DRAFT GUIDANCE OF EFSA

Revision of the joint AFC/BIOHAZ guidance document 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 intended for human consumption[1]

European Food Safety Authority[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

KEY WORDS

Decontamination, efficacy, antimicrobial resistance, environmental impact

ABSTRACT

The abstract will be added to the final document.

SUMMARY

The summary will be added to the final document.

1 On request of EFSA, Question No EFSA-Q-2009-196, adopted on DD Month YYYY, issued on DD Month YYYY.

2 Correspondence: biohaz@efsa.europa.eu

3 Acknowledgement: EFSA wishes to thank the members of the Working Group on the revision of the guidance on carcass decontamination for the preparation of this EFSA scientific output: Yvonne Agersø, Philippe Hartemann (invited member from SCENIHR), John Threlfall, John Sofos, Birgit Nørrung (chair), Miguel Prieto Maradona, Antonia Ricci, Fidel Toldrá (CEF panel member), Josè Tarazona (invited member from SCHER), Jean-Yves Maillard for his participation as Hearing Expert and EFSA's staff members Alessandro Broglia and Renata Leuschner for the support provided to this EFSA scientific output.

Suggested citation: EFSA Panel on Biological Hazards (BIOHAZ) Guidance on Revision of the joint AFC/BIOHAZ guidance document 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 intended for human consumption.

17 **TABLE OF CONTENTS**

54

BACKGROUND

Article 3(2) of Regulation 853/2004 of the European Parliament and Council, which lays down specific hygiene rules for foods of animal origin, constitutes the legal basis for the use of substances other than potable water or clean water to remove surface contamination from foods of animal origin intended for human consumption. The use of substance(s) for the removal of microbial surface contamination of foods of animal origin is authorised according to the legislative procedures of the European Commission (EC). The EC shall consult EFSA on any matter within the scope of Regulation 853/2004 that could have a significant impact on public health. Indeed, EFSA in its role as the EU risk assessment body in food safety is responsible for the evaluation of the safety and efficacy of substances to be used to remove microbial surface contamination of foods of animal origin.

Decontamination treatments involve the application of a substance at a given step during the slaughter process in order to reduce the microbial contamination level of carcasses. Therefore there are three main aspects to be considered when assessing the substances: i) safety of the intended substance itself, ii) its effect as to the development of antimicrobial resistance and iii) the efficacy i.e. does the use of the substance in practice decrease the level of contamination of pathogenic bacteria. For this purpose, EFSA issued a guidance document (EFSA, 2006) which points out the major components and data that a dossier/application should contain in order to demonstrate that the substance intended to be used for the removal of microbial surface contamination of foods of animal origin is both safe and efficacious.

So far, the only substances where both the safety and efficacy has been assessed are peroxyacids (EFSA, 2005b). In evaluating both the safety and efficacy of peroxyacids intended to be used to reduce the microbial surface contamination of foods of animal origin such as poultry carcasses, the EFSA Panel on additives, flavourings, processing aids and materials in contact with food (AFC) concluded that, based on the data available, there was no safety concern, within the proposed conditions of use (EFSA, 2005a). For its part, the Scientific Panel on Biological Hazards (BIOHAZ) concluded that, owing to lack of sufficient data available to the Panel, including those submitted by the applicant, it was unable to say if this substance effectively killed or reduced pathogenic bacteria on poultry carcasses (EFSA, 2005b).

The BIOHAZ Panel concluded that the use of substance(s) for decontamination treatments will be regarded efficacious when any reduction of the prevalence and/or numbers of pathogenic target bacteria is statistically significant when compared to the control (e.g. water) and, at the same time, this reduction has a positive impact on reduction of human illness cases (EFSA, 2008a). On the one hand efficacy depends on a range of factors such as concentration, contact time, temperature and mode of application, the microbial load of the surface and other conditions of application.

In addition, concern has recently been raised about the potential for microorganism(s) to develop resistance to substances used for decontamination of carcasses. In most cases, such resistance could be developed following the improper use or storage of the substances resulting in a decrease in their effectiveness (EFSA, 2008a).

The BIOHAZ Panel concluded that despite a long history of use, there are currently no published data to conclude that the application of the four substances - chlorine dioxide, acidified sodium chlorite, trisodium phosphate, peroxyacids (EFSA, 2008a) to remove microbial contamination of poultry carcasses at the proposed conditions of use will lead to the occurrence of acquired reduced susceptibility to these substances or to antimicrobial resistance (AMR). The Panel recommended that additional research on the likelihood of the emergence of acquired reduced susceptibility to substances used for decontamination treatments and resistance to antimicrobials should be encouraged (EFSA, 2008a).

The BIOHAZ Panel further recommended the revision of the guidance on the submission of data for the evaluation of the efficacy of substances for the removal of microbial surface contamination of foods of animal origin.

An assessment on the same four substances was conducted by the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), and the Scientific Committee on Health and Environmental Risks (SCHER) about the environmental impact of the above and their effect on AMR of the above mentioned four substances when used for the removal of microbial surface contamination of poultry carcasses (SCHER/SCENIHR 2008). In this opinion it was concluded that the discharge of these substances may pose an environmental risk, unless properly treated in waste water treatment plants. Concerning the risk of development of AMR, it was concluded that there is a lack of data, but there is an environmental concern about the possibility that resistant strains could be disseminated.

TERMS OF REFERENCE

To revise the joint AFC/BIOHAZ (EFSA Panel on Food contact materials, enzymes, flavourings and processing aids and Panel on biological hazards) guidance on the submission of data for the evaluation of the efficacy of substances for the removal of microbial surface contamination of foods of animal origin in the context of Article 3(2) of Regulation 853/2004. This revision should include:

- example(s) of study designs at the laboratory and at the slaughterhouse in order to demonstrate that a substance for which authorization is sought, demonstrates efficacy;
- the type of data/studies that a dossier/application should include for the evaluation of the potential occurrence of acquired reduced susceptibility to the substance(s) and/or resistance to antimicrobials[4];
- example(s) of study designs for the monitoring of the potential development of acquired reduced susceptibility to the substance(s) and/or resistance to antimicrobials when a substance has already been authorized and used;
- the type of data/studies that a dossier/application should address on the environmental impact of the disposal of the substances, with particular reference to the biological and chemical risk for the environment, the residues or their by-products in the carcasses and the potential development and dissemination of resistant strains;
- the factors that should be considered when monitoring the safety and efficacy of a substance that has already been authorized and used.

When revising the guidance document the following aspects should be taken into consideration: the target pathogens (prevalence and concentrations), the type of antimicrobials, the methods to be used, the frequency of testing, and the sampling plan.

[4] See chapter “Definitions” of the present document

PUBLIC CONSULTATION

In the Plenary meeting on 8th - 10th December 2009 of the BIOHAZ Panel the draft-guidance document was approved for public consultation on the EFSA website.

1. INTRODUCTION

The present document is intended to provide guidelines for dossiers of applications to be submitted to the European Commission, for authorisation of substances to be used for the removal of microbial surface contamination of foods of animal origin.

Article 3(2) of Regulation 853/2004 of the European Parliament and Council, which lays down specific hygiene rules for foods of animal origin, constitutes the legal basis for the use of substances other than potable water or clean water to remove surface contamination from foods of animal origin intended for human consumption (decontamination agents[5]). The Regulation became effective on 1 January 2006.

According to this Regulation, the use of any substance other than water to remove/reduce surface contamination from products of animal origin is not authorized in the EU, unless the use of the substances has been approved in accordance with the Regulation. The EC shall consult EFSA on any matter within the scope of Regulation 853/2004 that could have a significant impact on public health.

The EC informed EFSA that substance(s) intended to be used for the removal of microbial surface contamination of foods of animal origin should be used to reduce the numbers and/or prevalence of pathogenic microorganisms. These substances can be considered as processing aids, as defined in the recent EC Regulation 1333/2008, since they are not consumed as a food by itself, and "intentionally used in the processing of raw materials, foods or their ingredients, to fulfil a certain technological purpose during treatment or processing". According to this Regulation, these substances and/or their by-products may result in the unintentional but technically unavoidable presence of residues in the final product, provided they do not present any health risk and do not have any technological effect on the final product. Therefore, these substances should be rinsed off after the application.

Furthermore, it is a risk management policy that the use of substance(s) for the removal of microbial surface contamination of foods of animal origin should only be considered as an additional measure, to further reduce the load of pathogenic microorganisms, following the application of good hygienic/manufacturing practices, and not as a substitute for those good hygienic/manufacturing practices (SCVPH, 1998; SCVPH, 2003; EFSA, 2006).

From a risk management point of view, the use of substances other than potable water or clean water can only be considered if the toxicological safety for the consumers and the environment and the efficacy of the substance can be demonstrated.

The evaluation of the safety and the efficacy of such treatments falls within the remit of EFSA (Art. 13, Reg. 853/04). EFSA has been asked by the EC to consider the impact of the use of these substances on the environment and the risk of potential occurrence of acquired reduced susceptibility to the substances and resistance to antimicrobials. It should be noted that evidence for the development of AMR due to the use of formulated products is for the most part limited to laboratory experiments; the evaluation of this issue for untested formulated products will therefore follow a case-by-case approach.

[5] See chapter "Definitions"

Therefore, in order to perform a proper assessment of the safety and efficacy of the substances, the following aspects should be considered: i) the safety of the intended substance ; ii) the effect as to the development of resistance to therapeutic antimicrobials; iii) the efficacy, i.e. does the use of a substance in practice decrease the level of contamination of pathogenic bacteria and iv) the safety of the intended substance and its by-products for the environment and especially the receiving water bodies for the wastewaters issued from the plants using this kind of treatment.

Concerning the toxicological safety of the decontamination agents, the information and data requested in this guidance (chapter 6) reflect what previously indicated in the joint AFC/BIOHAZ guidance document published in 2006. The EFSA Panel on Food contact materials, enzymes, flavourings and processing aids (CEF) has been consulted for the revision of the present guidance, and in particular concerning the toxicological issues.

For the purpose of this document the use of decontamination agents, under defined conditions, will be regarded efficacious when a reduction[6] of the prevalence and/or numbers of pathogenic target bacteria, set according to determined criteria, is statistically significant when compared to a non-treated control group. At the same time this reduction should provide benefits in terms of public health impact (decrease of human disease prevalence). It is recognised that the best way to validate efficacy is to perform large scale in-plant studies. Other relevant considerations, as mentioned in the SCVPH report (1998), must be dealt with by other fora. These include the impact of the treatment on product quality, on worker safety, on the consumer acceptance.

In order to properly assess the environmental issues, aspects related to the development of AMR and/or acquired reduced susceptibility to decontamination agents, representatives of both Scientific Committee of SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), SCHER (Scientific Committee on Health and Environmental Risks), and from the Community Reference Laboratory for Antimicrobial Resistance have been involved in the revision of the present guidance document. SCENIHR and SCHER experts kindly provided the necessary expertise on this issue, in particular concerning the impact of the disposal of the substances, with reference to the biological and chemical risk for the environment, the residues and/or their degradation products in the wastes and the potential development and dissemination of resistant strains.

The data needed concerning the risk of potential development of reduced susceptibility to the formulated product and development of resistance to antimicrobials have been listed in this guidance thanks to the support of experts from the Community Reference Laboratory for Antimicrobial Resistance. This aspect is of critical importance due to the increasing antimicrobial resistance both in environmental and pathogenic microorganisms which is now a real challenge for public health; it is therefore crucial to evaluate the possible risk of decontamination agents in the induction of AMR. This assessment should be performed both for products in use for many years and for new decontamination agents under the specific conditions of use.

All the items below must be addressed for the dossier to be considered valid for the evaluation process. If the applicant submits data other than those required or considers a topic irrelevant in the case(s) of the formulated product in question, this must be clearly justified for each of those items required.

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), the Scientific Committee on Health and Environmental Risks (SCHER), and the Community Reference Laboratory for Antimicrobial Resistance are acknowledged for their valuable contribution to this document.

This guidance document will be revised in the light of any new legislation and the experience that EFSA develops in evaluating applications.

[6]The extent of reduction is a risk management decision

2. OBJECTIVE

The objective of this document is to provide guidance on the submission of data for the evaluation of the safety for consumers and environment and the efficacy of substances intended to be used for the removal/reduction of microbial surface contamination on foods of animal origin.

3. SUBMISSION OF AN APPLICATION

The applicant should provide all available data relevant for the evaluation by the EC, both on paper and in electronic format in IUCLID5 (http://iuclid.echa.europa.eu) on standard physical media (CD-ROM). It has to be declared by letter that the electronic and the paper version are identical. The dossier must be submitted to:

European Commission
Directorate General for 'Health and Consumers
B-1049 BRUSSELS

In addition to the complete version with the full information, applicants should provide a second version of the CD-ROM without the confidential information. This version will be made available to anyone who might submit a request to EFSA. Any specific literature reference (full length scientific papers) mentioned and used to support the application must be supplied in the dossier in electronic format. When reference is made to a book or to extensive publications, only the relevant parts need to be supplied. Applicants may deviate from the guidelines, provided that valid and documented scientific reasons are given in the dossier. In all cases, the EFSA may request additional data. Applicants shall note that competent authorities in member States will get full access to any dossier submitted to EFSA. It should also be noted that applications for authorisation, supplementary information from applicants and opinions from the Authority, excluding confidential information, shall be made accessible to the public. Confidential information in the dossier has to be clearly marked.

If an applicant would like to have some information kept confidential verifiable justification must be provided. Information relating to the following shall not be considered confidential:

- the name and address of the applicant and the chemical name of the substance;
- information of direct relevance to the assessment of the safety and efficacy of the substance;
- the analytical methods used to determine the above.

All procedures, materials and methods and data submitted should be of a quality suitable for publication in peer reviewed journals.

The results of post market monitoring should be submitted to the national competent authority, and then forwarded to the EC.

3.1. Information to be supplied with an application

The dossier shall be composed of three sections:

1. The summary document;

2. The administrative part;

3. The technical part (technical dossier).

To allow a complete safety assessment, sufficient information must be provided in all the above sections.

3.2. Summary document

The summary document should contain a summary of all information provided in the technical dossier (TD) and the safety evaluation, including:

- the principal and target function of the formulated product;
- the main relevant physic-chemical characteristics of the substance(s), and its manufacturing process, conditions of storage and shelf life;
- the intended use of the substance(s) with respect to the types of foods to be applied on and the conditions of time and temperature of use,
- the existing authorization in EU Member States and other countries,
- the toxicological data.

This should be a 'standalone' document. If a reference is made to other documents, a summary of the relevant information in these documents shall also be provided.

3.3. Administrative information

The data supplied shall identify the legal entities and the business involved, as well as the person in charge of the application:

1. Name of the applicant (company, organisation submitting the petition), address and other means of communication, e.g. telephone, e-mail.

2. Name of the business operator on whose behalf the petition is submitted (if different from above), address and others means of communication, e.g. telephone, e-mail.

3. Name of the person responsible for the dossier, address and other means of communication, e.g. telephone, e-mail.

4. Date of submission of the dossier.

5. Table of contents of the dossier.

4. TECHNICAL DATA

4.1. Identity of the substance(s) and specifications

Substances either single or in a simple or complex mixture, must be clearly identified giving respectively:

- Chemical names (IUPAC), CAS registry numbers, synonyms and trade names;
- EC numbers and REACH registration numbers;
- Molecular weight, molecular and structural formula;
- Solubility in water and/or organic solvents and in the food of contact;
- Purity, impurities present and their level, dosage method;
- Description of the product to be used, conditions of storage and shelf life.

4.2. Manufacturing process

Method of manufacture with description of the source (raw materials), the process used to produce the substance(s), production controls and quality assurance.

4.3. The treatment and its purpose

i. A statement of the purpose of the treatment, including a list of the type of foods of animal origin to be treated and the pathogenic microorganisms the substance(s) is (are) intended to target. Further specifications should be provided, concerning, all above, if the treatment is aimed to:

   a. target raw material before further transformation;

   b. reduce the global contamination of foodstuffs before consumption;

   c. reduce the contamination of food products by pathogenic microorganisms and thereby reduce the risk to public health;

   d. produce a bacteriostatic effect to prolong the shelf life of food products;

   e. increase the production performance;

ii. A list of the pathogenic microorganisms potentially occurring on the surface of foods of animal origin to be treated and a brief statement of associated public health risks should be provided.

iii. A description of the mode of application of the substance(s) to the surfaces of foods of animal origin, any recycling of the substance(s) and description of where in the processing lines the substance(s) will be applied. This includes the intended doses to be used, ways of application (e.g. dipping, spraying, etc.), conditions of use (e.g. time, temperature, pH, etc.), and subsequent rinsing. The description should be sufficient for allowing a quantitative estimation of the expected environmental releases of the substance and its by-products during the storage, handling, use and waste management.

4.4. Reactions and fate on the treated foods of animal origin after rinsing

The following information should be provided:

i. Quantification of residual levels of the substance(s) used in the treated food.

ii. Description and quantification of any degradation product(s) of the substance(s) used that may remain in the treated food.

iii. Description and, when feasible, quantification of any reaction by-products resulting from potential reactions with natural compounds in the food during and after treatment, e.g. proteins, peptides, free amino acids and lipid compounds.

4.5. Methods of analysis

All methods used for the microbial analyses and for the analysis of the substance(s), its (their) degradation products and major reaction by-products should be provided by the applicant (including detailed protocols, validity and performance parameters, etc.).

5. CONSUMER EXPOSURE ASSESSMENT

An estimate of potential daily exposure of the consumer to residues, degradation products and any relevant reaction by-products present in the treated food must be provided.

6. TOXICOLOGICAL AND ECOTOXICOLOGICAL DATA

Available toxicological and ecotoxicological data on each substance, including its potential degradation products and any identified reaction by-products, should be submitted. Depending on these data and on the chemical structure of the substances and the levels remaining in the treated food, further data might be requested following a first evaluation. In cases where a substance is already approved for direct addition to food in the EU (Reg. EC 1333/08), a reference to the previous toxicological assessments can be provided as supporting information regarding the safety for consumers. EFSA may consider that no additional toxicological assessment is required on the basis of comparative exposure estimation.

It should be noted that mammalian toxicological data may be also required for the environmental risk assessment, in particular for assessing the risk associated to secondary poisoning of mammals and other terrestrial vertebrates. This assessment is required for substances with bioaccumulation potential. The environmental assessment requires a reassessment of the toxicological studies. Preference should be given to oral studies where the chemical is applied within the food; gavage studies can also be used if needed. The environmental risk assessment should be based on endpoints with ecological relevance, such as effects on survival, growth or reproduction. Effects at the biochemical or histological level which do not results in ecologically relevant consequences should not be considered; as a consequence, the NOEL (No Observed Effect Level) and NOAEL (No Observed Adverse Effect Level) selected for the environmental assessment usually differ from those selected for human health protection.

7. INFORMATION REQUIRED TO ASSESS THE EFFICACY OF A FORMULATED PRODUCT

The proposal should be a coherent presentation of the arguments for use of the formulated product[7], supported by studies of the efficacy of pathogen reduction and of the potential acquired reduced susceptibility to the formulated product itself, performed according to the guidelines below and presented in a structured way. It is suggested that each of the items below is addressed briefly in a summary, cross-referenced to appropriate enclosures or annexes:

i. The dossier intended to assess efficacy should include full reports of all relevant experiments.

ii. Only studies conducted under conditions directly related to the intended conditions of use of the formulated product application will be considered. Such studies could be experiments performed specifically for the dossier or experimental work already performed or published.

iii. All studies should be made with the formulated product for which authorisation is sought. If various formulations are foreseen, all of them should be tested. The processing conditions used to evaluate the efficacy must be comparable with those for which the formulated product is intended. The study must include a comparison of the prevalence and/or numbers of the pathogenic microorganisms on the food of animal origin to which the formulated product will be applied and on the untreated control food. The only difference must be the presence or absence of the formulated product and not the method of application or other factors. The

[7] See chapter “Definitions”

study design should be as close as possible to the real conditions under which the formulated product is intended to be applied. Therefore, if the formulated product is intended, for example, to be used as a dip or spray on broiler carcasses with skin, then meat samples with skin should be dipped or sprayed in the experimental study.

iv. The prevalence and/or numbers of the target pathogenic microorganisms and other pathogens of concern in the product must be measured before and after application of the formulated product and at the end of the shelf life of the food product in question, in order to ensure that there is no repair of sub-lethally injured organisms. The same testing should also be followed for the control foods.

v. Although the application of the formulated product is intended to reduce the prevalence and/or numbers of target pathogenic microorganisms, data on the counts of non-pathogenic microorganisms, such as indicator microorganisms and total viable counts, should be provided and may also assist in the assessment of the overall efficacy of the proposed application.

vi. The study design must be justified in relation to the specific claim(s) made for the formulated product and must include a consideration of sound statistical methodology. All tests should be performed on a sufficient number of samples, depending on the actual prevalence and/or numbers of the target organisms. Any statistical analysis of data should describe the method applied and the statistical power.

vii. Firstly tests must be made with inoculated pathogenic bacteria, taking into account strain diversity. This can be achieved by using different strains or cocktails of strains, including standard reference strains (for comparison with other studies), strains isolated from the surface of foods of animal origin to be treated, and clinical strains. An inoculum should be tested at a range of levels including the level expected in the food product. In addition the efficacy of the formulated product must be validated by testing on naturally contaminated foods of animal origin.

viii. Available scientific information on natural or acquired reduced susceptibility to the formulated product should be provided.

ix. The determination of the efficacy of a formulated product must involve the use of an appropriate neutralization method or the removal of the formulated product by filtration (as described in CEN standard test).

x. Justification of the concentration of the product formulation proposed should be experimentally demonstrated, for instance by providing data, showing the effect of different concentrations of the product formulation on the target microorganisms reflective of the conditions of use.

xi. A description of the methods used to control and monitor the concentration of the active substance on the food product in the processing plant during operational time, including the identification of factors that may influence the efficacy of the active substance (e.g. organic load, pH, temperature etc), must be provided. Testing the development of possible acquired reduced susceptibility to the compound itself is suggested to be performed under conditions simulating the intended use in food.

xii. If a products is authorised and in use, a post-market monitoring of its efficacy should be performed and it is recommended to be incorporated in the HACCP implementation procedure. This would include an evaluation of the possible development of acquired reduced susceptibility to the formulated product.

An example of a study with the purpose of evaluating the efficacy of a decontamination agent in a formulation/product to reduce the number of *Campylobacter* on broiler meat experimentally in the laboratory and at slaughterhouse is shown in appendices A and B, respectively.

Similar study designs could be used to evaluate the efficacy of a decontamination agent in a formulated product to reduce the number of target pathogens, taking into account the different methods needed for detection of the target organisms. The study designs could also be applied to animal products other than broiler meat and broiler carcasses. Appropriate samples should be taken in accordance with standard procedures (e.g. ISO 17604: 2003).

The surface temperature of the food and/or the temperature of the dipping solution are some of the parameters that may affect the bactericidal efficacy of decontamination agents in a formulation/product. Temperature at the point of application is therefore an important factor to monitor and control during studies. Controls treated with potable water instead of formulated product should therefore be included.

An example of statistical approach needed for execution of these studies is described in Appendix C.

8. INFORMATION NECESSARY FOR THE EVALUATION OF THE POTENTIAL EMERGENCE OF ANTIMICROBIAL RESISTANCE (AMR)

In cases where the formulated product has already been in use previously as “processing aid” in food products or as a food additive and it does not appear that such usage has led to the development of, or selection for AMR, the applicant may apply for approval based on the history of apparent safe use.

When no prior knowledge is available concerning a proposed formulated product and its potential for development of AMR, additional tests would be required to address these issues.

The use of decontaminating agents may select for AMR as follows (EFSA, 2008a):

1. Cross-resistance: (i) selection for genes encoding resistance to both the formulated product and one or more antimicrobial classes or (ii) change the physiological response of the bacterium to become less susceptible to both formulated product and antimicrobials.
2. Co-resistance: selection for clones or mobile elements also carrying AMR.
3. Indirectly select for clones that are resistant to antimicrobials.
4. Enhance DNA uptake by e.g. activating a SOS response in bacteria.

In the generic context of a potential selection for AMR through the use of the formulated product it is necessary to be aware of these potential ways of resistance development (selection and dissemination).

The evaluation of untested formulated products will entail a case-by-case approach.

In order to assess the potential emergence of AMR, studies will be required to investigate if the use of the formulated product leads to development of resistance to such antimicrobials.

Following submission of the dossiers, the results of these studies will be evaluated by expert bodies.

In most cases the interpretation will be based on experimental studies, supporting information and published data. When a formulated product is taken into use the level of resistance to antimicrobials is expected to be negligible. Awareness should be high if resistance to antimicrobials develops due to the use of the formulated product.

The evaluation is divided into pre-market and post-market evaluation. A plan for the post-market evaluation should be provided when an authorization for a decontamination agent is sought.

8.1. Pre-market evaluation

The following points have to be addressed:

i. The pre-market evaluation should include laboratory experiments to examine the development and dissemination of resistance to antimicrobials following exposure to the formulated product at in-use and lower concentrations. As indicated above, existing information may be considered.

ii. The type and quality of data expected are indicated in the section 8.3.

iii. Target and indicator microorganisms have to be tested for resistance to therapeutic antimicrobials listed in earlier reports (EFSA 2008b,c,e). In general these antimicrobials are considered appropriate for most pathogens, although account should be taken of differences in the intrinsic resistance of Gram-negative and Gram-positive target and indicator organisms to certain antimicrobials.

iv. Development of resistance to therapeutic antimicrobials should be tested in:

- Target organisms: *Campylobacter* species, *Salmonella enterica*, *Listeria monocytogenes* and *Staphylococcus aureus*;
- Indicator organisms: *Escherichia coli*, enterococci.

For these investigations reference strains of target and indicator organisms should be included.

If the formulated product is neutralised before discharge of wastewater, then no tests about development and dissemination of AMR of environmental bacteria are required.

In the absence of neutralisation, environmental indicator bacteria isolated from sediment and wastewater treatment plants should be examined, taking into account the possible intrinsic resistance of such strains.

In such cases, a sampling procedure should be performed in order to specifically address the microbial flora upstream and downstream of the waste water efflux, preferably also from sediments and wastewater drains. These samples should be tested by viable counts of bacteria in the presence of the concentrations of the formulated product and/or degradation products which leave the processing environment.

8.2. Post-market evaluation

Development of resistance to therapeutic antimicrobials in pathogens or indicator bacteria in the food or processing environment should be examined simultaneously with verification of efficacy of the formulated product through HACCP.

If the product is released in the environment without neutralisation, a post-market monitoring and evaluation is recommended to determine the long-term effects of using the formulated product on selection and dissemination of AMR.

The following points have to be addressed, if the formulated product is not neutralised before discharge:

i. Any novel scientific information about the formulated product should be taken into account.

ii. A statistically significant number of environmental samples should be collected in the wastewaters and both upstream and downstream of the point of discharge. The sampling strategy should take into account seasonal changes and characteristics of the effluent.

iii. From the environmental samples taken, relevant bacteria should be isolated, identified and used for monitoring of resistance to antimicrobials as described above. All experimental data should be provided.

iv. These examinations could be performed in a structured follow-up during a minimum of three years in line with EMEA (2006).

8.3. Type and quality of data

i. The methods used should be reproducible and validated with the necessary controls and samples included. If available, standardised methods should be used.

ii. The data should be suitable for risk assessment and if possible quantitative.

iii. Susceptibility testing methods for antimicrobials and decontamination agents should be done using the most recent updated standardised methods (e.g. ISO and CLSI standards) for determination of the minimal inhibitory concentration (MIC). The determination of MBC should be performed according to a standard efficacy test (e.g. CEN standard).

iv. Information on the conditions of application of the formulated product must be documented, including the minimum concentration of the decontaminating agent achieved at the point of application, presence and nature of organic load, minimum exposure time, temperature, type of surfaces.

v. The interpretative criteria used to determine the level of AMR should be based on published recommendations from EUCAST and EFSA (EFSA 2008b, c, e).

vi. The interpretative criteria used to determine the level of resistance to a formulated product should be based on bacterial population distributions of MBC of the bacterial species in question.

9. INFORMATION NECESSARY FOR THE EVALUATION OF THE TOXICOLOGICALENVIRONMENTAL IMPACT OF THE SUBSTANCES[8]

In order to authorise the use of substances for the removal of microbial surface contamination of foods of animal origin, data set and information are required about the conditions of application and release of the substance and eventually by-products or degradation products in the environment.

9.1. Risk related to the release of the chemicals into the environment

The release of substances for the removal of microbial surface contamination of foods of animal origin may have a negative impact on the environment, and especially for some species living in the receiving water bodies. On 1st June 2007, the European REACH Regulation (EC) No 1907/2006 entered into force. This guidance for substances for the removal of microbial surface contamination of

[8] This chapter is attributable to contributions from SCHER (Scientific Committee on Health and Environmental Risks) and SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks).

foods of animal origin has considered the test requirement for the registration of substances under the REACH Regulation, additional test requirements may be necessary for conducting the risk assessment for this specific use.

Aquatic environmental risk is evaluated on the PEC/PNEC ratio between Predicted Environmental Concentration of the substance (PEC) and the highest concentration of the substance that it assumed to have not harmful effects in the environment (PNEC). Classically, risk is assumed to be low if the PEC/PNEC ratio is below 1 (some guidance documents require the PEC/PNEC ratio to be below 0.1 in certain cases for accounting for the additional uncertainty). Thus the environmental risk assessment of the substance and its by-products is necessary and the risk can be characterized as a PEC/PNEC ratio for the relevant compartments. This is conducted by classical international methodology taking into account a study of hazards, scenarios for their dissemination in the environment and assessment of the risk. Typically, a risk refinement should be conducted if the PEC/PNEC ratio is higher than 1; and, depending on the uncertainty of the assessment, in some cases where the ratio is between 1 and 0.1.

An initial worst case estimation of the potential environmental risk can be obtained through the adaptation of the default scenarios established by the Technical Guidance Document (ECB, 2003) and the guidance for Chemical Safety Assessment under REACH (ECHA guidance documents, available at http://echa.europa.eu/). The adaptation should follow the methods recommended by the EU Scientific Committees (SCHER/SCENIHR, 2008). If needed, the refinement of the exposure scenarios could be based on measured values, release estimations or ad-hoc models. Deviations from the default values should be scientifically justified. Considering that these compounds are expected to be particularly toxic for environmentally relevant microbial functions, the environmental impact assessment should contain enough ecotoxicological information for establishing at least, Predicted No Effect Concentrations (PNECs) for aquatic organisms ($PNEC_{water}$) and for Wastewater Treatment Plants ($PNEC_{WWTP}$). Following the SCHER recommendation (SCHER, 2007), if the PNEC for sediment and soil is estimated using the equilibrium partitioning method, the lowest PNEC (water or WWTP) should be used for the calculation.

In addition, an assessment of the PBT (Persistent, Bioaccumulative and Toxic) and vPvB (very Persistent and very Bioaccumulative) properties is needed. This environmental hazard assessment expresses the inherent characteristics of the substance for provoking long-term environmental damage. The PBT and vBvP assessment should be conducted following the criteria established in Annex XIII of the REACH Regulation. For substances fulfilling the PBT and/or vPvB criteria, the environmental impact assessment should be extended for considering long-term risks and risk associated to biomagnification through the food chain. Risk mitigation measures should be implemented for dealing with these potential environmental impacts.

9.2. Assessing environmental impacts via wastewater emissions (pre-market).

The release estimations of the different chemicals from the slaughterhouse production must be calculated using realistic scenarios. Screening assessment based on worst-case estimations and default values are also possible.

An example of generic worst-case scenario could consider that a slaughterhouse processes 50 tons/day of meat. This value is the threshold designated by the IPPC Directive (EC, 2008). The EPER database indicates that just a few slaughterhouses in the EU are above this limit. The very large facilities, exceeding this production level, have specific environmental controls through the IPPC Directive and specific wastewater treatment facilities should be implemented. The large majority of slaughterhouses in the EU are below this limit but the 50 tons meat per day limit may be considered appropriate for a generic assessment. It is assumed that slaughterhouses not covered by the IPPC may discharge wastewater from the production directly to the municipal wastewater treatment plant (WWTP) without pre-treatment at the production site, or directly in the receiving water body.

As the conditions in the effluent are unknown, a precautionary worst case approach would be selected, based on the maximum theoretical amount of decontamination agent and by-products that could be produced by the treatments.

Risk estimations are to be produced at least for the following three scenarios.

- Scenario 1: direct discharge of the slaughterhouse wastewater into aquatic environments.
- Scenario 2: the municipal wastewater treatment plant (WWTP) receiving the slaughterhouse wastewater.
- Scenario 3: the slaughterhouse wastewater discharged through a default municipal WWTP.

For each scenario it is necessary to calculate PEC/PNEC ratio (the scenario 2 does not consider the degradation within the WWTP).

The minimum requirements for the environmental fate assessments are assays covering the physical-chemical properties, including water solubility, K_{ow}, vapour pressure, surface tension, ionization potential, and reactivity. In addition a ready biodegradability study should be provided unless highly reactivity and/or rapid hydrolysis can be demonstrated. The information must cover the substance and all relevant by-products.

The ecotoxicity data should be included in the dossier. All available information should be submitted. The minimum requirements are ecotoxicity tests covering the three aquatic taxonomic groups (fish, invertebrates and algae) and an activated sludge respiration inhibition test. Regarding the algal test, assays with green algae and with cyanobacteria are required for a proper assessment, if a read-across or other method clearly indicate that one taxonomic group is expected to be more sensitive, the assay could be limited to the sensitive taxa. The assessment of persistent and bioaccumulative substances should always include chronic assays.

Whenever possible, the ecotoxicity tests should be conducted with the substance and with any relevant reaction/transformation product released or produced under the expected use patterns. The test protocols should be adapted for highly reactive substances, Direct Toxicity Assessment (DTA) methods applied to samples collected under real or simulated use conditions may offer a proper assessment method; deviations from the standardized protocols should be recorded and justified.

If the physical-chemical properties and/or environmental fate studies indicate a potential of the substance or its by-products to bind WWTP sludge and/or sediment, the assessment should be extended for covering soil and/or sediment dwelling organisms respectively.

Following the TGD criteria (ECB, 2003), an assessment of secondary poisoning is required for substances with potential for bioaccumulation.

Additional considerations should be presented for potential synergistic effects with other substances released simultaneously and with related mechanisms of action and/or environmental targets.

Thus for each substance the potential environmental impacts should be considered when assessing the use of this chemical as decontamination agents to treat carcasses including:

- The chemical risk associated with, at least, the releases of each chemical into the aquatic environment or into WWTPs, which can be estimated through the comparison of PNEC for aquatic organisms and for WWTP microbial communities respectively, with the PEC.
- A PBT and vPvB assessment, and if positive, the risk mitigation options and an assessment including the level of control expected by the proposed measures.

- The nature, toxicity and predicted concentrations of any by-products resulting from the interaction of each decontamination agent with water and with organic matter.

- The contribution from the use of each decontamination agent for carcass treatment to the total environmental load of decontamination agents in waste water treatment facilities and the wider environment.

9.3. Requirements related to the post-market monitoring of the environmental risk

The requirements related to the post-market monitoring of the environmental risk of decontamination agents should focus on the confirmation of the exposure estimations. If potential concerns are observed during the authorization process, the Predicted Environmental Concentrations should be confirmed by measuring the concentrations in the final effluent released to the environment. The measurement should cover the parent substance and any relevant metabolite. In some cases, chemical analysis could be replaced by Direct Toxicity Assessment, measuring directly the toxicity of the effluent; this alternative is particularly suitable for monitoring substances with complex or unknown metabolism/degradation patterns.

REFERENCES

Birk T, Rosenquist H, Brøndsted L, Ingmer H, Bysted A, Christensen BB. A comparative study of two food model systems to test the survival of *Campylobacter jejuni* at -18°C. J Food Protection, 69-2635–2639.

Boysen L and Rosenquist H, 2008. Reduction of thermotolerant *Campylobacter* species on broiler carcasses following physical decontamination at slaughter. J of Food Protection, 72, 497–502

Ciofu O, Riis B, Pressler T, Poulsen HE and Hoiby N, 2005. Occurrence of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis patients is associated with the oxidative stress caused by chronic lung inflammation. Antimicrob. Agents Chemother., 49, 2276-82.

Directive 2008/1/EC of the European Parliament and of the Council of 15 January 2008 concerning integrated pollution prevention and control

Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC

ECB, 2003. Technical Guidance Document in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Edition 2. Ispra, 2003.

ECDC, EFSA, EMEA, SCENIHR 2009. Scientific Opinion of the European Centre for Disease Prevention and Control; Scientific Opinion of the Panel on Biological Hazards; Opinion of the Committee for Medicinal Products for Veterinary Use; Scientific Opinion of the Scientific Committee on Emerging and Newly Identified Health Risks on antimicrobial resistance (AMR) focused on zoonotic infections. EFSA Journal 2009; 7(11):1372; European Medicines Agency Reference EMEA/CVMP/447259/2009.

EFSA 2005a. Scientific opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to Treatment of poultry carcasses with chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids. The EFSA Journal 297: 1-27.

EFSA 2005b. Scientific opinion of the Scientific Panel on Biological Hazards on the evaluation of the efficacy of peroxyacids for use as an antimicrobial substance applied on poultry carcasses. The EFSA Journal 306: 1-10.

EFSA 2006. Joint AFC/BIOHAZ guidance document on the safety and the efficacy of substances for the removal of microbial surface contamination of foods of animal origin. The EFSA Journal 388: 1-19.

EFSA 2008a. Scientific opinion of the BIOHAZ Panel on the assessment of the possible effect of the four antimicrobial treatment substances on the emergence of antimicrobial resistance. The EFSA Journal , 659 1-26.

EFSA, 2008b. Report from the Task Force on Zoonoses Data Collection including guidance for harmonized monitoring and reporting of antimicrobial resistance in commensal Escherichia coli and Enterococcus spp. from food animals. The EFSA Journal (2008) 141: 1-44.

EFSA, 2008c. EFSA Working group on Developing harmonised schemes for monitoring antimicrobial resistance in zoonotic agents, “Harmonised monitoring of antimicrobial resistance in Salmonella and Campylobacter isolates from food animals in the European Union, Clinical Microbiology and Infection, vol. 14, number 6, 522-33.

EFSA, 2008d. Scientific opinion of the BIOHAZ Panel on Foodborne antimicrobial resistance as a biological hazard. The EFSA Journal (2008) 765, 1-87.

EFSA 2008e. EFSA Working group on developing harmonised schemes for monitoring antimicrobial resistance in zoonotic agents, 'Harmonised monitoring of antimicrobial resistance in *Salmonella* and *Campylobacter* isolates from food animals in the European Union. Clin. Microbiol. Infect. 14, 522-533.

EFSA, 2008f. Scientific opinion of the FEEDAP Panel on update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. The EFSA Journal (2008) 732, 1-15.

EMEA 2006. Guideline on the environmental risk assessment of medicinal products for human use. London, UK

Erill I, Campoy S, Barbé J. Aeons of distress: an evolutionary perspective on the bacterial SOS response. FEMS Microbiol Rev. 2007; 31(6):637-56.

EUCAST, 2000. Terminology relating to the determination of the susceptibility of bacteria to antimicrobial agents (EUCAST definitive document E.Def 12) *Clin. Microbiol. Inf.* 6:503-508.

Foster KL, 2007. Stress-induced mutagenesis in bacteria. Crit. Rev. Biochem. Mol. Biol., 42, 373-97.

Griffith KL, Shah IM, Wolf RE Jr., 2004. Proteolytic degradation of *Escherichia coli* transcription activators SoxS and MarA as the mechanism for reversing the induction of the superoxide. (SoxRS) and multiple antibiotic resistance (Mar) regulons. Mol. Microbiol. 51, 1801-16.

Hastings PJ, Rosemberg SM, Slack A., 2004. Antibiotic-induced lateral transfer of antibiotic resistance. Trends Microbiol. 12, 401-4.

Helsel, DR, 2005. Nondetects and Data Analysis: Statistics for censored environmental data. Statistics in Practice. Wiley Interscience, chapter 6.

ISO 17604: 2003 Microbiology of food and animal feeding stuff- Carcass sampling for microbiological analysis

Lorimer MF, Kiermeier A., 2007. Analysing microbiological data: Tobit or not Tobit? Int J Food Microbiol. 116, 313-8.

Lorimer MF, Kiermeier A., 2008. A guide to using R for analysing microbiological data. http://www.sardi.sa.gov.au/__data/assets/pdf_file/0014/45050/guide_totobit_analysis_of_microbial_data.pdf

Lund M, Wedderkopp A, Waino M, Nordentoft S, Bang DD, Pedersen K, and Madsen M, 2003. Evaluation of PCR for detection of Campylobacter in a national broiler surveillance programme in Denmark. J Appl.Microbiol. 94, 929-935.

Mokgatla RM, Gouws PA and Brozel VS, 2002. Mechanisms contributing to hypochlorous acid resistance of Salmonella isolate from a poultry-processing plant. J. Appl. Microbiol.; 92, 566-73.

Oliver A, Canton R, Campo P, Baquero F and Blazquez J High frequency of hypermutable Pseudomonas aeruginosa in cystic fibrosis lung infection. Science, 2000; 288, 1251-4.

Pomposiello PJ, Bennik MH and Demple B Genome-wide transcriptional profiling of the Escherichia coli responses to superoxide stress and sodium salicylate. J. Bacteriol. 2001; 183(13):3890-902.

Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. Official Journal of the European Union L 226/22 of 26 June 2004.

Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Official Journal of the European Union L 396/01 of 30 December 2006.

Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. Official Journal of the European Union L 354/16 of 31 December 2008.

729 Riedel CT, Brøndsted L, Rosenquist H, Haxgart SN, Christensen BB, 2009. Chemical
730 Decontamination of *Campylobacter jejuni* on chicken skin and meat. J Food Protection, 72-1173-
731 1180.

732 Rosenquist H, Sommer HM, Nielsen NL, and Christensen BB, 2006. The effect of slaughter
733 operations on the contamination of chicken carcasses with thermotolerant Campylobacter.
734 International Journal of Food Microbiology 108, 226-232.

735 SCHER/SCENIHR 2008. Scientific opinion on the environmental impact and effect on antimicrobial
736 resistance of four substances used for the removal of microbial surface contamination of poultry
737 carcasses, April 2008.

738 SCHER, 2007. Scientific opinion on the risk assessment report on chloroform (CAS 67-66-3),
739 environmental part, 20 September 2007.

740 SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), 2009.
741 Assessment of the Antibiotic Resistance Effects of Biocides, 19 January 2009.

742 SCVPH (Scientific Committee On Veterinary Measures Relating To Public Health), 1998. Report on
743 the benefits and limitations of antimicrobial treatments for poultry carcasses. 30 October 1998.

744 SCVPH (2003) Opinion on the evaluation of antimicrobial treatments for poultry carcasses.
745 http://ec.europa.eu/fs/sc/scv/out63_en.pdf

746 Thrusfield M, 2007. Veterinary Epidemiology. Blackwell Science, Oxford, 2007.

747 U.S.Food and Drug Administration (FDA), 2001. Bacteriological Analytical Manual Online. Chapter
748 7: Campylobacter. http://vm.cfsan.fda.gov/~ebam/bam-7.html

749 Walsh TR, 2006. Combinatorial genetic evolution of multiresistance. Curr. Opin. Microbiol.;
750 9(5):476-82.

751

APPENDICES

APPENDIX A

EXAMPLE OF AN EXPERIMENTAL PROCEDURE FOR TESTING THE EFFICACY OF CHEMICAL SOLUTIONS IN REDUCING THE NUMBER OF *CAMPYLOBACTER* ON BROILER MEAT

Preparation of inoculum. From frozen stock (–80 °C in Brain Heart Infusion broth (BHI) containing 15% glycerol), strains are streaked onto Blood Agar Base No 2 plates (Oxoid CM271, UK) added 5% horse blood and incubated for 2-3 days in microaerobic conditions (6% O2, 7% H2, 7% CO2, 80% N2). One loop full of each culture is subsequently streaked onto new Blood Agar Base No 2 plates, which are incubated for 24 h. Cells are harvested from plates with 2 ml phosphate buffered saline (PBS) (Oxoid BR0014, UK) and mixing with a Drigalski spatula. The inoculum is diluted to OD600 = 0.1 which corresponds to approximately 8 log10 CFU/ml. Subsequently, the inoculum is diluted to approximately 7 log10 CFU/ml in Buffered Peptone Water (BPW, Oxoid CM0509, UK), (Birk et al., 2006).

Preparation of broiler meat samples. Frozen *Campylobacter* negative broiler breast fillets are thawed over night at 5 °C. The breast fillets covered with fascia are levelled to a thickness of 0.5 cm and cut into smaller samples using a stainless steel plug centre bit with a 35 mm diameter. Each piece of meat is placed on gauze in a Petri dish. Samples are stored at 5 °C ± 2 °C until use (maximum 2 h), while kept inside a plastic bag with a wet towel to prevent desiccation. (Riedel et al., 2009.)

I**noculation of meat samples**. An amount of 50 µl of inoculum (corresponding to approximately 5.7 $\log_{10}$ cfu) is added carefully with a pipette within seconds by letting the pipette gently touch the meat surface and leave a few microliters at a time (Riedel et al., 2009). To allow the settlement of the cells, the meat is left at room temperature for 20 min, before treatment.

Treatment. The model allows for test of all sorts of soluble chemicals. An example is given below.

Treatment with the formulated product. Formulated products of 40 ml and sterile water are kept in glass bottles at room temperature, and separate solutions are used for treatment of each meat sample. Meat samples are dipped into the solution or water (controls) with a pair of tweezers. These dipping treatments are conducted for 15 s (may vary depending on the reaction time of the chemical), immediately followed by microbiological analysis.

Microbiological analyses. Counts of thermotolerant *Campylobacter* are determined stomaching individual meat samples and gauze for 2 min in 100 ml Maximum Recovery Diluent (MRD) (BD 218971, USA) in a stomacher for 2 min followed by 10 fold serial dilutions in MRD. (The large rinse volume is applied to quickly dilute any chemical solution left on the surfaces of the skin or meat samples. For experiments where lower initial inoculation levels are applied, smaller amounts of MRD might be used to allow for easier detection). From appropriate dilutions, five times 10 µl are spotted onto *Campylobacter* selective Abeyta-Hunt-Bark agar plates (AHB) with 1% triphenyltetrazoliumchloride (Rosenquist et al., 2006). All plates are incubated under microaerobic conditions for 40 ± 4 h at 41.5 ± 1 °C and then the number of *Campylobacter* was counted.

Presentation of results. Concerning the data analysis, the bacterial counts (CFU per sample) are log transformed to fit a normal distribution of the data. Samples in which *Campylobacter* is present but below the detection limit are given a value of one-half of the detection limit. The analysis of variance is carried out using a statistical software. An α-value of 0.05 is used as the level of significance.

In the example above, a rinsing procedure is not included in the study design. The reason for this is that such procedures may vary and it was regarded meaningless to try to simulate such uncharacterized procedures.

APPENDIX B

EXAMPLE OF AN EXPERIMENTAL PROCEDURE FOR TESTING THE EFFICACY OF CHEMICAL SOLUTIONS IN REDUCING *CAMPYLOBACTER* ON BROILER CARCASSES AT SLAUGHTER

For testing the efficacy of decontamination agents in reducing the *Campylobacter* contamination of poultry carcasses, a sample size calculation has to be performed (see Appendix C). Considering a high within-flock prevalence (flocks fully contaminated by *Campylobacter* will be selected), a sample size of 50 carcasses is sufficient to obtain statistical sound results.

Broiler flocks. Carcasses or breast fillets (depending on the method) from *Campylobacter* positive broiler flocks processed on different days in a slaughter plant should be used. One week prior to slaughter, the flocks should be examined and found *Campylobacter* positive by sampling and analysis of sock-samples using a PCR-method (Lund et al., 2003).

Chemical solutions. Different chemicals and method of application can be investigated. Whole carcasses are treated with a chemical solution and a control group is treated with sterile water applied the same way as the chemical solution.

After treatment with chemical solutions or sterile water (controls) carcasses are washed in order to rinse of the chemical solutions and controls are washed similarly.

Sample preparation. Carcasses are prepared as described by the FDA (U.S. Food and Drug Administration, 2001) with minor modifications. Each carcass is placed in a 3500 ml stomacher bag with filter (Bie & Berntsen A/S, Denmark). An amount of 200 ml 0.1% buffered peptone water is added (BPW; consisting of 10.0 g peptone (BD 211677), 17.5 g sodium chloride (Merck 1.06404.1000), 3.5 g disodium hydrogen sulphate (Merck 1.06404.1000), 1000 ml distilled water). The bag is then sealed and the content manually massaged for 2 min. Next, the bag is tilted to let the liquid flow to one corner. The bottom corner is sanitized with 70% ethanol and cut off with a sterile scissor. Holding back the carcass and the filter, the rinse is poured into a 250 ml sterile centrifuge tube, which is kept at 4 °C for a maximum of 24 h before analysis. Finally, the rinse is centrifuged at 13,000 x *g* for 15 min, the supernatant is discarded, and the pellet resuspended in 10 ml 0.1% BPW (Boysen and Rosenquist, 2008).

Microbiological analysis. Naturally occurring thermotolerant *Campylobacter* in the chicken rinse are enumerated in accordance with the direct plating technique described by Rosenquist *et al*. *(*Rosenquist et al., *2006)*. Ten-fold dilutions of the chicken rinse are made in BPW, and 0.1 ml of the dilutions is plated onto Abeyta-Hunt-Bark agar containing 0.1% triphenyl tetrazolium chloride for red-staining of colonies (Rosenquist et al., 2006).

Presentation of results. Concerning the data analysis, the bacterial counts (CFU per sample) are log transformed to fit a normal distribution of the data. Samples in which *Campylobacter* is present but below the detection limit are given a value of one-half of the detection limit. The analysis of variance is carried out using a statistical software. An α-value of 0.05 is used as the level of significance.

APPENDIX C

STATISTICAL APPROACH FOR EFFICACY ASSESSMENT IN FIELD SITUATION OF A SUBSTANCE USED FOR DECONTAMINATING POULTRY CARCASSES

In order to demonstrate that a substance, for which authorisation is sought, has efficacy in reducing the contamination of pathogen microorganisms on treated poultry carcasses, two different aspects have to be evaluated: the effect on the prevalence of positive carcasses of slaughtered poultry (Part A), and the effect on the level of contamination (Part B).

In order to evaluate both these effects, we will consider two populations under study: chicken carcasses treated with a substance, and chicken carcasses treated with water. The study will be conducted in slaughterhouses, where a single batch of poultry will be randomly subdivided into two groups: treated with decontaminant and treated with water. Two conditions have to be fulfilled:

- it is necessary to select for the study batches of poultry likely to be positive at the slaughterhouse: this will be achieved selecting flocks that resulted positive in a control performed at the farm within the three weeks before the date of slaughter (as foreseen in national control programs);
- at the slaughterhouse, treated and non treated carcasses must be processed in the same way, in order to ensure that no variables other than the treatment are present in the two sub populations.

Among completely randomised designs, we will choose a superiority study, where one treatment (decontamination) is thought likely to be better than the use of water only, assuming a null hypothesis that there is no difference, which may then be disproved.

Part A

In order to assess the reduction in the proportion of positive carcasses, the following study design to be applied at the slaughterhouse is proposed.

We are in this case interested in evidencing a difference between proportions of presence of the event in treated (**T**) and non treated (**C**) chicken carcasses:

The sample size will be defined taking into account which level of error the study can tolerate. A sampling scheme is proposed, considering the following criteria:

- alpha= 0.05
- beta= 0.2 (power = 1-β = 0.8)
- prevalence reduction to be highlighted = 50% (at least)

The scheme will have to be adapted on a case-by-case basis, considering specific situations related to the compound under study, the processing plant, the sanitary situation of treated flocks.

Assumptions:

prevalence in C = 15.8% (CI=11.1-21.2; CL=95%) ;

prevalence in T = 8% (assumed that the treatment reduces the prevalence of at least 50%);

The sample size is calculated according to Thrusfield (2007), and the results are shown in Table 3.

Table 1: Table 3. Number of carcasses (ss) to be tested for each group according to the expected prevalence for C (p_c) and the expected (or desired) prevalence (p_t) according to the expected (or desired) prevalence reduction (Pr_50; Pr_60; Pr_70).

	Pr_50%		Pr_60%		Pr_70%	
p_c	p_t	ss	p_t	ss	p_t	ss
10	5	341	4	222	3	152
16	8	202	6.4	132	4.8	90
20	10	156	8	102	6	70
30	15	94	12	62	9	43
40	20	64	16	42	12	29
50	25	45	20	30	15	21
60	30	33	24	22	18	16
70	35	24	28	16	21	12
80	40	17	32	17	24	9

In conclusion, in the described example, 202 carcasses have to sampled for each group (treated and controls) in order to identify a 50% reduction in prevalence (from 16% to 8% of positive carcasses). All the carcasses will be submitted to a qualitative test for the detection of the pathogen under study. In case of higher prevalence in the control group, the number of carcasses to be sampled will be reduced according to table 3.

Part B: estimate differences between means

This part of the study is aimed at evaluating the efficacy of the formulated product in reducing the level of carcasses contamination, comparing treated (**T**) and non treated (**C**) chicken carcasses

According to Lorimer and Kiermeier (2007) in this kind of analysis it is important to consider both positive and negative samples, in order to avoid possible overestimation of the mean concentration of pathogens on the carcasses if only positive samples are considered. Negative samples in fact are the ones in which the concentration falls under the limit of detection (LoD) of the quantitative test, but their true concentration is not always zero, being comprised between zero and LoD. Consequently, the most appropriate statistical method to estimate the mean of the concentration in the two groups, and therefore the mean difference, is the censored regression approach.

On the basis of this approach, considering the situation described in part A (prevalence of group C~16%, prevalence of group T~8%), all the carcasses under study (202) will be included also in the quantitative evaluation. From the laboratory point of view, it will be possible to submit to quantitative examination only the carcasses that resulted positive in the qualitative test.

In different situations, with a higher prevalence of positive carcasses, the number of carcasses to be included in the quantative study will be smaller: e.g. 100 with a prevalence up to 50%, 50 with higher prevalences. In all this cases it will be possible to identify a difference of 0.5 $\log_{10}$ between the mean concentration of the two groups, with a percentage > 80% of tests found to be statistically significant using a significance level of 0.05 (table 4).

In any case, results will have to be elaborated using the censured regression model, as described by Lorimer and Kiermeier (2008). For the simulation of data with a high proportion of censored data (low expected prevalence), the study by Helsel (2005) has been taken into account.

906 **Table 2:** Table 4: number of carcasses to be sampled for different prevalence and different
907 differences to be estimated

	Other simulated scenarios												Lorimer results								
Expected prevalence in C	17,03			26,05			37,05			49,3			72,99			89,2			96,87		
Number of carcasses to be sampled	50	100	200	50	100	200	50	100	200	50	100	200	20	30	50	20	30	50	20	30	50
Estimated mean difference*	0,66	0,52	0,5	0,514	0,49	0,49	0,439	0,49	0,5	0,50	0,5	0,5	0,4849	0,5034	0,507	0,5047	0,503	0,508	0,4879	0,512	0,495
% **	49	73,1	96,1	57,7	85,8	99,1	68,7	92,6	99,7	75,60	95,1	99,9	45,8	62,5	83,16	49,81	66,6	87,08	51,4	64,6	86,36

* Estimated mean difference for each scenario for the censored approch, averaged over the 1000 simulations

** Percentage of tests found to be statistically significant (p<0.05) from 1000 simulations for each scenario

908
909

DEFINITIONS

ANTIBIOTIC

A substance produced by, or derived (chemically produced) from a micro-organism that selectively destroys or inhibits the growth of other micro-organisms (ECDC, EMEA, EFSA, SCENIHR, 2009).

ANTIMICROBIAL

An active substance of synthetic or natural origin which destroys bacteria, suppresses their growth or their ability to reproduce in animals or humans, excluding antivirals and antiparasites (ECDC, EMEA, EFSA, SCENIHR, 2009).

ANTIMICROBIAL ACTIVITY[9]

It is the inhibitory or lethal effect of a decontamination agent or an antibiotic.

ANTIMICROBIAL RESISTANCE

The ability of micro-organisms of certain species to survive or even to grow in the presence of a given concentration of an antimicrobial that is usually sufficient to inhibit or kill micro-organisms of the same species (ECDC, EMEA, EFSA, SCENIHR, 2009). Of primary concern is the emergence of resistance to therapeutic antimicrobials, defined as antimicrobials used for treatment of diseases in humans and animals.

CO-RESISTANCE

Genes conferring AMR are frequently contained in larger genetic elements such as integrons, transposons or plasmids, and as such may be linked to other, unrelated resistance genes. In such cases, multiple resistance genes may be transferred in a single event. When two or more different resistance genes are physically linked, this is termed “co-resistance”. Consequently, selection for one resistance attribute will also select for the other resistance gene(s), termed co-selection (ECDC, EMEA, EFSA, SCENIHR, 2009).

CROSS-RESISTANCE

It is the tolerance to a usually toxic substance as a result of exposure to a similar acting substance. Antimicrobials are a diverse group of molecules, commonly ordered in classes with similar structure and mode of action. Within a class, the target in the bacterial cell and the mode of action of the antimicrobial is the same or similar in each case. Some mechanisms of resistance will confer resistance to most or all members of a class, i.e. cross-resistance (ECDC, EMEA, EFSA, SCENIHR, 2009).

DECONTAMINATION AGENTS

These are substances applied to remove or reduce surface contamination of food. When decontaminants are used on food, the substance is considered a processing aid if removed following the application. If the substance is not removed, it will be classified as a food additive (it remains present in the food and has a technological effect, e.g. a preservative action; a food additive can also be applied on the surface of food e.g. glazing agents).

946 **DISINFECTION**[9]

947 The reduction, by means of chemical agents and/or physical methods, of the number of
948 microorganisms in the environment, to a level that does not compromise food safety on suitability.

949 **ECOTOXICOLOGICAL RISK**

950 The ecotoxicological risk is assessed by taking into account the hazards (substances discharged in the
951 environment) characterized by toxicological studies on different representative environmental species
952 and the exposure of these species depending on the chemical and physical properties of the substance
953 , environmental characteristics ,duration and route of exposure . The use of bio monitors is frequent
954 for the routine surveillance.

955 **ECOTOXICOLOGY**

956 Science dealing with the fate and effects of pollutants on ecosystems.

957 **FOOD ADDITIVES**[10]

958 Any substance not normally consumed as a food in itself and not normally used as a characteristic
959 ingredient of food, whether or not it has nutritive value, the intentional addition of which to food for a
960 technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or
961 storage of such food results, or may be reasonably expected to result, in it or its by-products becoming
962 directly or indirectly a component of such foods.

963 **FORMULATED PRODUCT**

964 The ready-to-use product for which authorisation is sought.

965 **PROCESSING AIDS**[10]

966 Processing aid shall mean any substance which (i) is not consumed as a food by itself; (ii) is
967 intentionally used in the processing of raw materials, foods or their ingredients, to fulfil a certain
968 technological purpose during treatment or processing; and (iii) may result in the unintentional but
969 technically unavoidable presence in the final product of residues of the substance or its derivatives
970 provided they do not present any health risk and do not have any technological effect on the final
971 product;

972 **MULTIDRUG RESISTANCE**

973 This term is used when a bacterial strain is resistant to more than one antimicrobial or antimicrobial
974 class. There is no standard definition, which makes the term problematic and comparisons difficult. It
975 is therefore important to define multidrug resistance in any document referring to 'multiple
976 resistance'. Traditionally multidrug resistance is regarded as resistance to at least three different
977 chemically-unrelated classes of antimicrobials, and is frequently transmissible. Strains exhibiting such
978 resistance are termed 'multidrug-resistant' (MDR) (ECDC, EMEA, EFSA, SCENIHR, 2009).

979

[9] CAC/RCP 1-1969, Rev. 4-2003: Recommended international code of practice: General Principles of food hygiene

[10] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives.

ABBREVIATIONS

AMR	Antimicrobial Resistance
CAS	Chemical Abstracts Service
CLSI	Clinical and Laboratory Standards Institute
EFSA	European Food Safety Authority
EPER	European Pollutant Emission Register
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GRAS	Generally Recognised As Safe
HACCP	Hazard Analysis and Critical Control Points
IPPC	Industrial Pollution Prevention and Control
IUCLID	International Uniform ChemicaL Information Database
IUPAC	International Union of Pure and Applied Chemistry
MBC	Minimal Biocidal Concentration
MDR	Multi Drug Resistance
MIC	Minimal Inhibitory Concentration
PBT	Persistent, Bioaccumulative and Toxic
PE	Population Equivalents
PEC	Predicted Effect Concentration
PNEC	Predicted No Effect Concentration
RAR	Risk Assessment Report
REACH	Registration, Evaluation, Authorisation and restriction of Chemicals (Reg. 1907/2006)
SCENIHR	Scientific Committee on Emerging Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SCVPH	Scientific Committee on Veterinary Measures Relating to Public Health
TGD	Technical Guidance Document
vPvB	very Persistent and very Bioaccumulative)
WWTP	Waste Water Treatment Plant