Section 5

In this guide

In this guide

10. EFSA Draft Guidance for Public Consultation: submission of data for the evaluation of the safety and efficacy of substances for the removal of microbial surface contamination - Annex A

Efficacy of pathogen reduction (Section 5, page 16)

35. Decontamination efficacy is established by demonstrating a statistically significant and consistent reduction in the prevalence and/or numbers of target pathogenic microorganisms compared to a control, while considering the inherent variation between experiments or batches of naturally contaminated foods. The control sample is either treated with water (if the decontamination method could have a rinsing effect, such as with meat carcasses) or left untreated (if the treatment doesn't replace a water treatment, such as with ready-to-eat food). It is crucial to consider the enumeration error, particularly when log reductions are below 1 log, as this can affect the interpretation of the results.

36. The dossier supporting the efficacy should include both existing experimental work from the literature and new experiments specifically conducted for the dossier ("in-house studies"). A comprehensive description of how existing studies were identified (search strategy, databases used, search limits, etc.) is essential for transparency and assessment of the evidence. Studies must be relevant to the intended treatment, focusing on the target pathogens and applying the decontaminating substance under the specified conditions. This includes details on the application stage (e.g., along the processing line), the specific application method (spraying, dipping, etc.), the concentration and temperature of the solution, and the pressure and duration of the treatment, all within the intended range of parameters. A proper control, treated with water or left untreated (with justification), is mandatory. The testing should assess at least one target pathogen, or relevant indicator microorganisms (such as Enterobacteriaceae, coliforms, or *E. coli*), immediately after treatment and optionally during storage and at the end of shelf-life. The study design and setting (laboratory, pilot-scale, or industrial) should be clearly defined.

37. The dossier should present a well-structured and coherent argument for the use of the decontamination solution. This argument must be supported by studies demonstrating the efficacy of pathogen reduction and an evaluation of the potential development of acquired reduced susceptibility to the formulated

product. All studies should be performed using the specific solution for which authorisation is sought, covering all intended formulations and concentrations. Processing conditions used in efficacy evaluations must mirror the intended use conditions, ensuring even distribution of the substance. Pilot or in-plant studies are preferred over laboratory-scale studies as they better reflect real-world conditions. Experimental data is required to justify the proposed concentration of the product formulation, showing the effect of different concentrations on the target pathogens. While the primary focus is on target pathogens, data on indicator microorganisms can be valuable for assessing overall efficacy.

38. Each study must include a comparison of pathogen prevalence and/or numbers between the food treated with the decontamination solution and the control group (water-treated or untreated). The control should ideally differ only in the presence or absence of the decontaminant. Comparisons between water-treated and untreated samples can provide supporting evidence of the rinsing effect. Artificial inoculation studies must use diverse strains or strain cocktails of the target pathogens, including reference strains and strains isolated from the target food. The inoculum should be evenly distributed, and sufficient time should be allowed for bacterial attachment before treatment. The inoculum level should be high enough to allow for quantification of log reductions. Sampling should occur at key time points: before treatment, immediately after treatment, and optionally during storage and at the end of shelf-life. Validated reference methods or other acceptable methods should be used for pathogen detection and enumeration, with recovery techniques for stressed cells. A validated neutralisation method (ISO 18593:2018) or removal of the formulated product is essential. For phage-based treatments, specific neutralisation methods such as centrifugation may be used. In pilot-scale or industrial settings, potential redistribution of organisms due to liquid treatments needs evaluation.

39. The study design must be justified in relation to the intended use of the product and should incorporate sound statistical methodology to test the efficacy hypothesis. The sample size must be justified, considering the expected effect size, significance level, study power, and measurement variance. Statistical tests (e.g., ANOVA, t-test) are required for in-house studies, using independent experimental trials with independent samples to increase statistical power. Factors that may influence efficacy (e.g., organic load, pH, temperature) must be identified, along with methods for controlling and monitoring these parameters during operation. The potential for acquired reduced susceptibility must be evaluated.

40. For each experiment, detailed information must be provided, including the experimental setting, contamination type, substance, application method, product type, treatment characteristics (concentration, temperature, duration, pH, pressure), contamination characteristics (bacterial group, strain origin, inoculum preparation), analytical methods (detection/enumeration method, sampling method, limits of detection and quantification), treatment and storage conditions, and outcome reporting (number of samples, microbial concentration, number of positive samples, log10 reduction calculations, and statistical analysis). Mean log10 reductions and their 95% confidence intervals should be reported, and the statistical methods used, including handling of negative samples, should be clearly stated.

41. This section outlines the requirements for evaluating the potential for decontamination substances (chemical or biological) to induce reduced susceptibility in target and non-target microorganisms, and the implications for resistance to other biocides and therapeutic antimicrobials. For bacteriophages, the focus is solely on resistance development in target species. If reduced susceptibility to the decontaminant is observed, further investigation is needed to determine if it promotes cross-resistance (where one mechanism confers resistance to multiple antimicrobials) or co-resistance (where resistance genes are linked). Priority for cross-resistance evaluation should be given to biocides used in the same industrial settings. The goal is to understand the relationship between reduced susceptibility to the decontaminant and resistance to other antimicrobials, including the potential for multiple resistance development. Due to the complexity of this issue, there are no universal guidelines, requiring case-by-case analysis using diverse sources of information. These requirements apply even to substances with a history of safe use.