufig mußten Sie erbrechen?				_____ pro Tag	

Hautsymptome	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen
Hautausschläge				___/___	_____
Hautgeschwüre / offene Stellen				___/___	_____
Jucken (Hautreizung)				___/___	_____
Andere Symptome					
Ausgeprägte Müdigkeit (unübliche Müdigkeit, Mattigkeit)				___/___	_____
Benommenheit / Schwindelgefühl				___/___	_____
Stechen / Kribbeln				___/___	_____
Muskelkrämpfe (in Armen u. Beinen)				___/___	_____
Harnwegsinfektion / Blasenentzüdung				___/___	_____

Ist irgendein Symptom aufgetreten, das nicht auf der Liste steht? Wenn ja, welches:

_____

_____

_____

Wann haben die Symptome angefangen? _____

Wie viele Tage dauerten sie ? _____

Keine Symptome seit dem Badetag festgestellt

Wenn **keine** Krankheit vorliegt, so gehen sie bitte weiter zu Frage 7, sonst fahren Sie bitte mit Frage 5 fort.

5. Haben Sie Ihren Arzt wegen dieser Symptome aufgesucht ?

Ja Nein Nicht sicher

Wenn **Ja**, wurde eine Krankheit diagnostiziert

Ja Nein Keine Angabe

Diagnose _____

6. An wie vielen Tagen konnten Sie wegen dieser Symptome nicht Ihre normale Arbeit / Tätigkeit ausüben?

_____Anzahl der Tage

Wurden Sie deshalb in ein Krankenhaus eingewiesen ?

Ja Nein Keine Angabe

Wenn **Ja**, in welches Krankenhaus?_____

_____

Kontakt mit Naturgewässern ?

7. Waren Sie seit dem Badetag dieser Studie wieder schwimmen oder baden, bzw. haben Sie Wassersport betrieben?

Ja Nein Nicht sicher

wenn ja, war dies

im Schwimmbad (Beckenbad)

im Naturgewässer (Meer, Fluß, See, Baggersee etc.)

bitte genauere Angaben (z. B. Baden, Bootfahren,... Datum, Ort, Name des Freibads, des Hallenbads, des Strandbads, des Sees, etc.):

_____

_____

Arztbogen - vom untersuchenden Arzt auszufüllen -

Name des Arztes:
_____

	Normal	Gerötet	Belegt
Rachen und Tonsillen			

	ja	Nein
Anhalt auf einen Ohreninfekt ?		
Anhalt auf einen Augeninfekt?		
Anhalt auf einen sonstigen Infekt?		
Temperatur >38°C		

Wenn Ja, bitte kurze schriftliche Angaben machen (Verdachtsdiagnose):

_____

_____

_____

	Ja	Nein
Empfohlen Hausarzt aufzusuchen?		

Annex 45. Questionnaire No. 4:

Interview three weeks after exposure

Badegewässerstudie des Umweltbundesamtes und des Hygiene-Instituts der Universität Tübingen

Fragebogen 3 Wochen nach dem Badetag

Bitte lesen Sie diesen Fragebogen durch und beantworten Sie sorgfältig die Fragen.
Bitte beantworten Sie jede Frage entweder durch einkreisen der Antwort oder durch ankreuzen eines Kästchens, oder beantworten Sie die Frage schriftlich.
Beispiele:

MONAT: August/September **Jahr: 2001**

Mo	Di	Mi	Do	Fr	Sa	So
					18	19
20	21	22	23	24	25	26
27	28	29	30	31	1	2
3	4	5	6	7	8	9
10	11	12				

JA (X) NEIN ( )

Beginn	Dauer
01/09	5 Tage

Angaben zur Person:

1. Name:
_____

2. Geburtsdatum:
_____/_____/_____
Tag Monat Jahr

3. Geschlecht:
männl. weibl.

4. Anschrift:

Straße: _____

PLZ /Ort: _____

Telefon: _____

5. Telefon am Arbeitsplatz:
_____

6. In der nächsten Zeit zu erreichen unter (Adresse in den nächsten 3 Monaten)

_____

7. Hatten Sie **in den letzten drei Wochen (seit dem Badetag)** eines der folgenden Symptome? Bitte antworten Sie auf alle Fragen mit Ja, Nein oder Nicht sicher. Wenn Sie mit **Ja** auf eines der Symptome antworten, so geben Sie bitte das **Datum** an, an dem das Symptom anfing, die **Dauer** der Beschwerden und ob Sie unter diesem **Symptom häufiger** (öfter als 1 mal pro Jahr) leiden.

Grippe / Erkältungsanzeichen

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer (Tage)	Häufigkeit: Häufig (>1/Jahr)	selten	Nicht sicher
Fieber (heiß u. kalt, Schüttelfrost)				___/___	___			
Kopfschmerzen				___/___	___			
Schmerzende Beine, Arme, Gelenke				___/___	___			
Rachenentzündung / Halsschmerzen				___/___	___			

Atemwegssymptome

Beschwerden im Brustkorb				___/___	___			
Trockener Husten				___/___	___			
Husten mit Abhusten von Schleim				___/___	___			
Keuchen / Kurzatmigkeit				___/___	___			
Schnupfen				___/___	___			

Ohren-/ Augensymptome

Ohreninfektion (Entzündung, Vereiterung)				___/___	___			
Augeninfektion (rote, entzündete Augen)				___/___	___			
Sehstörungen				___/___	___			

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen	Häufigkeit Häufig (>1/Jahr)	selten	Nicht sicher
Magen- / Darmsymptome								
Appetitlosigkeit				___/___	_____			
Verdauungsstörung				___/___	_____			
Magenschmerzen (Koliken, Krämpfe, Bauchschmerzen)				___/___	_____			
Lockerer Stuhlgang (weicher als normal)				___/___	_____			
Durchfall				___/___	_____			
Wenn ja, waren die Durchfälle:								
breiartig braun - gelb				___/___	_____			
flüssig braun - gelb				___/___	_____			
wässerig klar				___/___	_____			
blutig				___/___	_____			

Wieviel Stuhlgänge traten pro Tag maximal auf? _____ pro Tag

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen	Häufigkeit Häufig (>1/Jahr)	selten	Nicht sicher
Brechreiz (Übelkeit / unwohlsein)				___/___	_____			
Erbrechen				___/___	_____			

Wie häufig mußten Sie erbrechen? _____ pro Tag

	Ja	Nein	Nicht sicher	Beginn Datum	Dauer in Tagen	Häufigkeit Häufig (>1/Jahr)	selten	Nicht sicher
Hautsymptome								
Hautausschläge				___/___	_____			
Hautgeschwüre /offene Stellen				___/___	_____			
Jucken (Hautreizung)				___/___	_____			
Andere Symptome								
	Ja	Nein	Nicht sicher	Beginn Datum	Dauer (Tage)	Häufigkeit Häufig (>1/Jahr)	selten	Nicht sicher
Ausgeprägte Müdigkeit (unübliche Müdigkeit, Mattigkeit)				___/___	_____			
Benommenheit /Schwindelgefühl				___/___	_____			
Stechen / Kribbeln				___/___	_____			
Muskelkrämpfe (in Armen und Beinen)				___/___	_____			
Harnwegsinfektion / Blasenentzündung				___/___	_____			
Pilzinfektion				___/___	_____			

Wenn Sie irgendein Symptom haben, das nicht auf der Liste steht, so schreiben Sie es bitte in der nachfolgenden Spalte auf.

	Beginn Datum	Dauer (Tage)	Häufigkeit Häufig (>1/Jahr)	selten	Nicht sicher
_____	___/___	_____			
_____	___/___	_____			
_____	___/___	_____			
_____	___/___	_____			

Wenn Sie auf eines der Symptome mit **Ja** geantwortet haben,
so fahren Sie bitte mit **Frage 8** fort.

Wenn Sie keines dieser Symptome hatten, so gehen Sie bitte zu Frage 16..

8. Waren diese Symptome Teil einer einzigen Erkrankung ?

Ja (einzige Erkrankung)

Nein (ich hatte mehrere Erkrankungen gleichzeitig)

Keine wirkliche Erkrankung (ich fühlte mich nicht krank)

Unsicher

9. Kreisen Sie bitte auf dem unteren Kalender alle Tage ein, an denen Sie sich unwohl fühlten oder irgendeines dieser Symptome hatten.

August/September 2001

Mo	**Di**	**Mi**	**Do**	**Fr**	**Sa**	**So**
					18	19
20	21	22	23	24	25	26
		27	28	29	30	31
1	2	3	4	5	6	7
8						

10. Welches war das erste Symptom ?

_____

11. Wenn Sie mehrere Erkrankungen gleichzeitig hatten, so machen Sie bitte unten genauere Angaben dazu, besonders wichtig ist das Anfangsdatum der Krankheit.

_____

_____

_____

_____

12. Wurde die Erkrankung von Ihrem Arzt festgestellt ?

Ja Nein Nicht sicher

Wenn JA , wie lautete die Diagnose ?

_____

Bitte kreuzen Sie an, ob wir Ihren Arzt, falls wir mehr Informationen benötigen, fragen können. Falls ja, geben Sie bitte Name und Adresse an.

Ja Nein Keine Angabe

Name und Adresse des Arztes:

_____

_____

Hiermit entbinde ich meinen oben genannten Arzt bezüglich der oben genannten Erkrankungen von der Schweigepflicht gegenüber dem Projektleiter der Badegewässerstudie (Herr Dr. med. A. Wiedenmann).

Datum:_____

Unterschrift:_____

13. Wurden Ihnen auf Grund der Erkrankung Medikamente verschrieben?

Ja Nein Keine Angabe

Wenn **JA** welche, bitte notieren:

1._____

2. _____

3. _____

14. Mußten Sie wegen einer der Erkrankungen seit dem Badetag ins Krankenhaus ?

Ja Nein Keine Angabe

Wenn **JA,** wegen welcher Erkrankung und in welches Krankenhaus ?

_____

_____

15. Wie viele Tage konnten Sie wegen dieser Erkrankung Ihre Arbeit oder eine andere Tätigkeit nicht ausüben ?

Keinen | Nur einen Tag | 2 - 7 Tage | 8 - 14 Tage | Mehr als 14 Tage | Keine Angabe

16. Sind Sie jemals nach dem Baden in einem Gewässer in Deutschland krank geworden. ?

Ja Nein Nicht sicher

Wenn **JA**, welche der nachfolgenden Krankheiten waren es ?
(Sie können mehrere ankreuzen)

Kopfschmerzen	Zahnschmerzen	Ohrenschmerzen
Durchfall	Erbrechen	Fieber
Erkältungen	Rachenentzündung	Augenbeschwerden
Anderes*	Keine Angabe	

*Bitte genauere Angaben

_____

17. Sind Sie jemals an einen Badestrand gegangen, obwohl Sie sich schon krank gefühlt haben ?

Ja Nein Nicht sicher

Wenn **JA**, welche der nachfolgenden Krankheiten waren es ?
(Sie können mehrere ankreuzen)
Wenn **NEIN,** fahren Sie bitte mit Frage 18 fort

Kopfschmerzen	Zahnschmerzen	Ohrenschmerzen
Durchfall	Erbrechen	Fieber
Erkältungen	Rachenentzündung	Augenbeschwerden
Anderes*	Keine Angabe	

*Bitte genauere Angaben

_____

Hat Sie das davon abgehalten, ins Wasser zu gehen ?

Ja Nein Nicht sicher

18. Wie oft bekommen Sie beim Baden einen Sonnenbrand?

Immer Häufig Selten Nie Nicht sicher

19. Wie oft behandeln Sie Ihren Sonnenbrand anschließend (Cremes, Hausmittel etc.) ?

Immer Häufig Selten Nie Nicht sicher

20. Wird Ihnen vom Autofahren, Busfahren oder Bahnfahren leicht übel ?

Immer Häufig Selten Nie Nicht sicher

21. Hat sich eine Person, die mit Ihnen in Ihrem Haushalt lebt, während der letzten 3 Wochen unwohl gefühlt oder war eine Person in Ihrem Haushalt krank?
(Der Haushalt schließt nur Personen ein, mit denen Sie zusammenleben und mit denen Sie Einrichtungen wie z.B. Küche, Bad und Toilette teilen.)

Ja Nein Nicht sicher

Wenn **NEIN** (keine neu aufgetretene Erkrankung in den letzten 3 Wochen in Ihrem Haushalt), fahren Sie bitte mit Frage 24 fort.

22. Wenn **JA**, hat diese Erkrankung **vor** Ihrer eigenen Erkrankung begonnen?

Ja Nein Nicht zutreffend

23. Bitte machen Sie nachfolgend genaue Angaben zu den Erkrankungen Ihrer Haushalts-Mitglieder:
Je nach Erkrankung schreiben Sie:

D	für	Durchfall
Ü	für	Übelkeit
E	für	Erbrechen
O	für	Ohreninfektion (z.B. Ohrenschmerzen)
A	für	Augeninfektion (z.B. entzündete, rote Augen)
F	für	Fieber oder erhöhte Temperatur
R	für	Rachenentzündung
S	für	sonstige Symptome

z.B.: wenn ein Kind Durchfall, Erbrechen und Fieber hatte,
müssen Sie als Krankheitsbeschreibung folgendes schreiben: **D, E, F**

Initialen des Erkrankten	Alter	Krankheits-bezeichnung	Krankheits-beginn	Nahm diese Person ebenfalls an der Studie teil?	
				Ja	Nein
				Ja	Nein
				Ja	Nein
				Ja	Nein
				Ja	Nein

Fortsetzung der Frage 23

Bitte notieren Sie alle Details, wie zum Beispiel die vermutete Krankheitsursache, Besonderheiten oder andere Informationen zur Krankheit bzw. den Symptomen:

_____

_____

24. Haben Sie seit dem Badetag außerhalb Ihres Haushaltes übernachtet ? (z.B. in den Ferien / Dienstreisen / Besuche von Verwandten und Bekannten)

Ja Nein Nicht sicher

Wenn **JA**, war es in Deutschland oder im Ausland?

Deutschland Ausland Beides

Bitte geben Sie unten das Datum und die Orte –möglichst mit Postleitzahl- an, die Sie besucht haben:

	Ort	Datum	Dauer
1.)			
2.)			
3.)			

25. Waren Sie seit dem Badetag wieder Schwimmen?
(Nur **Beckenbäder** mit **gechlortem Wasser**)

Ja Nein Nicht sicher

Wenn NEIN, gehen Sie bitte zu Frage 26 weiter.

Wenn **JA** beantworten Sie bitte die nachfolgenden Fragen durch Ankreuzen der entsprechenden Kästchen.

	Ja	Nein	Nicht sicher	Wie oft, seit dem Badetag
In öffentliche Schwimmbädern?				_____
In anderen Schwimmbädern?				_____

26. Waren Sie seit dem Badetag im **Meer**, in einem **See** oder in einem **Fluß** schwimmen?

Ja Nein Nicht sicher wie oft?

_____

Wenn **JA**, notieren Sie bitte wo genau Sie schwimmen waren (z.B. auch welches Standbad), kreuzen Sie an, ob in Deutschland oder im Ausland und geben Sie möglichst das genaue Datum an.

	Ort -möglichst mit Postleitzahl-	Deutschland	Ausland	Datum
1.				
2.				
3.				
4.				

27. Haben Sie seit dem Badetag Strände besucht, ohne ins Wasser zu gehen ?

Ja Nein Nicht sicher wie oft?

_____

Wenn **JA**, schreiben Sie bitte jeden Strand auf den Sie besucht haben, kreuzen Sie an, ob in Deutschland oder im Ausland, und geben Sie möglichst das genaue Datum an.

	Ort –möglichst mit Postleitzahl-	Deutschland	Ausland	Datum
1.				
2.				
3.				
4.				

28. Wenn Sie einen Badestrand besuchen, gehen Sie dann auch ins Wasser *?*

Meistens Manchmal Selten Nie Nicht sicher

29. Wie oft tauchen Sie Ihren Kopf beim Baden unter Wasser ?

Meistens Manchmal Selten Nie Nicht sicher

30. Haben Sie seit dem Badetag irgendeinen Wassersport betrieben?
Kreuzen Sie bitte bei jeder der nachfolgenden Wassersportarten
JA, **NEIN** oder **NICHT SICHER** an.

	Ja	Nein	Nicht sicher
(1) Schlauchboot / Ruderboot / Kajakfahren			
(2) Schnellboot- / Motorbootfahren			
(3) Tauchen / Schnorcheln			
(4) Surfen / Wasserski / Jetski / Segeln usw.			
(5) Angeln			
(6) Planschen / Wassertreten			
(7) Anderes *			

*Genauere Angaben anderer Wassersportarten

_____

_____

31. Betrachten Sie Wassersport (bzw. Sportliche Aktivitäten im Wasser) als gefährlich ?

Ja Nein Nicht sicher

Wenn **JA**, welche der folgenden Aktivitäten betrachten Sie als gefährlich?
(Sie können mehr als eine ankreuzen)

Schlauchboot fahren | Kajak fahren | Windsurfen / Segeln | Tauchen / Schnorcheln | Wasserski

Surfen | Schwimmen / Baden | Anderes * | Nicht sicher

*Bitte genaue Angaben

_____

_____

32. Waren Sie seit dem Badetag in einem Vergnügungs- / Freizeitpark **und** haben Sie dort irgendwelche Wasseranlagen benutzt? (z.B. Wasserrutschen, Wasserfontänen, Rafting)

Ja Nein Nicht sicher

Wenn **JA**, war die Anlage im Inland oder im Ausland?

Inland Ausland

Bitte geben Sie den Namen der Anlage an:

_____

33. Haben Sie schon mal etwas darüber gehört wie die Badegewässer und Badestrände in Deutschland gepflegt und kontrolliert werden ?

Ja Nein Nicht sicher

Wenn NEIN gehen Sie bitte zu Frage 34 weiter.

Wenn **JA,** war diese Information eher positiv oder negativ ?

Positiv Negativ Nicht sicher

Wenn **NEGATIV,** hat Sie das beunruhigt ?

gar nicht etwas sehr nicht sicher

34. Haben Sie schon irgend etwas bezüglich der Sauberkeit von Badegewässern in Deutschland gehört ?

Ja Nein Nicht sicher

Wenn **NEIN,** gehen Sie bitte zu **Frage 35**
Wenn **JA,** war diese Information positiv oder negativ?

Positiv Negativ

Wenn **NEGATIV**, von welchen speziellen Problemen haben Sie gehört ?
(Sie können auch mehrere Kästchen ankreuzen)

Öl-Lache Treibgut Gesundheitsrisiko

Verschmutzung durch Chemikalien | Verschmutzung durch Abwasser | Anderes * | Nicht sicher

* Bitte genauere Angaben:

35. Haben Sie es jemals abgelehnt aus einem der folgenden Gründe an einer öffentlichen Badestelle baden zu gehen?

Strand zu dreckig		Wasser zu dreckig		zu starke Brandung / zu hoher Wellengang		Furcht vor Krankheiten		Nicht sicher
ja	nein	ja	nein	ja	nein	ja	nein	

36. Wodurch haben Sie zum ersten Mal von dieser Studie gehört?
(Bitte nur ein Kästchen ankreuzen).

Durch den Partner Freunde/ Bekannte | durch einen Anwerber | aus dem Fernsehen | aus einer Zeitung | Anderes* | Nicht sicher

*Bitte genauere Angaben:
_____

37. Haben Sie in den Medien irgendwelche Nachrichten und / oder Berichterstattungen über diese Studie gesehen?

Ja Nein Nicht sicher

38. Sind Sie Mitglied einer Umweltorganisation?

Ja Nein Keine Angabe

39. Welche Tageszeitung lesen Sie ? Wenn keine, schreiben Sie bitte KEINE

_____

40. **Kommentare:** Bitte schreiben Sie hier alles, wovon Sie denken, daß es für unsere Studie nützlich sein könnte

_____

_____

Unterschrift:_____

Ausgefüllt am Datum: _____/_____/_____/_

Vielen Dank, daß Sie sich die Zeit genommen haben, diesen Fragebogen auszufüllen.
Und vielen Dank für Ihre Teilnahme an der Badegewässerstudie!
Bitte schicken Sie den vollständig ausgefüllten Fragebogen so bald wie möglich in dem beigefügten Briefumschlag zurück.

Adresse:

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72074 Tübingen
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Annex 46. Retrospective molecular analysis of frozen samples for *Escherichia coli* and intestinal enterococci by quantitative real time PCR

Materials and methods

Sample preservation and storage

Four 45 ml aliquots of all water samples collected during the epidemiological trials were mixed each with 5 ml of glycerol and frozen at -20°C on the sampling day. These 50 ml samples were retrospectively analysed for *Escherichia coli* and intestinal enterococci by quantitative real time PCR (LightCyclerTM-PCR). Samples were stored at -20°C for a maximum of 31 months before DNA extraction. Extracted DNA was stored at -72°C for a maximum of 8 months before amplification.

Separation of bacterial cells from water samples

Two 45 ml aliquots of each of the original water samples were thawed and filtered through a 0.4 µm membrane filter (Type GTTP, Millipore).

Cell lysis and DNA extraction

The membrane was folded up and placed into a 1.5 ml micro-centrifuge tube. 300 ml of 0,2 M EDTA, pH 8.0, were added; the tube was vortexed, frozen in liquid nitrogen and subsequently thawed and boiled in a dry-block. The freeze-boil procedure was repeated five times. 300 ml binding buffer und 40 µl proteinase K were added to give a total volume of 600 µl. The reaction mix was incubated at 72°C for 10 minutes. The filter membrane and the 600 µl of reaction mix were placed onto the membrane of the filter unit of a Spin-X^{TM} micro-centrifuge tube filter (Costar). The tube was centrifuged for one minute at 5000g. DNA was extracted from the 600 µl of filtrate using a commecially available DNA extraction kit (High Pure PCR Template Kit; Roche). The final extraction volume was 50 µl, which is equivalent to 90 ml of the original water sample.

Sample volume equivalents used for PCR detection

Aliquots of 1 µl, equivalent to 1.8 ml of the original water samples, were used in the PCRs to detect *Eschericha coli* (EC), and aliquots of 5 µl, equivalent to 9 ml of the original water samples, were used in the PCRs to detect intestinal enterococci (IE). Thus the theoretical PCR detection limits were 56 EC/100ml and 11 IE/100ml, and 2 bacterial cells in a reaction mix would approximately correspond with the NOAELs of ca. 100 EC/100ml and 25 IE/100ml. It was considered that if quantification by PCR would fail, this might open up the chance to compare qualitative PCR results with NOAELs so that concentrations below NOAELs would correspond to negative PCR results and concentrations above NOAELs would correspond to positive PCR results.

PCR for *Escherichia coli*

Primer sequences (P1 = 5'-ATC ACC GTG GTG ACG CAT GTC GC-3' and P2= 5'-CAC CAC GAT GCC ATG TTC ATC TGC-3') were derived from the uidA gene of E. coli, encoding beta-glucuronidase, and were used to amplify a 486-bp fragment (Juck et al., 1996). Hybridization probes (EC1FL = 5'-CAT CCG GTC AGT GGC AGT--FL-3' and EC1LC = 5'-LC Red640--AAG GGC GAA CAG TTC CTG A--PH-3') were synthesised by TIB MOLBIOL, Berlin. The lightCycler protocol consisted of 4 programs. Program 1 (denaturation): Segment 1 (Temperature Target 95°C, Hold Time 600 sec, Slope 20°C/sec, Acquisition Mode: None). Program 2 (amplification): Segment 1 (Temperature Target 95°C, Hold Time 1 sec, Slope 20°C/sec, Acquisition Mode: None); Segment 2 (Temperature Target 55°C, Hold Time 10 sec, Slope 20°C/sec, Acquisition Mode: Single); Segment 3 (Temperature Target 72°C, Hold Time 12 sec, Slope 20°C/sec, Acquisition Mode: None). Program 3 (melting curve): Segment 1 (Temperature Target 95°C, Hold Time 0 sec, Slope 20°C/sec, Acquisition Mode: None); Segment 2 (Temperature Target 45°C, Hold Time 15 sec, Slope 20°C/sec, Acquisition Mode: None); Segment 3 (Temperature Target 90°C, Hold Time 0 sec, Slope 0.1°C/sec, Acquisition Mode: Continuous). Program 4 (cooling): Segment 1 (Temperature Target 40°C, Hold Time 30 sec, Slope 20°C/sec, Acquisition Mode: None). Quantification settings: F2/F1; Baseline Adjustment: Arithmetic; Analysis: Second Derivative Maximum. LightCycler capillaries containing 1, 10, 50, 100, 200,

500, 1000 and 10000 HPLC purified target molecules were purchased from Roboscreen, Germany, and used as quantification standards. Negative and positive extraction controls and negative reagent controls were applied with each LightCycler run. If any of the controls yielded an inappropriate result the complete run was excluded from analysis. This was the case in 1 of 21 PCR runs.

PCR for intestinal enterococci

PCR for intestinal enterococci was performed using a commercially available kit (LightCycler *Enterococcus* Kit M^{GRADE}; Cat. No. 3 375 048; Roche). The LightCycler settings were done according to the maufacturers instructions. Primer and probes sequences are not disclosed by the manufacturer. The kit detects *E. faecium* and *E. faecalis* online with specific LC Red640 and fluorescein labelled hybridisation probes. *E. durans*, *E. hirae* and *E. mundtii* are also detected. These species, however, have lower melting points than *E. faecium* and *E. faecalis* and can only be qualitatively detected by melting curve analysis. Quantification standards for *E. faecium* and *E. faecalis* are not included in the kit. They were produced by extraction of the DNA from a known number of colony forming units of *E. faecium*. The kit includes a synthetic internal control sequence which is spiked at a low concentration to each sample. The sequence is amplified by the primers and detected by a separate set of hybridisation probes in a different detection channel. If no signal can be detected in the target channel and no signal can be detected for the internal control then the PCR result can be considered to be invalid (inhibition of PCR reaction). Negative and positive extraction controls, negative reagent controls, and a positive control supplied with the kit were applied with each LightCycler run. If any of the controls yielded an inappropriate result the complete run was excluded from analysis. This was the case in 6 of 21 PCR runs. In addition, if the PCR result was negative and the result for the internal control was negative, too, the reaction was considered to be invalid and excluded from analysis. This was the case in 24 of 304 reactions.

Results

The results of the retrospective analyses of frozen sample aliquots by quantitative real time PCR for *Escherichia coli* and intestinal enterococci were disappointing. While nearly perfect standard curves could be achieved for given concentrations of target DNA, the quantitative results of the sample analyses did not correlate with the culture results. Moreover, the samples from certain locations seemed to contain inhibitory substances which could not be effectively removed by the applied DNA extraction procedure, and obviously caused large numbers of false negative results, although the internal control in the Enterococcus Kit indicated a failure of amplification in only 8% (24/304) of the samples.

Conclusions

The PCR-based quantitative molecular detection methods which were evaluated in this project cannot be considered to be a promising alternative to culture methods. However, the molecular analyses may have been hampered by the storage conditions (up to 31 months at -20°C for water samples and up to 10 months at -72°C for extracted DNA) and by the lower sample volumes which were applied in the PCRs (effective volumes: 1.8 ml for *Escherichia coli*; and 9 ml for intestinal enterococci).

In addition, PCR for intestinal enterococci did not detect the same species as the culture method. Quantification was restricted to *E. faecium* and *E. faecalis*, and qualitative results were restricted to *E. faecium*, *E. faecalis, E. durans, E. mundtii* and *E. hirae.*

In contrast to the standards used for E. coli detection, which were quantified by HPLC, the standards for intestinal enterococci were not absolute; they incorporated the potential losses of DNA which may occur during the extraction procedure. This may be an explanation for the lower detection limit for intestinal enterococci than for *E. coli* which was observed in comparison with the culture results.

For future epidemiological studies it is recommended to extract DNA from larger water sample volumes immediately after sample collection and to store extracted DNA at -70°C or in liquid nitrogen instead of storing water aliquots.

Literature

Juck, D., J. Ingram, M. Prevost, J. Coallier, and C. Greer. 1996. Nested PCR protocol for the rapid detection of Escherichia coli in potable water. Can. J. Microbiol. 42:862-866.

Acknowledgements

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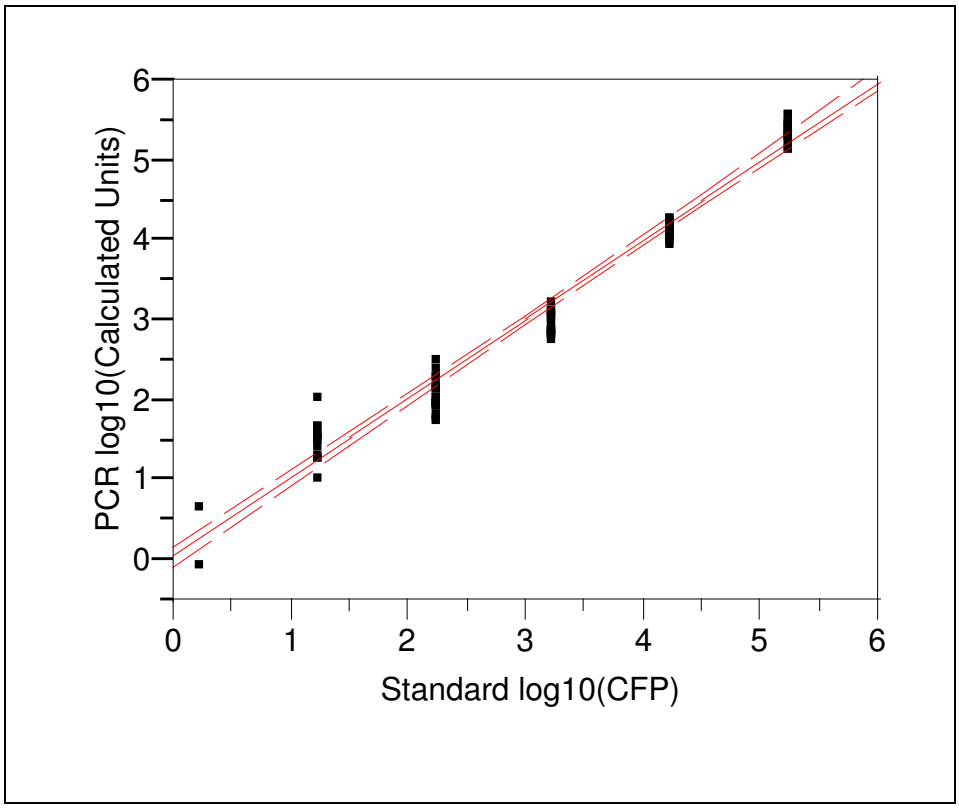


Linear Fit (x to y) and 95% Confidence Curves

R^2 = 0.97

n = 83

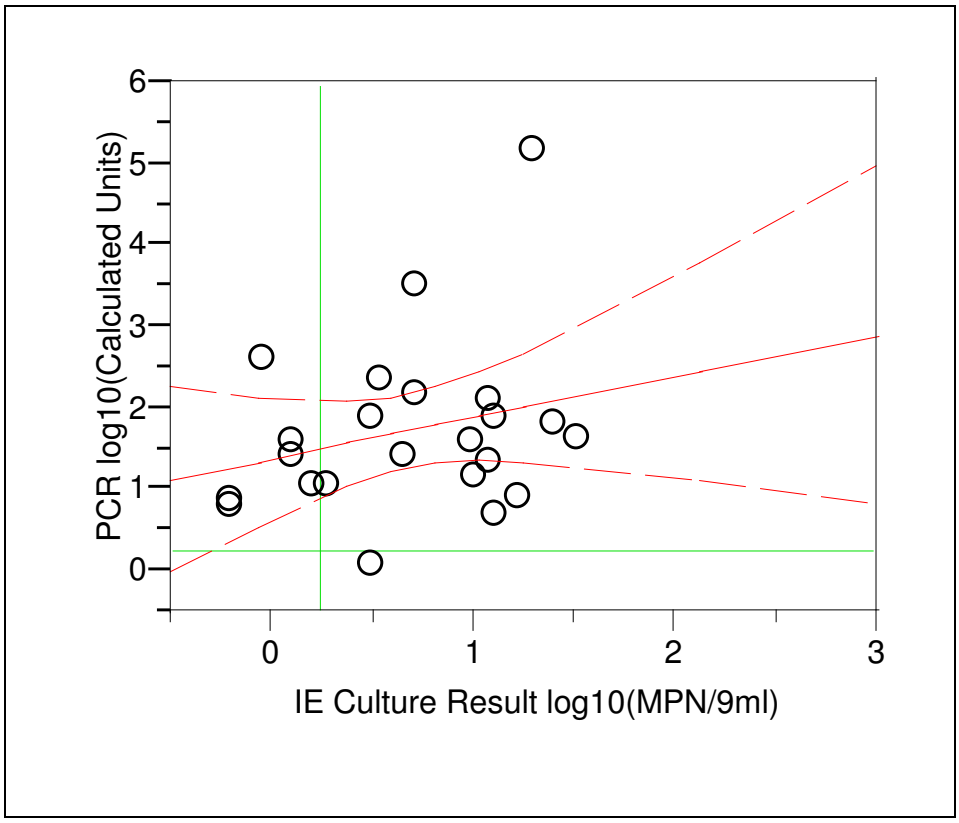
Combined results for the quantification standards in 15 runs of quantitative real time PCR for intestinal enterococci (IE)

Note: All individual R^2 values were >0.90.

Negative PCR results were excluded.

The standard was extracted from an equivalent number

of colony forming particles (CFP) of *Enterococcus faecium.*



Linear Fit (x to y) and 95% Confidence Curves

R^2 = 0.06

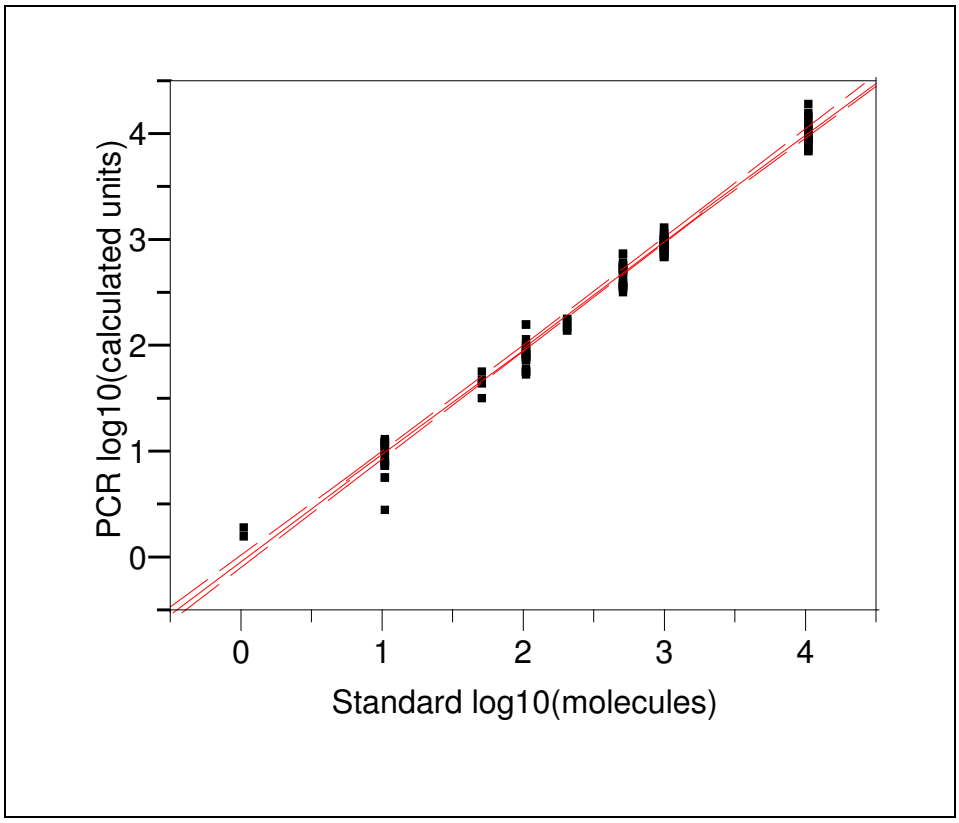
n = 23

NOAEL for culture results (ca. 25 IE/100ml)

Comparison of culture results (ISO 7899-1; MUD-hydrolysis) and quantitative real time PCR for intestinal enterococci (IE) in 23 bathing water samples

Note: PCR only detects *E. faecium and E. faecalis*

Negative PCR results were excluded.



Linear Fit (x to y) and 95% Confidence Curves

R^2 = 0.98

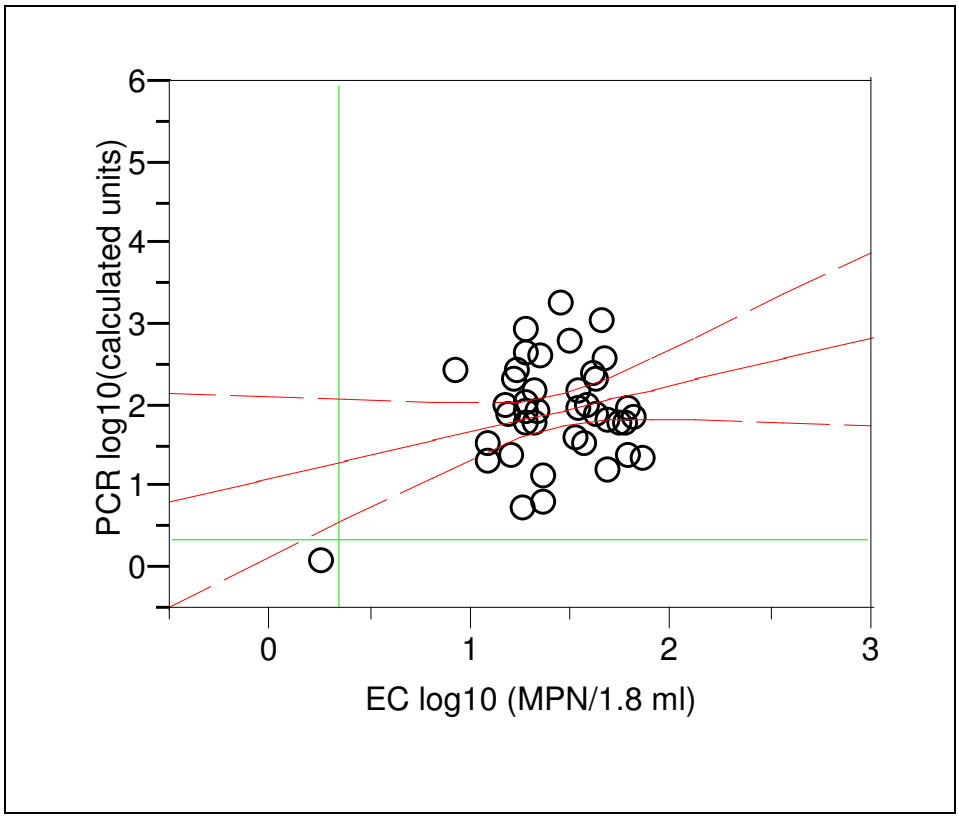
n = 115

Combined results for the quantification standards in 20 runs of quantitative real time PCR for *Escherichia coli* (MUG gene)

Note: All individual R^2 values were >0.90.

Negative PCR results were excluded.

Molecules were quantified by HPLC (Roboscreen, Germany).



Linear Fit (x to y) and 95% Confidence Curves

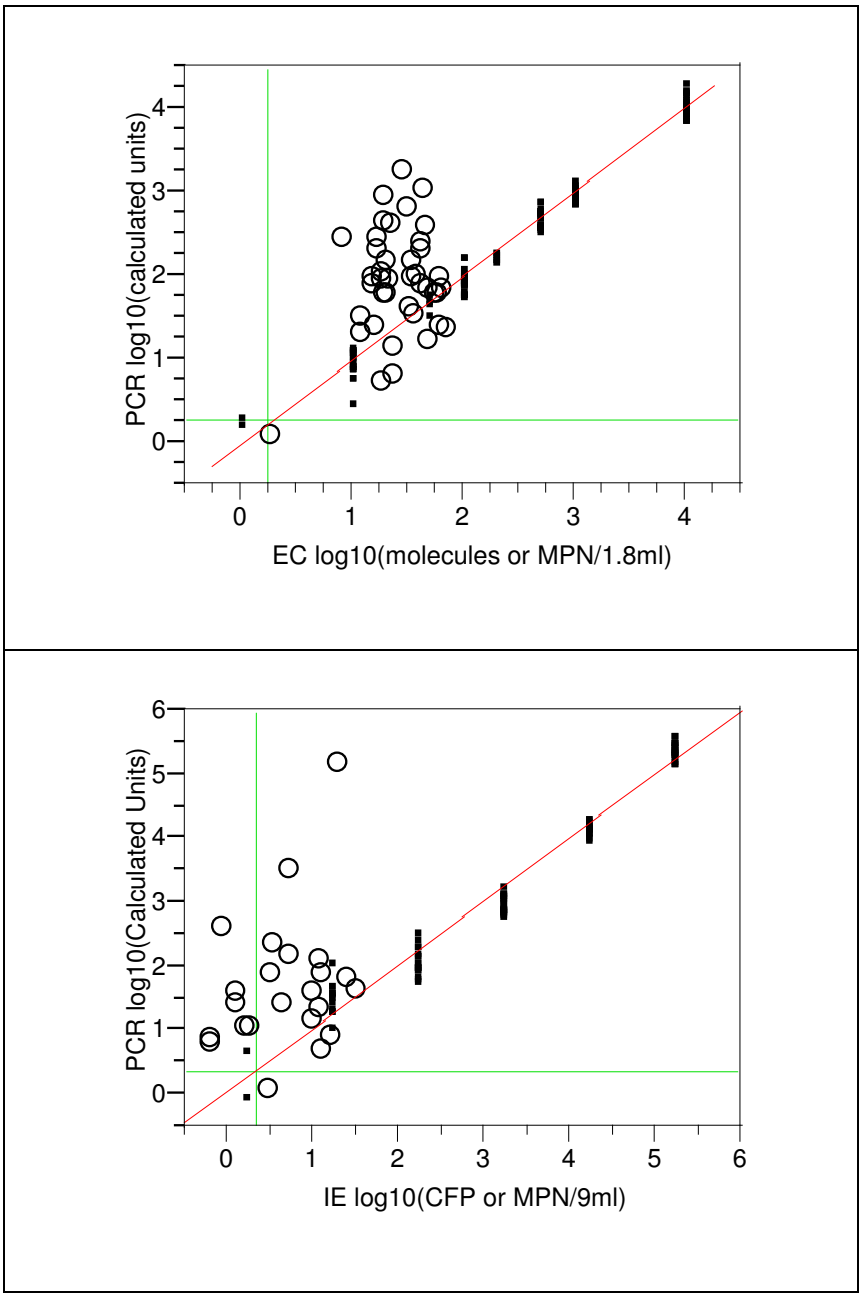
R^2 = 0.07

n = 41

NOAEL for culture results (ca. 100 IE/100ml)

Comparison of culture results (ISO 9308-3; MUG-hydrolysis) and quantitative real time PCR (MUG-gene) for Escherichia coli (EC) in 41 bathing water samples

Note: Negative PCR results were excluded.

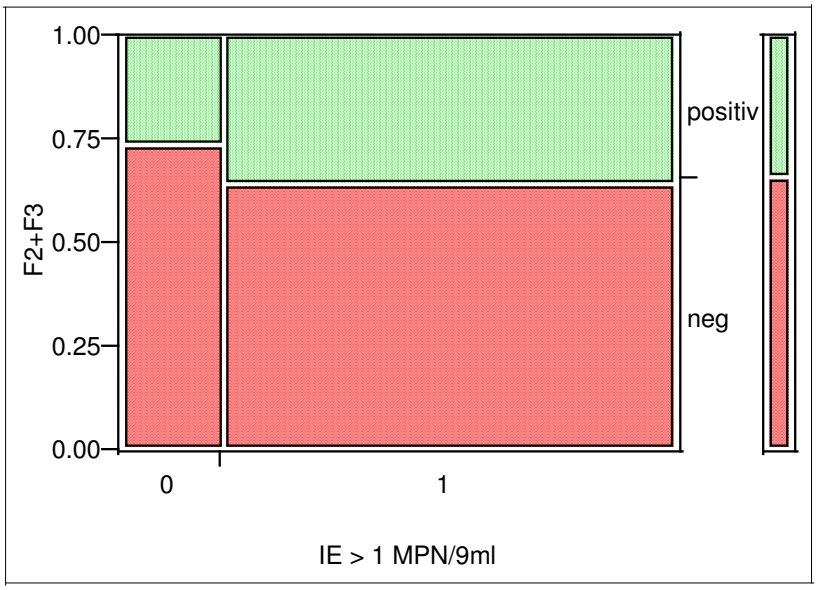


Comparison of quantitative real time PCR results and culture results for Escherichia coli (EC) and intestinal enterococci (IE)

o = water samples **:** = quantification standards

red line = linear fit for quantification standards (x to y)

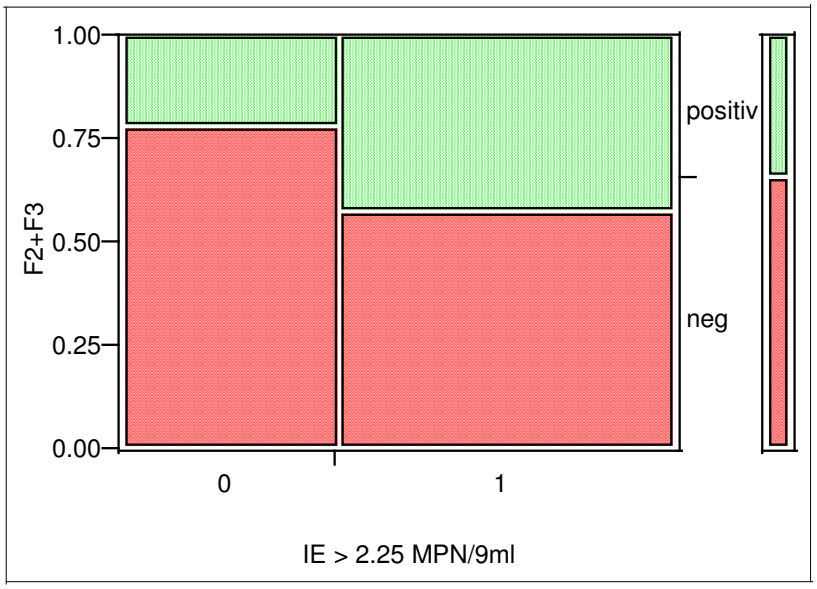
green lines = NOAELs for culture results (ca. 100 EC/100 ml and ca. 25 IE/100ml)



Culture result	PCR negative	PCR positive	Total
≤ 1MPN/9ml	36	13	49
> 1 MPN/9ml	138	78	216
Total	174	91	265

Comparison of culture results (ISO 7899-1; MUD-hydrolysis)
and qualitative real time PCR results for intestinal enterococci (IE)
at a cut-off level of 1 MPN/9ml
(= theoretical PCR detection limit; = ca. 11 MPN/100ml)

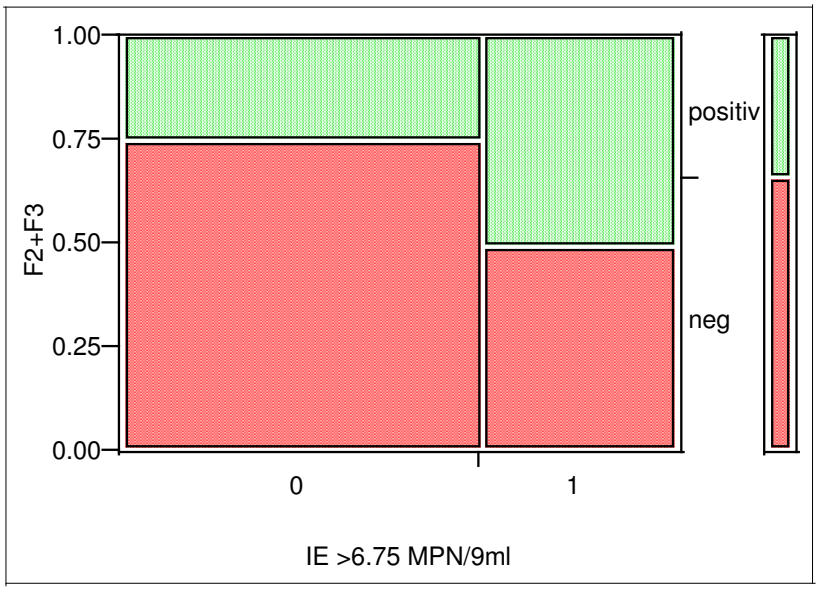
Note: PCR detects *E. faecium*, *E. faecalis, E. durans, E. mundtii and E. hirae.*
Discrepant results are underlined.



Culture result	PCR negative	PCR positive	Total
≤ 2.25 MPN/9ml	81	23	104
> 2.25 MPN/9ml	93	68	161
Total	174	91	265

Comparison of culture results (ISO 7899-1; MUD-hydrolysis)
and qualitative real time PCR results for intestinal enterococci (IE)
at a cut-off level of 2.25 MPN/9ml
(= NOAEL; = 25 MPN/100ml)

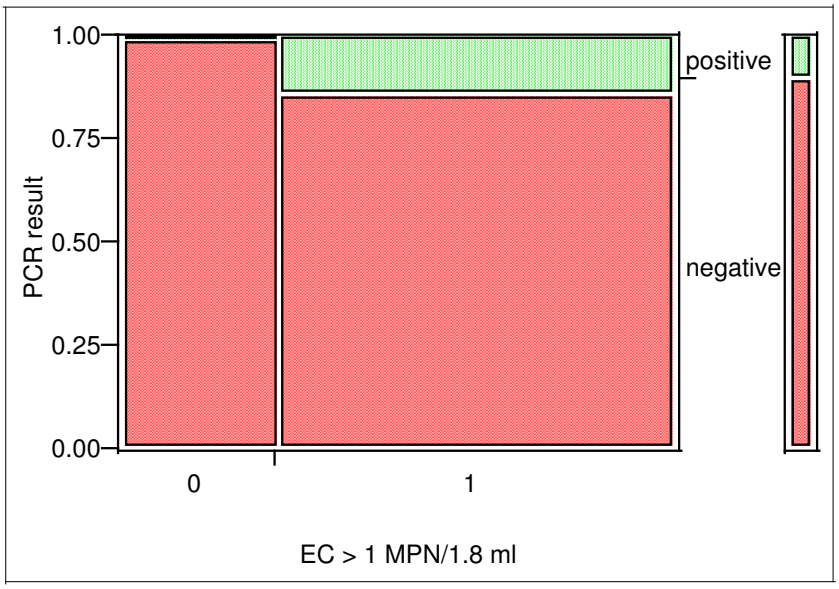
Note: PCR detects *E. faecium*, *E. faecalis, E. durans, E. mundtii and E. hirae.*
Discrepant results are underlined.



Culture result	PCR negative	PCR positive	Total
≤ 6.75 MPN/9ml	129	44	173
> 6.75 MPN/9ml	45	47	92
Total	174	91	265

Comparison of culture results (ISO 7899-1; MUD-hydrolysis)
and qualitative real time PCR results for intestinal enterococci (IE)
at a cut-off level of 6.75 MPN/9ml
(= level with equal numbers of discrepant results; = 75 MPN/100ml)

Note: PCR detects *E. faecium*, *E. faecalis, E. durans, E. mundtii and E. hirae.*
Discrepant results are underlined.

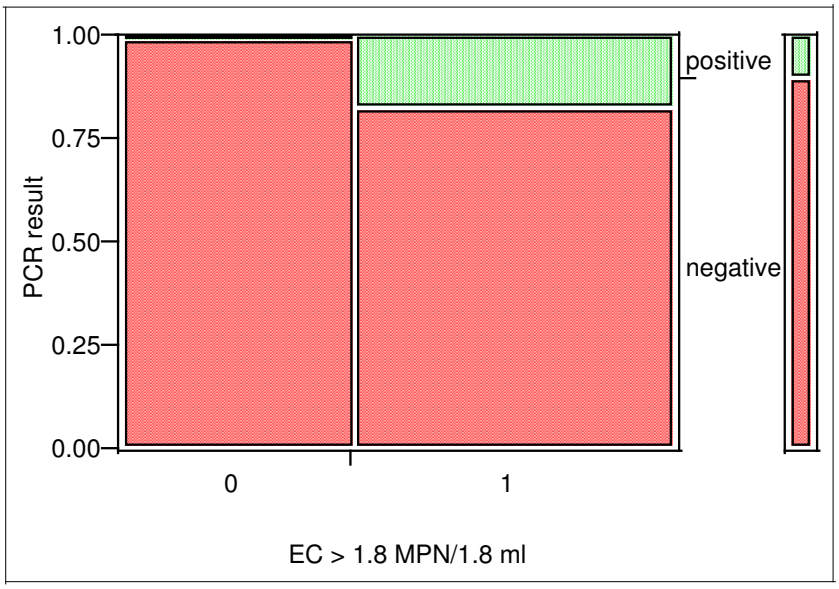


Culture result	PCR negative	PCR positive	Total
≤ 1 MPN/1.8ml	113	1	114
> 1 MPN/1.8ml	245	41	286
Total	358	42	400

Comparison of culture results (ISO 9308-3; MUG-hydrolysis) and qualitative real time PCR results for *Escherichia coli* (EC) at a cut-off level of 1 MPN/1.8ml (= theoretical PCR detection limit; = ca. 56 MPN/100ml)

Note: PCR detects MUG gene

Discrepant results are underlined.

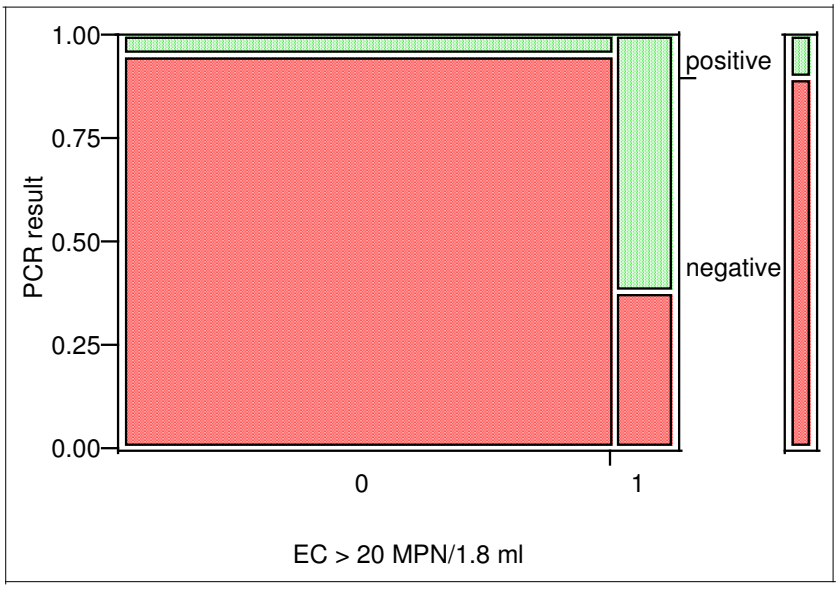


Culture result	PCR negative	PCR positive	Total
≤ 1.8 MPN/1.8ml	168	1	169
> 1.8 MPN/1.8ml	190	41	231
Total	358	42	400

Comparison of culture results (ISO 9308-3; MUG-hydrolysis) and qualitative real time PCR results for *Escherichia coli* (EC) at a cut-off level of 1.8 MPN/1.8ml (= NOAEL; = 100 MPN/100ml)

Note: PCR detects MUG gene

Discrepant results are underlined.



Culture result	PCR negative	PCR positive	Total
≤ 20 MPN/1.8ml	342	16	358
> 20 MPN/1.8ml	16	26	42
Total	358	42	400

Comparison of culture results (ISO 9308-3; MUG-hydrolysis) and qualitative real time PCR results for *Escherichia coli* (EC) at a cut-off level of 20 MPN/1.8ml (= level with equal numbers of discrepant results; = ca. 1100 MPN/100ml)

Note: PCR detects MUG gene

Discrepant results are underlined.