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Advice of the Director of the Office for Risk Assessment and Research

Advice on the suitability of VR 2827-3 as a disinfectant for slaughtering equipment in the meat processing industry

Background

During the slaughter process, there is a risk of the transfer of pathogenic microorganisms from one carcass to another if the slaughtering equipment used is contaminated with these pathogens. In order to avoid this cross-contamination, between the processing of the various carcasses, knives and other instruments must be disinfected with hot water at 82ºC, according to 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 (*Hygiëneverordening*). The duration of this disinfection is not defined in the Regulation. In practice, it means immersing (cleaned) tools in hot water in a single movement.

Slaughterhouses are required by law to have the necessary facilities for disinfecting slaughtering equipment with hot water at 82ºC, but an alternative with at least the same effectiveness can be allowed.

As there are disadvantages associated with immersion in hot water, namely steam and condensation formation and coagulation of proteins on the slaughtering equipment, in 2010 the then Food and Consumer Product Safety Authority (VWA: *Voedsel- en Warenautoriteit*) authorised a slaughterhouse to use the disinfectant INSPEXX 200, which had at that time not yet been authorised by the Dutch Board for the Authorisation of Plant Protection Products and Biocides (Ctgb: *College voor de toelating van gewasbeschermingsmiddelen en biociden*) as an alternative product. In 2014, INSPEXX 200, under the name ‘INSPEXX 210’, was authorised by the Ctgb as a biocide for use as a disinfectant for surfaces and materials in the food industry, with a legally prescribed 5-minute exposure time and a concentration of 0.5%. The authorisation of INSPEXX 210 by the Ctgb means that the product INSPEXX 200 may no longer be used on the basis of the authorisation previously granted by the Netherlands Food and Consumer Product Safety Authority (NVWA: *Nederlandse Voedsel- en Warenautoriteit*), but that application is only permitted according to the Ctgb’s instructions for use.

A manufacturer has recently introduced the product VR 2827-3, which is equivalent in composition to INSPEXX 210. The slaughterhouse/manufacturer wants to carry out a test with this product at a slaughterhouse, the idea being that all disinfection units in the slaughterhouse will use this product. The authorisation (dispensation) for such a test was granted by the Ctgb to the

slaughterhouse. However, the Ctgb indicates that the NVWA should determine whether it considers this product safe for use in a food business.

For this reason, the Director of the Directorate for Inspection of the NVWA asked the NVWA's Office for Risk Assessment and Research (BuRO: *Bureau Risicobeoordeling & onderzoek*) for an advice on the suitability of VR 2827-3 as a disinfectant for slaughtering equipment in the meat processing industry. This first of all concerns the use of VR 2827-3 during a test in a cattle slaughterhouse, where all disinfection units will use VR 2827-3.

Approach

BuRO used the 2008 advice as a starting point to assess the relevant developments in the last 10 years regarding use of the product in the meat processing industry. The current advice is limited to the risk of cross-contamination by contaminated knives and other slaughtering equipment. This means that only pathogens from the skin or intestines that can end up on the meat, or grow on slaughtering equipment, through the use of knives are taken into consideration. The reason for this is that although the transmission between carcasses of pathogens present *in* the meat via slaughtering equipment is not inconceivable, their transfer via the cutting surface is minimal, so that its contribution to the total human burden of disease from food-related infections is assumed to be negligible.

BuRO asked the RIVM-RIKILT Front Office for Food and Product Safety ('Front Office') questions about the expected effectiveness of the product VR 2827-3 (/ INSPEXX 200 /INSPEXX 210) under the proposed conditions, taking into account the relevant developments since 2008 and questions about the suitability of the trial design.

Documents provided by the Ctgb and the slaughterhouse/the manufacturer have been included in the assessment. Where necessary, additional information has been requested via the Directorate for Inspection. In addition, BuRO itself consulted additional sources and through the EFSA Focal Point asked foreign partners about their possible experience in testing alternative disinfection systems in slaughterhouses.

Findings

Disinfection of slaughtering equipment is intended to prevent carcass-to-carcass contamination. The legal standard for disinfecting slaughtering equipment is to use hot water at 82°C, and an alternative system must have a proven minimum equivalent effect. The slaughterhouse proposes VR 2827-3/INSPEXX 210 as an alternative disinfectant, with a shorter exposure time of ≤1 second and a lower concentration of 0.16% than the legal conditions for use (5 minutes and 0.5%) for which INSPEXX 210 has been approved by the Ctgb, in a field trial for a period of 2 years in all disinfection units of a bovine slaughterhouse. In this process, the slaughtering equipment used is not additionally disinfected with hot water at 82°C, or with an approved alternative, before coming into contact with the following carcass. This entails risks of a microbiological and toxicological nature with regard to the product VR 2827-3 and methodological nature with regard to the trial design, which may make it impossible to guarantee food safety. Toxicologically, on the basis of EFSA scientific opinions (EFSA, 2005, EFSA, 2014), no risk of residues of VR 2827-3 on the meat or of interaction of the product with organic material are expected.

There is not enough information available, for example from laboratory experiments, to determine whether with a short contact time of ≤1 second and concentration of 0.16% VR 2827-3 is as effective as hot water at 82°C against

pathogens that can be transmitted during slaughter and boning of animals. In addition, the contact time of ≤1 second for both 0.16% VR 2827-3 and hot water at 82°C may be too short to sufficiently disinfect slaughtering equipment. There are indications from BuRO's Red Meat Supply Chain Risk Assessment (*Risicobeoordeling Roodvleesketen*) that the current method in slaughterhouses is not always adequate to prevent carcass-to-carcass contamination. A scientific study by Toarmina & Dorsa (2007) shows limited efficacy with hot water at 82°C with a contact time of less than 1 second and improved efficacy with a longer contact time. If the short exposure time is not sufficient to reduce the numbers of relevant pathogens, there is a risk of cross-contamination and therefore a risk to public health.

There are still unanswered questions about the trial design. It is not clear how it is ensured that sampling represents daily practice. The statistical analysis and substantiation of the number of samples to be taken have not yet been described in detail. The duration of two years for the test is unnecessarily long. A more intensive sampling frequency may provide the same data over a shorter period. This is important, given the fact that the meat is placed on the market during the test. In addition, as also highlighted by the EFSA (EFSA, 2005) and RIVM (Appendix I), a good outcome of the test does not yet demonstrate that the results apply to slaughterhouses of other animal species.

The responses of 16 of 30 EFSA Focal Points to our data request show that one country, namely Ireland, has experience in assessing field trials with VR 2827-3 or a similar product as an alternative disinfection system in cattle slaughterhouses. The Irish authorities prescribe the necessary phased steps to be taken by food businesses in order to be allowed to test alternative methods in the meat processing industry. Since the next phase can only start once the results from the previous phase have been approved, food safety is guaranteed for the products produced during the field trials.

Advice of BuRO

To: the Inspector-General of the Netherlands Food and Consumer Product Safety Authority (NVWA)

- Use a phased method for the slaughterhouse/manufacturer and other slaughterhouses, in which the following elements are important for testing alternative disinfectants in slaughterhouses in accordance with the Hygiene Regulation (EC/853/2004):
    - Appropriate validation data, demonstrating the efficacy of the alternative to hot water at 82 C, for the process-related parameters (viable cell count for aerobic mesophilic microorganisms, *E. coli, Enterobacteriaceae*, *Salmonella*) *and* relevant pathogens per animal species (for cattle: *Campylobacter, Listeria*, pathogenic *E. coli*). In addition, for parameters aimed at the specific efficacy of the product (for VR 2827-3: microorganisms capable of producing catalase and/or peroxidase, e.g. *S. aureus*). Simulate practice by, for example, 'spiking' knives that are similar to those contaminated in the slaughterhouse with fat and organic material of the animal species concerned. If agreed, the design of the validation test can be started.
    - A detailed trial protocol for the validation test in a slaughterhouse, including the following considerations:
        1. Selection of at least 4 points requiring disinfection including the most heavily fat and organic contaminated points, possibly prior to the disinfection step a cleaning step in case of visible contamination. If agreed, Phase I of the field trial can be started.

2. Phase I: the establishment of a baseline for hot water disinfection at 82°C, where i) immediately after use and prior to disinfection and ii) immediately after disinfection, the presence of process-related parameters (viable cell count for aerobic mesophilic microorganisms, *E. coli, Enterobacteriaceae, Salmonella)* on the slaughtering equipment is determined, keeping track of the contact time. If approved, Phase II of the field trial can be started.
3. Phase II: the determination of the effectiveness of the alternative system, where i) immediately after use and prior to alternative disinfection and ii) immediately after alternative disinfection, the presence of process-related parameters (viable cell count for aerobic mesophilic microorganisms, *E. coli, Enterobacteriaceae, Salmonella)* on the slaughtering equipment is determined and the contact time is maintained. In this phase, double disinfection takes place by disinfecting with hot water at 82°C. If approved, Phase III of the field trial can be started.
4. Phase III: testing use of the alternative system, where i) immediately after use and prior to alternative disinfection and ii) immediately after alternative disinfection, the presence of process-related parameters (viable cell count for aerobic mesophilic microorganisms, *E. coli, Enterobacteriaceae, Salmonella)* on the slaughtering equipment is determined and the contact time is maintained. If approved, NVWA will consider approval of the system, as well as the conditions attached to it.

- Sampling, subsequent treatment and microbiological measurements should take place according to recognised standards, i.e. ISO, and accredited tests. Samples should be taken at each disinfection point in each Phase of the test on 2 production days throughout the day, as well as before the start of the day, and at the end of the day. Take a sample frequency of at least 1 sample per 100 carcasses.

-Enforce with respect to application of VR 2827-3 in other slaughterhouses, because results from a particular slaughterhouse cannot be extrapolated.

Yours faithfully,

Prof. Antoon Opperhuizen
Director of the NVWA Office for Risk Assessment and Research (BuRO)

SUBSTANTIATION

Background

Many different bacteria and viruses occur on or in slaughter animals. Some of these can end up on the meat through contaminated slaughtering equipment and cause disease in humans through consumption of the meat. Disinfection of slaughtering equipment is intended to prevent carcass-to-carcass contamination. The slaughtering equipment used varies between the different types of slaughterhouses for pigs, cattle and poultry, and possibly also for other animals such as horses, goats, sheep and game. Traditionally used slaughtering equipment for slaughtering cattle, calves, pigs, horses, lambs or sheep in abattoirs include (Baretta *et al.*, 1955): bolt device for stunning, sticking knife for cutting the throat, splitting knife, skinning knife, meat hooks for skinning, saw, cleaver, spinal and breast irons, sheath for storing knives, butcher's steel for sharpening knives, collecting troughs for blood and gut material, trolley for abdominal organs, 'stootbeen' for removing the skin, handbarrow. Specifically for calves, pigs and sheep a spreader, and specifically for pig scrapers. For cattle, calves, horses, lamb and sheep, slaughtering in slaughterhouses is still largely done manually nowadays. For pigs and poultry, a large proportion is done with machines and robots. For pigs, slaughter robots, rectal guns and carcass splitters are used. For chickens, almost the entire slaughtering process is automated, with cooling baths also being used to cool carcasses.

Regulation (EC) No 853/2004, i.e. the Hygiene Regulation, stipulates in Annex III, Section I (Chapter II, point 3): *'They* [i.e. slaughterhouses] *must have facilities for disinfecting tools with hot water supplied at not less than 82ºC, or an alternative system having an equivalent effect.'* In 2007, the slaughterhouse made use of this possibility within the Regulation to test the efficacy of the product INSPEXX 200 as an alternative system. In 2007, the slaughterhouse was granted dispensation from the then VWA to use the product for one month during a trial in its pig slaughterhouse in Helmond. The slaughterhouse has drawn up a confidential report on this trial. It describes the potential public health risks and the effectiveness of the use of INSPEXX 200, with short exposure time, on slaughter robots in a pig slaughterhouse under typical conditions. The effectiveness was determined for indicator organisms *Salmonella, Enterobacteriaceae* and the viable cell count for aerobic mesophilic microorganisms, and was at least equal to hot water at 82°C.

In its 2008 advice, the NVWA indicates that the effectiveness of disinfection and any toxicological risks to humans did not prevent the use of INSPEXX 200 for disinfection of slaughter robots (carcass chopper and anus drill) during the slaughter process. Before INSPEXX 200 could be used for the described application, it first had to be registered and authorised according to the biocides authorisation policy implemented by the Board for the Authorisation of Plant Protection Products and Biocides (Ctgb). In January 2010, the NVWA authorised the implementation of the alternative method provided that the concentration of the product is included as a critical point in the HACCP system.

In 2014, INSPEXX 210 was authorised by the Ctgb as a biocide against bacteria and yeasts including a legal prescription for use, namely a 5-minute exposure time and a concentration of 0.5%. An certificate of equivalence has been issued by the manufacturer stating that the active substances and formula of INSPEXX 200 and INSPEXX 210 are the same. The authorisation by the Ctgb of INSPEXX 210 had the legal consequence that the product INSPEXX 200, i.e. the product with the same composition but with a short exposure time, could no longer be used on the basis of the authorisation previously granted.

The slaughterhouse indicates, for no specified reason, that a 5-minute exposure time is not workable for a slaughterhouse and that the legal conditions for use are based on the reduction of the number of yeasts. The slaughterhouse now wishes to examine this in a field trial using the product VR 2827-3, for which a certificate of equivalence has also been issued indicating that VR 2827-3, INSPEXX 200 and INSPEXX 210 are the same. Although the authorisation (dispensation) for such a test was granted by the Ctgb to the slaughterhouse, the Ctgb indicates that the NVWA should consider whether it considers this product safe for use in a food business.

Hazard Identification

Disinfection of slaughtering equipment is intended to prevent carcass-to-carcass contamination. If an alternative method of disinfection is used, there is a risk that it will be less effective than hot water at 82°C. This results in there being a risk that pathogens might have a higher survival rate and may be able to pass from one carcass to another via slaughtering equipment. This effectiveness may vary per animal species to be slaughtered and per pathogen. The contribution to the disease burden thus varies between pathogens and species to be consumed. There is also a chance that the product itself may have a toxicological effect, where the application per animal species to be slaughtered may differ. In accordance with the law, when applications are made for a test with a biocide, the Ctgb assesses only the risks to man and the environment, but may, if necessary, impose conditions on the conduct of the test. The Ctgb indicates that food safety remains the responsibility of the slaughterhouse and other laws and regulations, such as the Hygiene Regulation. This advice therefore considers the potential microbiological and toxicological hazards per species and pathogen, taking into account public health, since it concerns a field trial in a food business whose products are placed on the market for consumption.

Hazards of insufficient neutralisation of pathogens on slaughtering equipment
In case of visible contamination, slaughtering equipment is cleaned and disinfected. This will in any case be done if an infectious disease or abscess in the slaughtered animal is only detected during slaughter, or if in a step in the slaughtering process the slaughtering equipment or carcass is contaminated with, for example, intestinal contents or stomach contents. The standard method for disinfecting slaughtering equipment is to use hot water at 82°C. The advice of BuRO's *'*Red Meat Supply Chain Risk Assessment*' (Risicobeoordeling Roodvleesketen)* (NVWA-BuRO, 2015)states that immersing knives in hot water of at least 82°C after each use on a carcass is a minimum requirement. When using a so-called '2-knife system', where the first knife is disinfected and work is continued with the second knife, the contact time will be longer than with immersion.

BuRO's *Red Meat Supply Chain Risk Assessment* and a report from the RIVM on the burden of disease of food-related pathogens in the Netherlands in 2017(Mangen, 2018) describe the following pathogens as a hazard for infection via beef, lamb, pigs and poultry: the Gram-negative *Campylobacter* spp., STEC O157, *Salmonella* spp.; the Gram-positive *Listeria monocytogenes*, *B. cereus* (toxin), *C. perfringens* (toxin), *S. aureus* (toxin); the parasites *Cryptosporidium* spp., *Giardia* spp., and *Toxoplasma gondii*. In addition, rotavirus and hepatitis E viruses are also relevant as pathogens in pork. Yeasts are not listed as relevant pathogens with regard to the burden of disease of food-related pathogens; no cases of disease are known through this route. No specific disease burden figures are available for horse meat, goat meat, and lamb and mutton.

For carcass-to-carcass contamination from the skin or intestines to the meat via knives or other slaughtering equipment, the following pathogens have been considered as dangerous: *Campylobacter* spp., pathogenic *E. coli* such as STEC, *Salmonella* spp., *Listeria monocytogenes*, hepatitis E virus.

- *Campylobacter* spp.: there are several species, the most quantitatively important of which being *Campylobacter jejuni*. Animal reservoirs include poultry, pigs, cattle, sheep and pigs. Disease symptoms during 5–7 days are mild gastrointestinal complaints, flu, (bloody) diarrhoea and abdominal pain. Complications that may occur are intestinal inflammation and joint problems. In exceptional cases, permanent symptoms such as Guillain Barré syndrome may occur. In vulnerable groups, the infection can be life-threatening. Risk groups are people with reduced resistance, pregnant women, young children and the elderly. The main source of infection is via poultry or poultry products, but infection can also be acquired through insufficiently heated pork or beef.
- STEC: STEC O157 is one of the most pathogenic shigatoxin producing *E. coli*, but other serotypes can be equally pathogenic. In particular, cattle and sheep are reservoirs. It causes symptoms of mild to bloody diarrhoea. When the toxin enters the bloodstream, it may cause haemolytic uraemic syndrome (HUS) and in some cases permanent kidney damage. A serious course of infection can be fatal. Risk groups are people with reduced resistance, pregnant women, young children and the elderly. Contamination is mainly caused by the consumption of undercooked beef.
- *Salmonella* spp.: There are many different *Salmonella* types (approx. 2,500), of which *Salmonella* Typhimurium and *Salmonella* Enteritidis are most common in the Netherlands. Animal reservoirs include chickens, pigs and cattle. Pathology consists of symptoms of gastroenteritis, which persist for 3 to 7 days. In exceptional cases, the bacteria may enter the bloodstream and affect organs, bones and joints. A serious course of infection can be fatal. Risk groups are people with reduced resistance, pregnant women, young children and the elderly. Contamination is contracted through insufficiently heated poultry meat, veal and pig meat.
- *Listeria monocytogenes*: This is the *Listeria* species that can cause disease in humans. It is an environmental bacterium that occurs everywhere in the environment and often causes recontamination. Many animal species, including pigs, are reservoirs. With normal immunity it results in flu-like symptoms. If the immune system is weakened, the infection may result in a serious course of blood poisoning, meningitis, or inflammation of the inner lining of the heart. In pregnant patients, it can cause miscarriage or a premature birth. Healthy people don't usually get sick from this bacterium. Pregnant women, newborn babies, the elderly and people with a weakened immune system are more likely to be infected. Contamination is mainly caused by chilled products of animal origin, such as cold cuts, minced meat, steak tartare and paté, especially if chilled for lengthy periods. In the case of pigs, the bacteria may be present in the tonsils and may constitute a source of contamination of the meat and slaughter equipment at the time of slaughter.
- Hepatitis E virus: Especially genotype 3 occurs in intestines and blood of pigs, and can cause disease in humans. Disease symptoms are generally mild with fever and liver inflammation. In vulnerable groups, chronic hepatitis and cirrhosis of the liver can develop. The risk groups are not yet fully clear, but pig farmers and staff of pig slaughterhouses have an increased risk of infection. The route of infection of genotype 3 is not yet fully clear, but is likely to occur through the consumption of undercooked pork.

In addition to the relevant pathogens mentioned above, yeasts are also included in this risk assessment because they are the subject of the trial and, as indicated in the Ctgb assessment, may not be disposed of at low concentration or short contact time with VR 2827-3.

- Yeasts, together with fungi, form a separate group of microorganisms. Yeasts can be an indicator of spoilage of a product, but they can also be used to produce food. In general, yeasts are not pathogenic; however, yeasts may produce mycotoxins. Some mycotoxins may cause acute food poisoning, others may have long-term carcinogenic effects. The yeast Candida occurs in cattle, and is carried by people in places including the mouth and intestine. If the immune system is weakened or if certain antibiotics are used, the yeasts may form filaments, which will lead to a fungal infection with white, painful spots or 'plaques' in the mouth. Infection is generally caused by host-related factors and it is unknown whether beef consumption plays a role in this. As far as known, no cases have been reported via the route.

Hazard of disinfection of slaughtering equipment with a short contact time
The degree of 'sufficient reduction of the numbers of pathogens' should be based on comparison with the reduction of the numbers of pathogens with hot water at 82°C. Regulation (EC) No. 853/2004 (Hygiene Regulation) indicates that an alternative with equivalent effect is allowed. The minimum duration of this disinfection with hot water at 82°C is not mentioned. According to the Regulation, this may therefore involve disinfection by immersion of knives where the contact time with water and disinfectant may be less than one second. Observation studies on disinfection moments at the time of slaughter of pigs have shown that the contact time used in pig slaughterhouses is less than 1 second (Taormina & Dorsa, 2007). Taormina and Dorsa (2007) compared the effectiveness of contact time of ≤1 second with a contact time of 15 seconds with different disinfection methods (hot water at 82°C, water at 48.9°C, peroxyacid solution). A contact time of ≤1 second appears to result in an *average* reduction of less than 1 log and in a part less than 0.5 log of the investigated pathogens. A contact time of 15 seconds showed a significantly higher effectiveness in both methods (1.5-3 log reduction).

The contact time will influence the extent to which pathogens can be reduced in number with hot water at 82°C. In addition, slaughtering equipment are disinfected at times when they are visibly contaminated, and sufficient contact with the surface must be possible. Here too, a short contact time will have consequences for the extent to which the material can be disinfected of the (visible) contamination. In the description of the slaughterhouse it is indicated that the cleaning of slaughter robots to disinfect them is automatic. It is unclear whether, and, if so, how, the knives and the slaughter tools are cleaned prior to disinfection in other slaughterhouses. All types of slaughtering equipment are covered by equipment' and 'materials and surfaces in the food industry'. The instructions for use for INSPEXX 210 as included at Ctgb is as follows, and does not explicitly indicate that the surface must first be cleaned:

A. Legal conditions for use
Only use as a means of combating bacteria (excluding mycobacteria and bacterial spores) and yeasts on materials and surfaces:
– in places where food and beverages are prepared, treated or kept, with the exception of farm milk extraction equipment;
– in the food industry;
The instructions for use set out under B. must be followed.
The product is intended for professional use only.

B. USAGE INSTRUCTIONS
INSPEXX 210 is applied directly to the surfaces to be disinfected and objects must be immersed in a solution with the correct concentration.

- Dosage: dilute INSPEXX 210 with water of minimum drinking water quality to a concentration of 0.5% (5 ml per litre water). The dilution is preferably done by means of an automatic dosing device.
- Minimum exposure time: 5 minutes. In the case of immersion, always change the solution daily or – if contaminated – more frequently.

Hazard of using hot water at 82°C for disinfection
Disinfection of slaughtering equipment with hot water at 82°C means that this water is used at the low ambient temperature within a slaughterhouse, which may be 15°C. This leads to steam formation and possible condensation on the carcass in which bacteria can grow. At a temperature of 82°C, proteins coagulate. This means that, when a knife is disinfected without first being cleaned, the remaining proteins in, for example, blood residues can form a biofilm on the knife, in which bacteria have a higher chance of survival and the knife becomes less sharp.

Hazard of using VR 2827-3 as disinfectant
According to the legal conditions for use, INSPEXX 210 is for use in combating bacteria and yeasts, and not for combating viruses. While disinfection with hot water at 82°C may inactivate viruses, this effect remains unknown when using VR 2827-3. This may result in a greater chance of survival for viruses.

Using VR 2827-3 as disinfectant, instead of hot water at 82°C, also means adding a chemical substance to a disinfection step in the process that normally takes place with water. The product – containing the components peracetic acid, peroctanoic acid, hydrogen peroxide, acetic acid, octanoic acid and hydroxyethyl diphosphonic acid (HEDP) – is added to water to the required concentration. The ready-to-use peroxyacetic acid solution is formed in water. The risk from the use of peroxyacetic acid solutions in reducing the number of pathogens in poultry carcasses and poultry meat has been extensively evaluated by the EFSA (EFSA, 2005, EFSA, 2014), taking into account an evaluation of the JECFA (JECFA, 2006) (Joint FAO/WHO Expert Committee on Food Additives) with regard to the possible addition of HEDP as an auxiliary material. Possible hazards of use of the product include:

i) Toxicological hazard of VR 2827-3 due to ingestion of residue on final product, or interaction of VR 2827-3 with the organic material, namely oxidation of lipids and amino acids, peptides or proteins, i.e. production of transformation products by reaction with meat protein or meat fat that may impair quality (rancid decay).
ii) Microbiological hazard when the effectiveness of VR 2827-3 in the reduction of the number of pathogens is not sufficient
iii) Development of bacterial resistance to the product
iv) Risk of the product when it enters the environment via wastewater.

Re iv) this point has been included in the assessment by Ctgb of INSPEXX 210, together with possible risks for exposure to workers, and in the authorisation of a trial with a biocidal product not yet authorised, and has not been found to be objectionable. BuRO has obtained a declaration that INSPEXX 200, INSPEXX 210 and VR 2827-3 have the same composition.

Hazard of the trial with VR 2827-3 in the industrial setting of a cattle slaughterhouse
The trial design is intended to measure the microbiological effectiveness of the product VR 2827-3 against yeasts and bacteria when used as a disinfectant, with short exposure time, of slaughtering equipment in a cattle slaughterhouse. The aim of the trial design is for meat products to be placed on the market for consumption.

According to the protocol, a concentration of 0.16% at an exposure time of approximately 1 second (immersion) is maintained in the trial. This is different from the legal requirement for a concentration of 0.5% and 5-minute exposure time. The product is therefore used during the proposed trial at shorter exposure time and lower concentration than the legal conditions for use. Disinfection with short exposure time (i.e. immersion) and lower concentration (i.e. 220 ppm, 0.022%) has previously been investigated for bacteria in a pig slaughterhouse, and not previously in a cattle slaughterhouse. According to the Ctgb, the effectiveness against bacteria has been convincingly demonstrated on the basis of the document supplied to them and based on various studies at a 5-minute exposure time and concentration of 0.15%; the effectiveness against yeasts at a 5-minute exposure time and concentration of 0.5%; the NVWA does not know which trials the findings of Ctgb are based on. The trials known to the NVWA differ in design, so that the results are not fully comparable.

The trial protocol of the requested trial of the slaughterhouse states that samples of potentially contaminated knives are to be taken. A lower limit of 3 sampling sites shall be maintained at the beginning, middle and end of a shift in the slaughter process, taking 5 samples at each of the 3 sampling sites. A sampling round will cover 15 samples. During the first month, 1 sampling round per week will take place. In the second through sixth month 1 round per month; and in the 7th through 12th month every 2 months, in the second year every quarter. Thus, at least 180 samples will be taken in the first year; in the second year 60; in total 240 samples over a period of 2 years, equivalent to 1 sample per 1,000 bovine animals slaughtered, when 2,500 bovine animals slaughtered per week are taken. It is not clearly stated whether sampling after 1 second of exposure time is compared with sampling after 5 minutes of exposure time, i.e. whether the slaughtering equipment is always ultimately exposed to 5 minutes of exposure time. It is also not mentioned whether it is compared to immersing in hot water at 82°C, and whether visibly contaminated knives are cleaned first. It is therefore possible that visibly contaminated knives are being disinfected with a short exposure time and lower concentration than required by law. If it is compared to the 5-minute exposure time, the concentration used is still lower than prescribed by law.

The question is whether the intended number of samples is sufficient to indicate the efficacy of the product in case of low prevalence, as is expected for yeasts, for example. This is also noted by the RIVM (Appendix I), and could be calculated using a so-called ‘power calculation’. A prevalence of 2.5% with confidence interval 1.5–2.5% already requires a sample of 900 samples. In addition, the test design cannot show whether the product is at least as effective as disinfection by immersion in hot water at 82°C, as comparison is not included in the protocol. Even if it is compared to 5 minutes of exposure time, the concentration is still lower than the requirement for the equivalent of hot water at 82°C.

Danger of limiting trials to a cattle slaughterhouse
In the Ctgb description, slaughtering equipment fall under the broad term ‘materials and surfaces in the food industry’. The danger is that after a positive outcome of the trial, the product can then be registered more widely, namely for

use of materials and surfaces in the food industry, with this short exposure time. The trial has been requested as an efficacy trial for a future application. The product may thus also be used in slaughterhouses for other animals without further testing in these slaughterhouses. The application for a biocidal product authorisation does, however, require a comprehensive set of efficacy data from Ctgb and is assessed by Ctgb before the authorisation is issued. However, the application regarding the effectiveness of VR 2827-3 is only aimed at bacteria and yeasts, while hot water at 82°C may also be effective against viruses.

Hazard characterisation

Adverse effects of pathogens on slaughtering equipment

When slaughtering animals, the pathogens described below may end up on the cutting surface, possibly survive the next steps in the meat processing process and ultimately cause disease to consumers (Source: LCI Directive, Mangen, et al. 2017). In principle, 1 pathogen can already cause infection. The chance of infection increases with an increasing dose but also depends on the pathogen itself, and on the characteristics of the individual. The extent to which pathogens are infectious indicates the chance of low dose infection:

- *Campylobacter* spp.: very infectious.
- *E. coli* O157: very infectious.
- Re *Salmonella* spp.: the extent to which they are infectious is highly dependent on the type of food, where fatty food can already cause disease symptoms at a low dose.
- Re *Listeria monocytogenes*: low chance at low dose, and actually only weak target groups. It is a microbiological hazard especially after the slaughter phase.
- Hepatitis E virus: The extent to which genotype 3 occurring in pigs is infectious to humans is unknown. It seems to be of especial relevance re vulnerable target groups.
- There are many different types of yeasts; the extent to which they are infectious is unknown.

Adverse effects of using VR 2827-3 as disinfectant

<u>In the case of slaughter of poultry</u>

The effects of the use of mixtures of peroxide compounds, such as VR 2827-3, as disinfectant in slaughterhouses, has been assessed by an expert panel of the EFSA for use in poultry slaughterhouses, where the product is used for carcass decontamination (EFSA, 2014). This occurs in different ways:

- by spraying on carcasses (15 seconds)
- by adding to cooling baths for chilling of hot carcasses (1–2 hours)
- by adding to cooling baths for chilling of cold carcasses (dipping, 3 minutes)

In all these cases, residue remains on the carcass (with any subsequent action of the agent). The JECFA (JECFA, 2006) furthermore estimated the hazard of ingestion of the ingredient HEDP. It should be noted that trials with visible contamination have not been included in the EFSA assessment because decontamination should not be at the expense of hygienic working methods.

Toxicology:

- due to the instability of the product, only acetic acid and octanoic acid and HEDP remain on the end product as residue
- the quantities of residues of acetic acid and octanoic acid do not present a toxicological hazard as regards human consumption
- no decay occurs due to interaction of peroxide compounds with the organic material, because only low numbers of amino acids on the carcass surface are present on young animals such as broilers.
- following the evaluation by the JECFA (JECFA, 2006), no toxicological effect is expected for HEDP.

Microbiology:

- Data for the pathogens relevant to poultry, namely *Campylobacter* spp., *Salmonella* spp. and human pathogenic *E. coli* strains, is scarce.
- When dipping cold carcasses (3 minutes), relevant reduction of *E. coli* and coliforms has been convincingly demonstrated with 1–3 log reduction compared to the control. For *Salmonella* and *Campylobacter*, too little data are available to determine log reduction. For *Salmonella*, a significant reduction in prevalence has been observed (i.e. qualitative, present or not), but these studies were weak in design.
- Spraying (10–15 seconds) of the agent on the carcass proved less effective than dipping (3 minutes)
- In cooling baths (1–2 hours), relevant reduction of *E. coli* was observed, while reduction of coliforms was less evident. For *Salmonella* and *Campylobacter*, too little data was available on log reduction. However, a significant decrease in prevalence was observed for *Salmonella* and *Campylobacter*.
- Whether the effectiveness at the end of the shelf-life date is still perceptible, or whether only a sub-lethal effect is achieved, was not clear from the two studies on naturally contaminated products.
- The development of reduced sensitivity to VR 2827-3 (i.e. resistance) is not likely.
- The various trials did not look at viruses or yeasts.

Environment:

- The components in VR 2827-3 are neutralised before they are discharged into wastewater. Thus, no environmental impacts are anticipated.
- As a result of this neutralisation, resistance development in the environment is not plausible.
- For HEDP, a preliminary (conservative) guideline indicates that the safety of emissions of HEDP from a poultry slaughterhouse depends on specific factors and therefore cannot, a priori, be considered safe. This forms part of the Ctgb's assessment.

<u>In the case of slaughter of pigs</u>

The effects of the use of a mixture of INSPEXX 200, and thus VR 2827-3, as disinfectant in slaughterhouses was experimentally tested and assessed in the Netherlands in 2007–2008. It was tested whether the disinfectant INSPEXX 200 has an equivalent or better bacterial disinfectant effect than hot water at 82°C when disinfecting the slaughter robots (Holtslag, 2008, Verkaar & Hutter, 2008). To this end, the following was looked at:

- Impact of knife immersion (several seconds)
- Spraying on slaughtering robot (several seconds).
- Viable cell count for mesophilic microorganisms (i.e. both Gram-positive and Gram-negative bacteria)
- *Enterobacteriaceae* viable cell count (Gram-negative bacteria)
- Control samples with hot water at 82°C

In a later manufacturer's test (2013), the following was looked at:

- Impact of knife immersion on 5 points in the slaughter process
- Testing of naturally contaminated knives for bacteria and yeasts (total)
- Control samples also with INSPEXX 210, in lab spiked with *S. aureus* (Gram-positive) and *Candida albicans* (yeast).

Toxicology:

- -VR 2827-3 is not applied directly to pork, but only indirectly via equipment disinfection. The residue, including HEDP, on the pig carcass will therefore be lower than in poultry.
- -Since in poultry the direct use on carcasses with longer contact time was found not to have a toxicological risk, this assessment can also be considered to apply to indirect short-term use in pigs.
- -Although the study of the slaughterhouse in 2007–2008 was not considered sufficient to demonstrate residues on pig carcasses, it was concluded that this is a non-serious deficiency of the study because no increased health risk was expected from residues.
- -The occurrence of decay by interaction of the product with the organic material was not tested in the 2007–2008 test. The EFSA indicates that transformation products are not expected in young animals such as meat pigs, given the low amount of free amino acids and peptides on this meat.

Microbiology:

- -The slaughterhouse demonstrated in 2007–2008 that the bacterial contamination of slaughtering equipment and pig carcases when using INSPEXX 200 is reduced at least as much with short exposure times as with hot water at 82°C. This applies directly to Gram-negative bacteria and indirectly to Gram-positive bacteria (namely as part of the mesophilic plate count).
- -This does not adequately demonstrate the effect on Gram-positive bacteria such as *Listeria*, as the RIVM also concludes (Appendix I). If the ratio is 90% Gram-negative and 10% Gram-positive, it is possible that the reduction is significant, but entirely due to the reduction of the number of Gram-negative bacteria.
- -It is striking, also according to the RIVM (Appendix I), that in control samples in the second test the choice was rather made for comparison with a Gram-positive bacterium. Since the difference in reduction is significant, the question is how well the product works against the, generally more resistant, Gram-positive bacteria.
- -In one of the tests carried out by the slaughterhouse, 1 out of 15 samples were positive for yeasts, indicating that yeasts occur in pig slaughterhouses. It is not known whether yeasts play a role in carcass-on-carcass contamination in slaughterhouses and cause disease burden in humans by that route.
- -The agent may be effective against yeasts, but on the basis of 1 positive sample no judgement can be made about this.
- -Data on the most relevant pathogens for humans in pigs, namely *Salmonella* spp., is not sufficiently available.
- -Effectiveness of VR 2827-3 (compared to hot water at 82°C) against hepatitis E virus has not been tested. As the RIVM (Appendix I) indicates, this virus has become a pathogen relevant to pig slaughterhouses over the past 10 years.

Environment:

- -The effects on the environment of use in pig slaughterhouses has not been investigated, but will be comparable to those of poultry slaughterhouses.
- -It is assumed that the components in VR 2827-3 will also have been neutralised in pig slaughterhouses before they are discharged into wastewater. Therefore, no environmental impacts are anticipated. Furthermore, as a result of this neutralisation, resistance development in the environment is not plausible.

- Given the situation dependence of the safety of emissions of HEDP, for pig slaughterhouses too, emissions into the environment cannot, a priori, be considered safe. This forms part of the Ctgb's assessment.

<u>In the case of slaughter of bovine animals</u>
No tests are known with the product VR 2827-3 or similar substances in cattle slaughterhouses, or with pathogens relevant to the slaughter of bovine animals. The documentation provided by the slaughterhouse / manufacturer contains a notice from the 'Bundesinstitut für Risikobewertung' (BfR). Enquiries at BfR show that this is an evaluation of the documents supplied, including the pilot test in pig slaughterhouses in 2009–2010. Another notice provided by the slaughterhouse / manufacturer indicates that the product will be implemented in 3 cattle slaughterhouses and 1 pig slaughterhouse in Germany. Upon enquiry, the BfR indicated that it has no knowledge of current use in practice in German slaughterhouses.

Toxicology:
- VR 2827-3 is not applied directly to beef, but only indirectly via equipment disinfection. The residue, including HEDP, on the beef carcass will therefore be lower than in poultry.
- Since in poultry the direct use on carcasses with longer contact time was found not to represent a toxicological risk, this assessment can also be considered to apply to indirect short-term use in cattle.
- The JECFA (JECFA, 2006) reports in 2006 that the formation of transformation products (rancid decay) has not been demonstrated for fresh beef.

Microbiology:
- No data are available on the effectiveness of VR 2827-3 against bacteria or yeasts in areas in the cattle slaughtering line.
- The findings of effectiveness in the pig slaughtering line cannot simply be extrapolated to the bovine slaughter line, as also concluded by the RIVM (Appendix I).
- For cattle, different pathogens are relevant than for pigs. As the RIVM (Appendix I) indicates, in cattle, the Gram-positive bacteria are more often a possible source of infection in humans, and the effectiveness of VR 2827-3 (INSPEXX 200/210) in the reduction of the number of Gram-positive bacteria has not been convincingly demonstrated.

Environment:
- The effects on the environment of use in cattle slaughterhouses has not been investigated, but will be comparable to the effects on poultry slaughterhouses.
- It is assumed that the components in VR 2827-3 will also be neutralised in cattle slaughterhouses before they are discharged into wastewater. Therefore, no environmental impacts are anticipated. Furthermore, resistance development in the environment is not plausible.
- Given the situation dependence of the safety of emissions of HEDP, for cattle slaughterhouses too, emissions into the environment cannot, a priori, be considered safe. This forms part of the Ctgb's assessment.

<u>In the case of slaughter of other animals</u>
No tests are known with the product VR 2827-3 or similar substances in other slaughterhouses.

Toxicology:

- For each situation, the method of use will have to be compared with the situation in the poultry sector. If the use is with less residue and shorter contact time on the carcasses, and there are few amino acids on the cutting surface, no toxicological hazards can be expected.

Microbiology:

- The pathogens that are important during slaughter must be determined for each species and the effectiveness against these pathogens must be sufficiently demonstrated.
- For each species, the slaughtering line should be examined in order to apply any extrapolation of other tests and, if necessary, to collect additional data.

Environment:

- It is assumed that the components of VR 2827-3 will have been neutralised before they enter the environment and no environmental effects are expected. Furthermore, resistance development in the environment is not plausible.
- Given the situation dependence of the safety of emissions of HEDP, for other slaughterhouses too, emissions into the environment cannot, a priori, be considered safe. This forms part of the Ctgb's assessment.

Adverse consequences of trial with VR 2827-3 in the industrial setting of a cattle slaughterhouse
If the proposed application VR 2827-3 does not disinfect sufficiently, meat products containing more pathogens may be placed on the market for 2 years than if disinfected with hot water at 82°C. This is especially true for the Gram-positive bacteria for which the effect of the product has not yet been sufficiently convincingly demonstrated, i.e. *Listeria monocytogenes*. This also applies to microorganisms capable of producing the catalase and/or peroxidase enzymes, such as *S. aureus*, and thereby rendering the peroxide activity of VR 2827-3 harmless, as the RIVM notes (Appendix I).

Adverse effects of limiting trial to a cattle slaughterhouse
The suitability for use of VR 2827-3 as a slaughtering equipment disinfectant in a cattle slaughterhouse does not guarantee the suitability of VR 2827-3 as a disinfectant in slaughterhouses for pigs, poultry, sheep, goats, horses. This will have to be assessed per slaughterhouse for the pathogens relevant to the animal species and for the slaughter equipment to be used for the animal species. The EFSA previously indicated that extrapolation to other situations is not appropriate, given the sometimes large differences in efficacy of a product under different conditions. These are not differences in meat but differences in equipment and all related parameters. As the EFSA (EFSA, 2005) also noted in decontamination of poultry carcasses:

'*It must be emphasised that, in general, decontamination treatments are able to reduce the contamination level but do not completely eliminate pathogens. Their effectiveness depends on the initial microbial load and treatment conditions. Regarding treatment conditions, there are many factors affecting the efficacy of these antimicrobials including concentration of the substance, time of exposure, temperature, pH and hardness of water, strength of bacterial adhesion to the carcasses, biofilm formation and the presence of fat or organic material in water.*'

The points to be sampled are insufficiently described. A previous assessment by RIVM of the trial carried out in a pig slaughterhouse in 2007 stated that*:* '*The sites sampled on the equipment have been chosen in such a way that they can be considered as the critical areas that come into contact with the carcass and are*

therefore presumed to be a source of contamination with microorganisms.' As indicated in the findings of the RIVM (Appendix I), the current description of the trial design for which permission is now requested does not provide this information. The NVWA's enquiry at the slaughterhouse for them to provide a more detailed trial protocol did not produce the required protocol. This means that the trial design provided cannot be assessed on this point. Since the procedure in pig slaughterhouses differs substantially from that in cattle slaughterhouses, the results as found for the slaughter robot for pigs cannot be extrapolated to those for the slaughtering equipment of the cattle slaughterhouse.

Although tests were carried out in pig slaughterhouses about 10 years ago, with a short exposure time of a similar agent, the findings of the RIVM (Appendix I) indicate that the pathogen hepatitis E virus relevant to pigs could not be taken into account at the time. This virus has appeared as a pathogen in the Netherlands over the past decade, probably as a result of consumption of contaminated pork products. Also, the effectiveness with respect to yeasts is insufficiently clear, as also stated in Appendix I. The RIVM notes that the effectiveness against Gram-positive bacteria was not tested separately at the time. In the case of pigs, the bacteria may be contained in the tonsils and may constitute a source of contamination of the meat and slaughter equipment at the time of slaughter.

Based on experience in poultry slaughterhouses, scientific opinions are available on the use of similar means of decontamination. These did not look at a contact time of 1 second, nor at disinfection of slaughtering equipment. Therefore, extrapolation of the data to the situation in a poultry slaughterhouse is also not possible.

The likelihood of the product passing the test and still being insufficiently effective
It is possible that the contact time of both VR 2827-3 and hot water at 82°C is too short to sufficiently disinfect a heavy contamination of the slaughtering material. VR 2827-3 may work better than hot water at 82°C when a contact time of 1 second is used for both. However, in both cases, it may lead to a non-relevant or selective reduction of pathogens. If the short exposure time is not sufficient to reduce the numbers of relevant pathogens, there is a hazard for infection with associated disease burden in the population.

The trial design does not clearly describe how the contact time of 1 second of the product is compared to a standard. It is mentioned how sampling takes place with 1 second contact time, but not whether and how sampling takes place after 5 minutes of operation, or after disinfection with hot water at 82°C. Furthermore, the number of samples to be taken is said to depend on the size of the slaughterhouse, but it is also describes that the product should be used in all disinfection units.

The number of samples collected is too low to demonstrate the effectiveness at low pathogen prevalence; assuming that the prevalence per pathogen on the blade will be <1%, 240 samples cannot significantly demonstrate that the prevalence deviates from 0%. This means that, on completion of the test, the efficacy of VR 2827-3 against relevant pathogens will not necessarily have been demonstrated yet.

Risk: probability (exposure estimate)

Chemical and toxicological
For poultry, pig, cattle slaughterhouses and slaughterhouses for other animals, the following applies:

- The chemical and toxicological exposure is low when the product VR 2827-3 is used for disinfecting the slaughtering equipment and not for decontamination of the carcass itself. Only a small quantity of the ingredients end up on the meat product.
- No toxicological effect is expected from the low amount of residue of HEDP and octanoic acid (JECFA, 2006, EFSA, 2014). The other components of the product are neutralised before the product is consumed. Consumer exposure to the product is minimal.
- Workers may be exposed to the product and are advised to take protective measures. It is intended for use by professionals. Safety for workers has been assessed by the Ctgb and has not been found to be objectionable.

Microbiological

Poultry (RIVM, 2016, NVWA-BuRO, 2018):

- In the Netherlands, about 600 million chickens and broilers are slaughtered each year.
- Based on the NVWA monitoring programme regarding retail products, *Campylobacter* was present in 37% of the chicken and 12% of the turkey in 2016.
- The NVWA's monitoring programme for long-term refrigerated non-perishable products demonstrated *Listeria monocytogenes* in 5% of 4,100 batches of food. Of these 5% positives, 40% was poultry meat. In some cases the standard of 100 CFU/g was exceeded.
- The NVWA's monitoring programme aimed at raw meat in shops shows *Salmonella* in 2–4% of chicken meat.
- Poultry meat is not normally considered a source of STEC (Xia *et al.*, 2010); in 1999, STEC was not detected in any of 744 raw chicken meat samples tested.
- Hepatitis E virus is not found in poultry.
- Pathogenic yeasts are not known to be found in poultry.

Pigs (NVWA-BuRO, 2015, RIVM, 2016):

- In the Netherlands, about 15 million pigs are slaughtered each year.
- Based on the NVWA monitoring programme regarding retail products, *Campylobacter* was present in 0% of pigs in 2016.
- *Listeria monocytogenes* was found on 2% of the ready-to-eat pig meat products examined by the EFSA (EFSA, 2012). The bacteria may be present in the tonsils of pigs and may constitute a source of contamination of the meat and slaughtering equipment at the time of slaughter.
- The NVWA's monitoring programme aimed at raw meat in shops shows *Salmonella* in 1–4% of the pork.
- The NVWA's monitoring programme makes no separate mention of STEC in pig meat.
- Pig meat is a primary reservoir of hepatitis E virus genotype 3. The virus has been detected in 50% of pig farms. Of the pigs for slaughter examined, 67% were recently infected. This percentage is higher on organic pig farms than on regular farms, namely 89% vs 72% in 2004. The virus can occur viraemically in pigs for approximately 10 days, this period may be longer in case of a co-infection with 'Porcine Reproductive and Respiratory Syndrome Virus' (PRRSV). Since blood contact is not a reason to disinfect slaughtering equipment, both VR 2827-3 and hot water at 82°C have no influence on this. If no disinfection is carried out between carcasses, carcass-to-carcass contamination may occur. Excretion via faeces can take place for weeks to several months.
- Pathogenic yeasts are generally not found in pig meat.

Cattle (NVWA-BuRO, 2015, RIVM, 2016):

- In the Netherlands, about 2 million bovine animals are slaughtered each year.
- Based on the NVWA monitoring programme regarding retail products, *Campylobacter* was present in 1% of cattle and calves in 2016. Some 3.9% of carcasses were found to be positive for *Campylobacter* during post-mortem inspection.
- The NVWA's monitoring programme for long-term refrigerated non-perishable products demonstrated *Listeria monocytogenes* in 5% of 4,100 batches of food. Of these 5% positive batches, 17% was beef and 14% was meat products (e.g. steak tartare or Dutch raw beef sausage called *ossenworst*). In some cases the standard of 100 CFU/g was exceeded. If found, it can often be attributed to biofilms on contaminated equipment.
- The NVWA's monitoring programme aimed at raw meat in shops shows *Salmonella* in 0–2% of the bovine meat. Some 0.2% of carcasses were found to be positive for *Salmonella* during post-mortem inspection.
- In carcasses during meat inspection, 2.3% was positive for pathogenic *E. coli* (STEC/VTEC). The NVWA's STEC monitoring programme found 1% of beef positive in the retail sector.
- Hepatitis E virus is not found in cattle.
- During the drying of beef, potentially harmful yeasts and fungi (*Candida* sp., *Cladosporium* sp., *Rhodotorula* sp.) were present at the start of the drying process of dried sausage, and these disappeared the longer the drying process lasted (Ryu *et al.*, 2018). It is possible that pathogenic yeasts and fungi play a role in the beef production chain, but it is unclear whether this is during the slaughter phase.

Other animals:

- In the Netherlands, about 1 million sheep, 140,000 goats and 4,000 horses or ponies are slaughtered each year.
- Based on the NVWA monitoring programme regarding retail products, *Campylobacter* was present in 11% of the sheep and 8% of the goats in 2016. This is not known for horses.
- The NVWA's *Listeria monocytogenes* monitoring programme on long-term refrigerated non-perishable products did not mention this bacterium in products incorporating lamb and mutton, goat meat or horse meat.
- The NVWA's monitoring programme aimed at raw meat in shops shows *Salmonella* in 0–2% of lamb.
- The NVWA's STEC monitoring programme found 4% of small ruminant meat positive in the retail sector in 2016.
- Hepatitis E has been demonstrated in 12% of tested wild boar.

What is the likelihood of exposure during the trial?
The knives may not be disinfected sufficiently after contamination.
If the product is not sufficiently effective, or at least less than the effect that hot water at 82°C would have, this could have an impact on public health. The EFSA opinion on meat inspections (EFSA, 2011, EFSA, 2013) indicates that the risks of *Salmonella* and *E. coli* in particular are not covered by the current meat inspections. Incision and touching are mentioned as a risk of cross-contamination. Experience from inspections shows that generally in slaughterhouses the speed of the conveyor belt is high, which means that a thorough inspection is not always possible, with possible consequences for the microbiological safety of the product.

Risk: impact
Estimated frequency of disease in the Netherlands due to pathogens that can survive on slaughtering equipment
Around 700,000 cases of food-related pathogens are reported annually in the Netherlands (Mangen, 2018). Of these, 15% are attributed to beef, lamb or

mutton, 21% to pork, and 18% to poultry (Bouwknegt *et al.*, 2013, Bouwknegt *et al.*, 2013).

- *Campylobacter* spp.: In the Netherlands, approximately 80,000 cases of *Campylobacter* gastroenteritis occur annually, with 18,000 GP visits, 600 hospital admissions, and 30 deaths among – especially elderly – patients (Source: LCI).
- STEC O157: There are 30 to 60 laboratory confirmed cases per year in the Netherlands (Source: LCI).
- *Salmonella* spp.: In the Netherlands, approximately 37,000 cases of *Salmonella* spp. gastroenteritis occur annually, with 1,100 hospital admissions, and 35 deaths (Source: LCI).
- *Listeria monocytogenes*: In the Netherlands, approximately 100 cases of listeriosis occur annually (Source: LCI).
- Hepatitis E virus: In the Netherlands, approximately 800 cases of hepatitis E virus infection occur annually.
- The number of cases of illness per year in the Netherlands due to yeasts is unknown.

These cases now occur with the current practice of disinfection of slaughtering equipment. It is not known what proportion of these cases can be attributed to carcass-to-carcass contamination.

Estimated effect in case of insufficient effectiveness of VR 2827-3 against specific pathogens
If the product VR 2827-3 is not found to be effective for Gram-positive bacteria or bacteria capable of producing catalase and/or peroxidase, and the current method is, this would increase the number of cases of disease caused by these pathogens, such as *Listeria monocytogenes*, in meat products attributable to contamination via slaughtering equipment. Because it is not known which part of the diseases can be attributed to carcass-to-carcass contamination, the number of additional cases or possible deaths is unknown.

Estimated effect in case of insufficient effectiveness of VR 2827-3 against yeasts
No data is available on the prevalence of human pathogenic yeasts in slaughterhouses and at times in the slaughtering line where disinfection is required. On the basis of the assessment of the Ctgb, it can be assumed that the lower concentration is not sufficiently effective for reducing yeasts. This is already the case with a 5-minute exposure time. The effects on public health are unknown, as no data on diseases are available along this alimentary route.

Estimated effect in case of insufficient efficacy of both hot water at 82°C and VR 2827-3 in case of short exposure time
The result of the test may be that the product VR 2827-3 appears to work as well as water, but that this is based on the fact that no significant difference is observed due to the short exposure time. As a result, it may not work as well as the current practice, especially if the current practice means disinfecting more than 1 second. Because it is not known which part of the diseases can be attributed to carcass-to-carcass contamination, the number of additional cases or possible deaths is unknown.

Individual risk due to change of disinfectant for slaughtering equipment in slaughterhouses

<u>Microbiological</u>
The individual risk of infection from pathogens depends on:

- The number of portions per individual per year
- The higher level of pathogenic contamination by using the short contact time of the disinfectant

- The viability and pathogenicity of the pathogens after preparation of the product
- The risk of exceeding a limit of pathogenicity at this higher level of infection. On the one hand, in the event of a transition to a new disinfectant with potentially less effectiveness, in case of pathogens with low infectious dose, the limit of pathogenicity may be exceeded. On the other hand, in case of pathogens with high infectious doses, it can promote the chance of growth. This limit will be exceeded sooner in people with poor health.

Chemical and toxicological

Individual risk of ingestion of VR 2827-3 depends on the residue on the finished product. When previously hot water was used for disinfection, an increase in individual exposure to a new disinfectant to be introduced is inherent. VR 2827- 3 is a product whose components are neutralised. At the time of consumption, the effect of a minimum residue on public health is negligible. The risk of residues of HEDP by using VR 2827-3 on slaughtering equipment is many times lower than for carcass decontamination, where the risk of HEDP intake was found to be negligible.

Environmental

The individual risk of VR 2827-3 via the environment is negligible.

Experience in other countries

The request made by BuRO through the EFSA Focal Point to 30 European partners on their possible experience in testing alternative disinfection systems in slaughterhouses resulted in 16 responses.

- Ireland has implemented a comprehensive approach through the government. All companies wishing to test an alternative system should demonstrate the efficacy compared to hot water at 82°C by means of a standardised trial protocol (Appendix II), which incorporates an evaluation at different times. This method prescribes how the test must be carried out. The method (Appendix IIIa) comprises several phases, namely (i) establishing a baseline with hot water at 82°C; (ii) a double disinfection period, namely disinfection with the alternative followed by disinfection with hot water at 82°C and, only when this is demonstrably equivalent to baseline (iii) disinfecting only with the alternative without post-disinfection with hot water at 82°C. Since the next phase can only start once the results from the previous phase have been approved, food safety is guaranteed for the products produced during the field trials. One of the steps is to provide specific advice at company level (Appendix IIIb). In Ireland there are 2 tests running with INSPEXX 210.
- Bulgaria indicates that INSPEXX 210 is used in cattle slaughterhouses for disinfecting knives, but that the use of hot water at 82°C is more common. No risk assessment was carried out for this purpose.
- The further information provided to the NVWA by BfR from Germany shows that the BfR has no knowledge of the practical application of the product INSPEXX 210 in German slaughterhouses. The slaughterhouse / manufacturer had submitted information to the NVWA on 6 September 2018 containing an evaluation by the German Federal Institute for Risk Assessment (BfR) of INSPEXX 210 regarding a possible transfer of the active substances to. The BfR indicated that this is not a risk assessment with regard to intake of INSPEXX 210 nor effectiveness against pathogens. It concerned an evaluation based on the available documents, such as the EFSA's 'scientific opinion' of 2005 and the JECFA statement of 2006, and available information provided by the manufacturer, i.e. information on the 2009–10 pilot test in pig slaughterhouses. The effectiveness against viruses and yeasts was not included because no information was available

on this. A doctoral research project is currently being conducted by the BfR in which INSPEXX 210 is being tested in a laboratory as a decontaminant of surfaces; the results are expected in half a year. The BfR adds: '*An assessment of the agent as a biocidal product in Germany according to Regulation (EU) No 528/2012 will only take place after the authorisation of the respective active substance following an application of the applicant. In 2017, the active substance in INSPEXX 210, peroxyacetic acid (CAS-No.: 79-21-0), was authorised according to Regulation (EU) 2016/672 as biocidal active substance for the product categories inter alia: 3 (hygiene in the veterinary field), 4 (food and feed safety). Applications for authorisation for peroxyacetic acid containing biocidal products have to be accorded until 01.10.2020. The BfR is not aware, if an application for authorisation or mutual recognition for INSPEXX 210 was submitted in Germany.*'

- Greece states that it has experience with the product INSPEXX 210 authorised there. The system was recently installed in four slaughterhouses for pigs, cattle, sheep and goats: '*The installation of the system in these 4 slaughterhouses is recent. The main purpose during this period is to familiarize staff with the system and to control concentrations during work so as to define a precise program to replace the solution in real working conditions. The fact that the slaughterhouses do not have a day-to-day function but also during the day the process is not continuous creates additional difficulties in controlling the concentration. The solution was adjusted to a concentration of 0.16% v / v at ambient temperature. The goal was to keep the solution around 220 ppm PAA continuously. Checks were made / hour or /45 minutes, measuring the levels of peracetic acid (PAA). PAA test strips (MERCK) ranging from 100 to 500 ppm were used for testing. At the same time, the temperature of the solution and the pH were checked. Note that there were no fluctuations in the concentration of the solution during the work related to temperature. Levels of active substance remained at the first two hours of work at levels within specifications (200 - 250 ppm). After two hours and as the solution was dulled, the levels of active substance were reduced (150-200 ppm) and had to be renewed (emptying and refilling). It was also noticed that this was happening faster when pigs were slaughtered and less in the case of sheep or cattle. It was also noticed that the level of active substance in the presence of organic matter (dirt) although initially set at the desired level of 220 ppm was decreased, but then this decrease remained constant until the end of the work (the longest duration was 5 hours) without falling under the level of 150 ppm. The immersion of the knives lasts 3 seconds. The instruction of rinsing the knife immediately after the immersion with potable water was not always followed (practical difficulties). As far as the microbiological data is concerned, tests will be conducted at a following stage and will involve swabs from the surface of the knives. So far only slaughterhouses collect data of the routine tests carried out by in the framework of own-checks.*'
- Iceland indicated it recently received a request for the use of an alternative product based on lactic acid ($C_3H_6O_3$) instead of peroxyacetic acid ($C_2H_4O_3$) and states the following: '*In that application there were: field trials in slaughterhouses, conductivity graphs, simulation tests, all made in –XXX– laboratory in 2008. We have not allowed use of this disinfectant yet, because of lack of training and language explanation <u>but mostly because of the time factor, for knives in the solution</u>. The last factor has not been solved yet by FBO´s.*'
- The Czech Republic states that alternative systems are used in their country and states the following: '*When an alternative disinfection system is used, the company is obliged to have a procedure in place for evaluating of*

> *efficacy of disinfection method (for example microbiological testing of samples taken from the equipment before and after disinfection). However, the detailed overview about the used alternative systems is not available as the information is not being collected. Therefore it is also impossible to answer all additional questions.*'

The remaining 10 countries (Switzerland, Estonia, Sweden, Malta, Croatia, Hungary, France, Austria, Latvia, Montenegro) indicate that they have no experience in testing alternative systems for disinfecting slaughtering equipment in slaughterhouses in their country.

Conclusions

Toxicological

Toxicologically, the use of VR 2827-3 cannot be expected to pose risks due to the residue of the product in meat or meat products or by transformation products after contact between the product and organic material meat or meat products. Since the product under the name 'INSPEXX 210' with 5 minutes' exposure time has been authorised by the Ctgb as a biocide for use as a disinfectant on hard surfaces, no additional toxicological risk can logically be expected in case of use of the same product with a short exposure time. Since the residue of HEDP was not found to be objectionable when used for carcass contamination in poultry, no additional toxicological risk can logically be expected for the use of VR 2827-3 for disinfection of slaughtering equipment.

Microbiological

For all relevant pathogens, the prevalence will be very variable, but will generally be low, so it is unlikely that a field trial will provide the necessary responses regarding effectiveness. The effectiveness of VR 2827-3 against the pathogens relevant to each animal species should therefore be demonstrated in a laboratory experiment, simulating practice.

Because it is not clear which part of the human infections can be attributed to transmission via slaughtering equipment, particular attention has been paid to pathogens that can end up on the meat via skin or intestines. Based on the low infectious dose, the risk of growth, the possibility of transmission via cutting surface, or the possibility of carcass-to-carcass contamination, effective action when disinfecting slaughtering equipment with VR 2827-3 with short exposure time is particularly important for:

- *Campylobacter* in the slaughter of poultry, cattle, sheep and goats
- Pathogenic *E. coli*, STEC, in the slaughter of cattle, sheep and goats
- *Salmonella* in the slaughter of poultry, pigs, cattle and lambs
- *Listeria monocytogenes* in the slaughter of poultry, pigs and cattle.

These pathogens are known to cause significant disease burden in humans through the consumption of meat. A less effective disinfection of slaughtering equipment presents the highest public health risk for these pathogens. Especially for these pathogens, it must be demonstrated that the efficacy of the alternative system is at least equivalent to hot water at 82°C, as this hot water is intended to be used. This means that the efficacy of a new product with a short exposure time should be at least equivalent to the use of hot water at 82°C with a longer exposure time, such as with a '2-knife system', where the first knife is disinfected and work is continued with the second knife.

In addition, effective action is desirable for:

- Hepatitis E virus in the slaughter of pigs and swine.

This is a pathogen that has recently been recognised as a cause of disease burden due to pig meat consumption. Since much is still unknown with respect to this pathogen, it is recommended to examine the effectiveness of the product against it, as also concluded in the RIVM report.

In the case of VR 2827-3, the action of which is based on the peroxide activity of a number of ingredients, an additional effective action is desirable for bacteria capable of producing catalase and/or peroxidase, as these enzymes may render the mechanism of action harmless.

The prevalence of yeasts in slaughter animals is unknown. Yeasts are mainly linked to spoilage. Although human pathogenic yeasts can be found on beef, no cases are known along this transmission route. The effectiveness of VR 2827-3 against yeasts is also unknown. The Ctgb has based the legal conditions for use of 5 minutes and a concentration of 0.5% on yeasts, so it is plausible that yeasts are not reduced in number. It is also unclear to what extent the effectiveness against yeasts is related to the effectiveness of hot water at 82°C against yeasts. There is no known food-related disease burden caused by yeasts.

The field trial

The field trial proposed by the slaughterhouse / manufacturer cannot guarantee the microbiological safety of beef products for 2 years. Experience abroad also does not provide the information needed to guarantee food safety. The efficacy of VR 2827-3 with a short exposure time and low concentration has not yet been convincingly demonstrated in a laboratory for all pathogens relevant to cattle. Also, the efficacy of the product has not previously been demonstrated in a cattle slaughterhouse. The results from pig slaughterhouses cannot be extrapolated to cattle slaughterhouses. In pig slaughterhouses, different pathogens are also relevant than in cattle slaughterhouses. For example, the trials in pig slaughterhouses have not demonstrated the effectiveness against Gram-positive bacteria, such as *Listeria monocytogenes*. Especially in the case of *Listeria*, biofilm on the carcass splitter is considered to be a risk of contamination in cattle. It is unclear whether slaughtering equipment is disinfected as standard at certain times, and whether this slaughtering equipment is cleaned first. As the RIVM report indicates, the degree of contamination is important to the extent to which a disinfectant can be effective. The shorter the contact time, the greater the chances the purpose of disinfection will not be met.

In order to determine whether a disinfectant has a similar effectiveness to hot water at 82°C, it is desirable to standardise the trial design, based on a baseline level where hot water at 82°C shows sufficient effectiveness. The contact time of ≤1 second is too short for water, and possibly too short to show significant differences between methods, so that a product is likely to emerge as at least as effective from the trial. In addition, it is important to demonstrate this similar effect for the relevant pathogens in the relevant disinfection step. The product VR 2827-3 has the great advantage compared to hot water at 82°C that no protein coagulation takes place on the slaughtering equipment. This limits the formation of a biofilm. This reduces the chances of survival of pathogens, and keeps the blades sharp. This aspect can also be incorporated into a standardised trial design.

Current practice:
The law prescribes a standard method for disinfecting, namely with hot water at 82°C, without attaching a minimum contact time to it. In practice, this translates into disinfecting knives by immersion, where a contact time of less than 1 second is possible. A contact time of 1 second has been found to be moderately effective in reducing pathogens. There are indications from the BuRO's Red Meat Supply Chain Risk Assessment (*Risicobeoordeling Roodvleesketen*) that the current method in slaughterhouses is not always adequate to prevent carcass-to-carcass contamination. The law allows for an alternative system with an equivalent effect to this (possibly moderately effective) standard. The EFSA emphasises that the use of decontamination agents should not lead to an unsanitary process by masking it. For this reason, these products have long been kept out of slaughterhouses. Only after a thorough assessment can products be allowed. The method proposed in the advice, based on experience from Ireland, contributes to this in terms of disinfectants.

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Appendix I. Risk assessment of the RIVM-RIKILT Front Office for Food and Product Safety of the suitability of VR 2827-3 as a disinfectant for slaughtering equipment in the meat processing industry.

Appendix II. Trader Notice MH 07/2012 – Alternative system for disinfecting tools in meat plants. Department of Agriculture, Food and the Marine, Ireland.

Appendix IIIa. Overall recommendations upon completion of INSPEXX trial 384.

Appendix IIIb. Overall recommendations upon completion of INSPEXX trial at EC350