National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

FRONT OFFICE FOOD AND PRODUCT SAFETY

Assessment of the safety of disinfectant VR2827-3 intended for use in the meat industry

Risk assessment requested by:	Office for Risk Assessment & Research (BuRO)
Risk assessment performed by:	National Institute for Public Health and the Environment (RIVM)
Date of request:	14 August 2018 10 September 2018 (additional questions)
Date of risk assessment:	24 August 2018 (draft) 27 August 2018 (final version) 19 October 2018 (revised version following supplementary questions, final version) 4 December 2018 (anonymised version) 29 January 2019 (English verion)

Subject

An advice drawn up by the Office for Risk Assessment and Research (BuRO: Bureau Risicobeoordeling & onderzoek) was issued in 2008 on the use of Inspexx 200 by a slaughterhouse. This advice was necessary because Inspexx had 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 a biocide (disinfectant for surfaces and materials in the food industry). In 2010, the former Food and Consumer Product Safety Authority (VWA: Voedsel- en Warenautoriteit) authorised the slaughterhouse to use this product. No exposure time is mentioned because, as with use of water at 82°C, immersing the blades in a single movement (short exposure) was considered to be sufficient.

Inspexx 200 was later replaced by Inspexx 210. A certificate of equivalence has been issued by the manufacturer stating that the active substances of both biocides are the same. Since 2014, Inspexx 210 has been authorised by the Ctgb as a biocide with legal conditions for use as a disinfectant against bacteria (excl. Mycobacteria and bacterial spores) and yeasts on materials and surfaces in the food industry. These legal conditions for use state a 5-minute exposure time, which is not practicable for a slaughterhouse. According to the slaughterhouse, this long exposure time seems to be based on the killing of yeasts. In order to submit an application for a disinfectant in the meat industry with a shorter exposure time (1 sec), the manufacturer has now proposed the product VR2827-3, which is said to be equivalent in composition to Inspexx 210.

The slaughterhouse and the manufacturer want to carry out a trial with this product at the cattle slaughterhouse, the idea being that all disinfection units in the slaughterhouse will use this product. This authorisation (dispensation) for such a trial has since been granted by the Ctgb. The Netherlands Food and Consumer Product Safety Authority (NVWA: Nederlandse Voedsel- en Warenautoriteit) should consider the question whether VR2827-3 can be considered safe for use in a food business.

Question

The NVWA asks the Front Office the following questions:

1.1 Is disinfection of slaughtering equipment in the meat processing industry for 5 minutes or a few seconds with the product VR2827-3 (/Inspexx 200/Inspexx 210) sufficient or at least equivalent to the application of water at 82°C to inactivate microorganisms, including viruses?
1.2 In the microbiological field, have there been developments since 2008 that should be taken into account when using the product Inspexx 200/Inspexx 210/VR2827-3 in the meat processing industry?
1.3 Is the trial design as proposed adequate to determine the suitability of VR2827-3 as a disinfectant for slaughtering equipment in the meat processing industry?

The answer should incorporate the following aspects:
Re 1.1: Take into account the different pathogens that are relevant for each species (in particular pigs and cattle) during slaughter and the subsequent steps. For example, in pigs, hepatitis E virus infection is possible when small parts of the livers of the liver are transferred to food products by the use of a diaphragm.
Re 1.2: For example in the field of antimicrobial resistance.
Re 1.3: Can the agent, for example, continue to work in the samples taken, thus still achieving a 5-minute exposure time?

Additional questions dated 10 September 2018
The above questions were answered in the Front Office assessment of 27 August 2018. It concluded that additional information was necessary in order to assess the microbial safety of the use of the product in the food industry, in particular with regard to its effect on yeasts and its functioning in a cattle slaughterhouse. The slaughterhouse and the manufacturer provided additional documents on 6 September. Therefore, the NVWA asked the Front Office the following additional questions on September 10, 2018. The answers to these questions have been added to this revised version of the Front Office assessment.

Additional questions:
A. Has the additional information demonstrated the effectiveness of the product VR2827-3 with regard to yeasts and, if so, at what exposure time?
B. Has the additional information shown the effectiveness of the product VR2827-3 in cattle slaughterhouses, and if so, at what exposure time?
C. Does the additional information provide an adequate trial design to determine the suitability of VR2827-3 as a disinfectant for slaughtering equipment with a short exposure time in the beef processing industry?

The answer should incorporate the following aspects:
Re A: It is indicated that yeasts are a minimal problem in a slaughterhouse environment (ref. 4 Practical test), namely 1 sample positive before disinfection, but this means that it does occur. Furthermore, it is indicated that the assessment was carried out according to EN1276, EN1650 and EN13697.
Re B: The general text states that the efficacy has been demonstrated in cattle slaughterhouses, but can this also be sufficiently concluded from the documents supplied?
Re C: Previously there were comments regarding the control samples and the description of the knives.

Conclusions 27 August 2018

1. Assuming that the chemical formulation of VR2827-3 is equal to Inspexx 200/210, the effectiveness of VR2827-3 with regard to bacteria in the slaughter robot for pigs has been demonstrated.
2. The effectiveness of VR2827-3 with regard to yeasts and viruses is unknown.
3. It cannot be established with the available information that the application of VR2827-3 with a contact time of 1 second at locations other than the slaughter robot for pigs is effective.
4. Since the beginning of this decade, hepatitis E virus has been a problem in pig farming. If future use is foreseen in the slaughter of pigs, it is recommended to investigate the effectiveness of VR2827-3 with regard to the hepatitis E virus. The current trial design does not take into account possible prevalent viruses.
5. The microbiological methods that will be used to quantify bacteria and yeasts are adequate. The way in which the control samples are treated does not correspond to the way in which knives are treated. There is a risk that the contact time is longer for the control samples than for the knives.
6. In view of points 2 and 3, the present trial design does not guarantee the safety of meat and/or meat products to be placed on the market.

Conclusions based on supplementary information, 19 October 2018

A. The efficacy of VR2827-3 against yeasts has not been demonstrated.
B. The additional information provided has not demonstrated the efficacy of VR2827-3 in cattle slaughterhouses.
C. The trial design is not adequate to determine the effectiveness of VR2827-3 with a short exposure time as a disinfectant for slaughtering equipment in the beef processing industry. In particular, because the efficacy against Gram-negative and Gram-positive bacteria, and against bacteria capable of producing catalase and/or peroxidase, which are representative of the flora that can be found on slaughtering equipment for pigs and cattle, is not specifically tested. Moreover, insufficient data on the prevalence and concentration of yeasts are available to adequately test the effectiveness of VR2827-3 against yeasts.

Comments on previous conclusions
In the assessment of August 27, 2018 and the previous assessment of INSPEXX, it was concluded that the effectiveness of INSPEXX or VR2827-3 with regard to bacteria in the slaughtering robot for pigs has been demonstrated. This conclusion is based on the results of studies that looked at the effect of INSPEXX or VR2827-3 on bacteria in general (aerobic mesophilic plate count, total viable count) and the effect on Enterobacteriaceae. The effectiveness on specific groups of bacteria, Gram-negative and Gram-positive bacteria, and bacteria capable of producing catalase and/or peroxidase was not tested. Since this has also not been specifically tested for use in pig slaughterhouses, it is generally not clear how effective INSPEXX or VR2827-3 is with regard to these specific groups.The effectiveness of VR2827-3 has been demonstrated to a limited extent with regard to bacteria in the slaughtering robot for pigs as the effectiveness against specific groups of bacteria has not been specifically demonstrated.

In the assessment dated 27 August 2018, conclusion 5 erroneously stated that: 'The way in which the control samples are treated does not correspond to the way in which knives are treated. There is a risk that the contact time is longer for the control samples than for the knives.' However, the treatment method is correct. Although the trial design shows that after transport the disinfectant is inactive, it does not show whether the neutralising action is direct. Furthermore, the use of S. aureus as a test organism in the neutralisation control is questionable, as has already been noted. This is because effectiveness of VR2827-3 as a disinfectant of knives in the slaughterhouse is tested using Enterobacteriaceae.

Introduction

Responses to the questions
In view of the present equivalence statements, the responses to the questions assumed that Inspexx 200, Inspexx 210 and VR2827-3 share the same active chemical composition. The previous assessment of Inspexx 200 specifically concerns the efficacy on bacteria in the slaughter robots in the pig slaughterhouse. The current question concerns the efficacy of VR2827-3 for cattle and poultry slaughterhouses, as well as for viruses and yeasts regarding pig slaughterhouses.

1) Holtslag (2008) describes the results of an experiment carried out by the slaughterhouse and the manufacturer to improve slaughter robot hygiene. The purpose of the experiment was to determine whether the disinfectant Inspexx 200 has an equivalent or better disinfecting effect for bacteria than water at 82°C when disinfecting the slaughter robots. The experiment was carried out at ambient temperature at which Inspexx 200 was diluted with tap water at a temperature of approximately 13°C. In these earlier tests, the viable cell count for mesophilic microorganisms and the viable cell count for Enterobacteria were examined with respect to microbiology. Determining the viable cell count for mesophilic microorganisms theoretically includes all bacteria, Gram negative and Gram positive. Determination of the viable cell count for Enterobacteria covers part of the Gram-negative bacteria. In principle, therefore, the effectiveness of the product against all bacteria has been demonstrated. This is supported by the earlier conclusion of the Front Office in 2008 that the experiment has shown that Inspexx 200 has a sufficient disinfecting effect with respect to bacteria with a contact time of several seconds at a temperature of 13°C.

In the experiment described by Holtslag (2008), only the effectiveness with regard to bacteria was investigated. The effectiveness against viruses and yeasts was not investigated at the time. The Front Office now finds that the effectiveness with regard to bacteria in the slaughtering robot for pigs has been demonstrated, but that it is not known whether the efficacy of VR2827-3 for viruses and yeasts is sufficient. No data are available on the effectiveness of VR2827-3 in areas in pig slaughtering lines other than the slaughter robot. Furthermore, it has not been investigated whether VR2827-3 is effective in cattle slaughterhouses and poultry slaughterhouses. Therefore, it cannot be established whether VR2827-3 is effective at locations other than the slaughter robot for pigs.

2) *Microbiological developments since 2008: hepatitis E in pig meat*
The problem of hepatitis E in pig meat has been recognised since the beginning of this decade. For example, an NVWA advice on hepatitis E virus in pig blood and other products was published in 2016. The reason for this opinion was:

'A possible increased incidence of human infections with hepatitis E virus genotype 3 has been observed in the Netherlands and our neighbouring countries since 2011. The main source (reservoir) of this virus appears to be pigs' (NVWA, 2016).

As stated in Response 1.1, the efficacy of VR2827-3 with regard to hepatitis E virus is not known and it is therefore appropriate, in the light of the development mentioned above, to investigate this further if application in pig slaughterhouses is envisaged. Data on HEV stability at higher temperatures are scarce. Exposure of HEV to 60°C in liquid for 1 hour results in a reduction of 96% (Emerson et al. 2005). In a trial design aimed at reducing HEV, comparison with the effectiveness of water at 82°C is desirable. In addition, data on HEV stability when exposed to chemicals are not known. However, given the limited time available to answer this Front Office question, no comprehensive literature study has been carried out.

3) *Microbiological developments since 2008: antibiotic resistant bacteria*
The Front Office concluded in 2008 that Inspexx 200 has a sufficient disinfecting effect with respect to bacteria at a contact time of several seconds. There is no reason to assume that the die-off of antibiotic-resistant bacteria by the active substances in VR2827-3 is different from that of antibiotic-sensitive bacteria.

4) According to the present trial design, a contact time of 1 second is considered between VR2827-3 and unspecified knives at 'pre-defined locations'. Total aerobic plate count and total yeast count will be considered using methods that are standard for bacteria and yeasts in terms of sampling and detection. It has previously been described that the contact time of 1 second, to be used in a new trial, results in a significant reduction in the total aerobic plate count (Holtslag, 2008; Heres and Verkaar, 2011).
The present trial design should clarify the effects on total yeast number, although it is not possible to compare it with the standard method, immersed in water of at least 82°C. This trial does not look at viruses in general or hepatitis E Virus (HEV) in particular. If future use is foreseen in the slaughter of pigs, it is recommended to also investigate the effectiveness of VR2827-3 against the hepatitis E virus.
The treatment of the control samples does not correspond to the contact time to be examined. For the control samples, the contact time may be longer. A better picture of the effect of VR2827-3 would be obtained if slaughtering equipment were contaminated with the relevant microorganisms and then sampled in the same way as real-life slaughtering equipment. It is also striking that for the control samples, only the effectiveness for a Gram-positive microorganism is considered, and not also a Gram negative ones.

Conclusions
1. Assuming that the chemical formulation of VR2827-3 is equal to Inspexx 200/210, the effectiveness of VR2827-3 with regard to bacteria in the slaughter robot for pigs has been demonstrated.
2. The effectiveness of VR2827-3 with regard to yeasts and viruses is unknown.
3. It cannot be established with the available information that the application of VR2827-3 with a contact time of 1 second at locations other than the slaughter robot for pigs is effective.
4. Since the beginning of this decade, hepatitis E virus has been a problem in pig farming. If future use is foreseen in the slaughter of pigs, it is recommended to investigate the effectiveness of VR2827-3 with regard to the hepatitis E virus. The current trial design does not take into account possible prevalent viruses.
5. The microbiological methods that will be used to quantify bacteria and yeasts are adequate. The way in which the control samples are treated does not correspond to the way in which knives are treated. There is a risk that the contact time is longer for the control samples than for the knives.
6. In view of points 2 and 3, the present trial design does not guarantee the safety of meat and/or meat products to be placed on the market.

References
Heres en Verkaar[i] 2011 Safepork –Alternative method for knife disinfection with Inspexx 200 is more efficient than 82 °C water.

NVWA (2016) https://www.nvwa.nl/documenten/dier/dierziekten/overige-dierziekten/risicobeoordelingen/hepatitis-e-bij-varkens

Holtslag J.B. (2008). April 2007, revision April 2008, Improving slaughter-robot hygiene. The efficiency of Inspexx© 200 as a disinfectant to improve hygiene of slaughter-robots regarding Salmonella, Enterobacteriaceae and mesophilic aerobic counts.

Emerson et al. 2005. Thermal stability of Hepatitis E Virus. J Infect. Dis. 192;930-933.

Responses to the additional questions

Response to question A
From the newly supplied Reference 4 (Microbial efficacy of INSPEXX210 against bacteria and yeasts) it can be concluded that yeasts were hardly present on the blades used in the test to evaluate the effectiveness of INSPEXX. In the only sample (1/15) found to be contaminated with yeasts (730 CFU/swab), treatment with INSPEXX 210 (contact time approximately 1 sec) resulted in a complete reduction in the number of yeasts. It can be inferred from the information provided that VR2827-3 is identical to INSPEXX 210 (Reference 2). Due to the limited amount of usable data (only 1 sample contaminated with yeast) however, it cannot be reliably established whether VR2827-3 is effective in removing yeasts at all, and certainly not in relation to other types of meat and carcasses such as cattle. Yeasts are mainly linked to spoilage. However, human pathogenic yeasts can be found on food including beef (Quintillate al., 2018; Rajkowska and Kunicka-Styczynska, 2018; Wirth and Goldani, 2012).

Response to Question B
The additional references 3 and 4 are studies carried out exclusively in pig slaughterhouses. In these it is tested whether VR2827-3 is effective under practical conditions (contact time: approximately 1 sec.) in the killing of bacteria in general (total viable count), *Enterobacteriaceae*, and yeasts. Since the microbial intestinal flora of pigs and cattle are not equal, the extent to which these findings can be translated into a cattle slaughterhouse is unclear. Testing of the effectiveness of VR2827-3 on cutting equipment infected with bovine intestinal bacteria could therefore result in different results. In addition, the effectiveness has not been specifically tested for representative Gram-positive bacteria, such as *Listeria (L.) monocytogenes* (Reference 3), *Clostridium* spp., and *Staphylococcus (S.) aureus*. These are potential pathogens and are generally less often associated with pigs than with cattle (Bouwknegt et al., 2015). By not testing for Gram-positive bacteria, VR2827-3 can appear to have a strong microorganism-reducing effect in samples with predominantly Gram-negative bacteria, although the Gram-positive bacteria which are present in small numbers might be resistant to it.

The effectiveness of VR2827-3 is based on the peroxide activity of a number of ingredients. Peroxide activity can be rendered harmless by microorganisms capable of producing the enzymes catalase and/or peroxidase (Rios-Castillo et al., 2017). However, not all microorganisms produce these enzymes. In testing the effectiveness of VR2827-3, it is recommended to use not only microorganisms which are generally representative of the flora that can be found on slaughtering equipment for pigs and cattle, but also *explicitly* to test with microorganisms producing catalase and/or peroxidase.

The manufacturer's response of September 5, 2018 refers to References 6 and 7. These references state that there is a consensus on the effectiveness of INSPEXX 210 in slaughterhouses for cattle and pigs. However, information is lacking on how the effectiveness has been determined (e.g. exposure time). These references also contain no research results with which this statement is or can be substantiated.

Response to Question C
The trial design described in Reference 10 (Microbiological efficacy of VR2827-3 against bacteria and yeasts) is identical to the trial design assessed earlier on August 27 2018.The trial design described in Reference 10 also does not deviate substantially from the trial design described in Reference 4 (Microbiological efficacy of INSPEXX 210 against bacteria and yeasts) and is unfit for determining the suitability of VR2827-3 as a disinfectant for slaughtering equipment with a short exposure time in the beef processing industry. Efficacy against bacteria in general (total viable count) and yeasts is tested. Because the total viable count is tested for, it is not clear to what extent the product is effective against specific Gram-negative bacteria (e.g. *Enterobacteriaceae*), Gram-positive bacteria (e.g. *L. monocytogenes*, *S. aureus* and *Clostridia*), and microorganisms

producing catalase and/or peroxidase. A trial design aiming to test the effectiveness against these groups of microorganisms separately is lacking.

Although the number of samples in the trial design described in Reference 10 is greater than the number of samples tested in the experiment described in Reference 4, still (as in the case of pigs) the prevalence of yeasts in a typical cattle slaughterhouse is not clear. Moreover, if yeasts are present, the concentration at cutting points of cattle carcasses in order to test the effectiveness of VR2827-3 remains unknown

Cross-contamination of carcasses via slaughtering equipment is particularly relevant in the event of a high level of cutting surface contamination. It is not clear what the efficacy of VR2827-3 is with a high degree of contamination, since it has now mainly been tested (Reference 4) or possibly will be tested (Reference 10) with cutting surfaces with a low degree of contamination. It is also unclear to what extent the effectiveness against yeasts is related to the effectiveness of hot water at 82°C against yeasts.

In the trial design described in Reference 10, the effectiveness of VR2827-3 is determined by sampling a knife used in practice before disinfection (one side of the blade) and after treatment of 1 sec in a solution of VR2827-3 (the other side of the blade). No description has been given of a comparative test with water at 82°C. Results of a previous trial in which the efficacy of INSPEXX 210 is compared to that of water at 82°C have been added. The trial design (Reference 10) or other additional information provided does not address previously noted deficiencies: the same control test is carried out and the description of the knives is also the same as described in Reference 4. It is good that the neutralisation check has been carried out but it would have been better to carry out the neutralisation check at the slaughterhouse instead of waiting until in the lab. The neutralising effect must be direct because otherwise the disinfecting activity will continue in the transport medium and thus no clear picture will be given of the disinfecting effect within 1 second that is used in practice for disinfecting slaughtering equipment.

Additional conclusions

A) The efficacy of VR2827-3 against yeasts has not been demonstrated.

B) The additional information provided fails to demonstrate the efficacy of VR2827-3 in cattle slaughterhouses.

C) The trial design is not adequate to determine the effectiveness of VR2827-3 with a short exposure time as a disinfectant for slaughtering equipment in the beef processing industry. In particular, because the efficacy against Gram-negative or Gram-positive bacteria, and against bacteria capable of producing catalase and/or peroxidase, which are representative of the flora that can be found on slaughtering equipment for pigs *and* cattle, is not specifically tested. Moreover, insufficient data on the prevalence and concentration of yeasts is available to adequately test the effectiveness of VR2827-3 against yeasts.

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In the assessment of August 27, 2018 and the previous assessment of INSPEXX, it was concluded that the effectiveness of INSPEXX or VR2827-3 with regard to bacteria in the slaughtering robot for pigs has been demonstrated. This conclusion is based on the results of studies that looked at the effect of INSPEXX or VR2827-3 on bacteria in general (aerobic mesophilic plate count, total viable count) and the effect on *Enterobacteriaceae*. The effectiveness on specific groups of bacteria, Gram-negative and Gram-positive bacteria, and bacteria capable of producing catalase and/or peroxidase was not tested. Since this has also not been specifically tested for use in pig slaughterhouses, it is generally not clear how effective INSPEXX or VR2827-3 is with regard to these specific groups. The effectiveness of VR2827-3 has been demonstrated to a limited extent with regard to bacteria in the slaughtering robot for pigs as the effectiveness against specific groups of bacteria has not been specifically demonstrated.

In the assessment dated August 27, 2018, conclusion 5 erroneously stated that: 'The way in which the control samples are treated does not correspond to the way in which knives are treated. There is a risk that the contact time is longer than in knives in the control samples.' However, the treatment method is correct. Although the trial design shows that after transport the disinfectant is inactive, it does not show whether the neutralising action is direct. Furthermore, the use of *S. aureus* as a test organism in the neutralisation control is questionable, as has already been noted. This is because effectiveness of VR2827-3 as a disinfectant of knives in the slaughterhouse is tested using *Enterobacteriaceae*.

Additional references

Bouwknegt, M, M-J Mangen, IHM Friesema, W van Pelt and AH Havelaar. 2015. Disease burden of food-related pathogens in the Netherlands, 2013. RIVM briefrapport 2014-0115/2015, Bilthoven.

Fabrikant. To whom it may concern. 5 September, 2018, and references:

- Reference 3: Trial Report: INSPEXX 210 on knives. 2008
- Reference 4: Microbiological efficacy of INSPEXX 210 against bacteria and yeasts. 2013.
- Reference 6: Bestätigung des Einsatzes von INSPEXX. Tönnies, 2017.
- Reference 7: Bestätigung INSPEXX Deutschland, 2017.
- Reference 10: Microbiological efficacy of VR2827-3 against bacteria and yeasts.

Quintilla, R, A Kolecka, S Casaregola, HM Daniel, J Houbraken, M Kostrzewa, T Boekhout and M Groenewald. 2018. MALDI-TOF MS as a tool to identify foodborne yeasts and yeast-like fungi. International Journal of Food Microbiology 266: 109-118. Online: https://doi.org/10/1016/j.foodmicro.2017.11.016

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Wirth, F and LZ Goldani. 2012. Epidemiology of *Rhodotorula*: an emerging pathogen. Interdisciplinary Perspectives on Infectious Diseases 2012, article ID 465717. Online: doi:10.1155/2012/465717.

[i] The document by Heres and Verkaar (2011) is an abbreviated publicly accessible report of the findings in Holtslag (2008).