EFSA Draft Guidance for Public Consultation: on the submission of data for the evaluation of the safety and efficacy of substances for the removal of microbial surface contamination of foods of animal origin

Section 6

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 EFSA Draft Guidance for Public Consultation: submission of data for the evaluation of the safety and efficacy of substances for the removal of microbial surface contamination -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

Chemical Substances (section 6.1, page 23)

42. A literature review is required to assess the potential for the chemical substance and formulated product to induce reduced susceptibility to itself, other biocides, and/or resistance to therapeutic antimicrobials in target and non-target species (gram-positive and gram-negative, including *E. coli* and *enterococci*) at working and sub-inhibitory concentrations. If concerns are identified, further testing is required. If no or acceptable concerns are identified, no further testing is needed at this stage, but a post-market evaluation plan is required.

43. Laboratory tests are needed to evaluate the potential for the chemical substance and formulated product to select for reduced susceptibility. Initial screening should mimic real-world conditions, including sublethal dilutions, short exposure times, and relevant environmental factors. Testing should use control strains, publicly available target pathogens (e.g., *Campylobacter*, *Salmonella*, *Listeria*, *Staphylococcus*), indicator bacteria (e.g. *E. coli*, *enterococci*), and non-target microorganisms. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) determinations should be performed, along with data supporting permanent changes in bacterial susceptibility. *In vitro* evolution studies are also required. This initial screening should ideally be validated in the processing plant or a similar setting. Recovered strains should be tested for MIC and MBC against the chemical substance and formulated product.

44. If reduced susceptibility is observed, further testing is required to assess cross-resistance and co-resistance to other biocides and therapeutic antimicrobials. For human pathogens, this should specifically address co-resistance and cross-resistance to biocides and representative antimicrobial classes used in human infection treatment. If concerns are identified, genetic analysis is required. If no or acceptable concerns are identified, no further testing is needed at this stage, but a post-market evaluation plan is required.

45. Genetic analysis is crucial for understanding the molecular mechanisms of decreased susceptibility, including point mutations, resistance gene acquisition, and changes in gene expression. If no molecular mechanisms of concern are found, a post-market evaluation and long-term monitoring plan are required.

46. A post-market resistance surveillance plan is required, including aspects of the in-plant screening. Data from other markets should also be submitted. If the decontaminant is released into the environment, a post-market monitoring plan is required to assess long-term effects on resistance development in the environment, including sampling of wastewater and relevant indicator microorganisms.

Biological Agents (section 6.2, page 23)

47. For biological agents, the EFSA guidance on the characterisation and risk assessment of microorganisms used in the food chain (EFSA Scientific Committee, 2024) should be followed, particularly regarding antimicrobial resistance genes and antimicrobial production. For bacteriophages, the search should cover both the phage genome and the host bacterial strain. The potential for bacterial resistance to phages and co-selection of resistance to therapeutic antimicrobials must be addressed.

48. A literature review is required on phage-bacteria coevolution, fitness costs of resistance, risk of resistance emergence, and the molecular basis of resistance, including *in vitro* and *in situ* studies under working conditions. If unacceptable concerns are identified, the evaluation is discontinued. If knowledge is insufficient, further testing is needed. If no or acceptable concerns are identified, no further testing is needed at this stage, but a post-market evaluation plan is required.

49. Laboratory tests are required to evaluate the potential for resistance emergence in target strains to the biological agent under various conditions. Initial screening should simulate realistic processing environments, examining the interaction of the bacteriophage and its formulated products under diverse conditions. Screening for phage-resistant bacteria should be performed. The stability and persistence of resistant strains should be demonstrated. Phenotypic characterisation of resistant isolates is needed. The initial screening should be validated in the processing plant or a similar setting.

50. Phenotypic characterisation of resistant isolates should include assessing cross-resistance and co-resistance to therapeutic antimicrobials and chemical biocides. If concerns are identified, genetic analysis is required. If unacceptable concerns are identified, the evaluation is discontinued. If no or acceptable concerns are identified, no further testing is needed at this stage, but a post-market evaluation plan is required.

51. Mechanisms of bacterial resistance to phages, therapeutic antimicrobials, and reduced susceptibility to biocides should be examined using whole genome sequencing or other appropriate methods. The potential for horizontal gene transfer should be assessed. If no molecular mechanisms of concern are found, a post-market evaluation and long-term monitoring plan are required.

52. A post-market resistance surveillance plan is required, including regular sampling and testing of target bacterial strains for susceptibility to the phages. Data from other markets should be submitted.