SCIENTIFIC REPORT OF EFSA

Available data on notified biocides[1] efficacy under field conditions (compared to sodium hydroxide and sodium carbonate)[2]

European Food Safety Authority[3]

European Food Safety Authority (EFSA), Parma, Italy

1 Listed in Annex II of Regulation EC 2032/2003 (amended by Regulation EC 1048/2005 and 1849/2006).
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BACKGROUND AS PROVIDED BY EFSA

On 20 November 2006 the French Food Safety Agency (AFSSA) sent a letter to the European Food Safety Authority (EFSA) expressing serious concerns with regard to the exclusion of sodium hydroxide from the notified substances under biological regulation (Annex II of Regulation EC 2032/2003, amended by Regulation EC 1048/2005 and 1849/2006). Particularly, in its letter AFSSA emphasised the high virucidal efficacy of sodium hydroxide in case of epizootic diseases outbreaks and expressed some doubts concerning the efficacy of the biocides listed in Annex II for animal disease control and prevention. Furthermore, consequent to a negative response from the European Commission (DG Environment) to the French request for essential use of sodium hydroxide for veterinary hygiene in case of diseases transmissible to humans, in the same letter AFSSA informed the Authority and its Panel on Animal Health and Welfare (AHAW) on the need for a scientific and technical support at the European level.

This being the premise, after a consultation with both AFSSA and DG Environment (January 2007) EFSA replied to the French Food Safety Agency on 24 July 2007 stating its availability for working on the issue on the base of an official request submitted either by the French Authorities or by the European Commission.

On 26 September 2007 the French Food Safety Agency provided EFSA with a request letter for thorough investigation on the risk related to the non-notification of sodium hydroxide as active substance. The letter was also accompanied by background information.

Having considered the request in detail, AFSSA correspondence was circulated to the members of EFSA's Animal Health and Welfare Panel for information and consultation. On 5-6 December 2007 the request was discussed during the 31st plenary meeting of the AHAW Panel.

The Assessment Methodology Unit of EFSA was asked to prepare the present document in response to the following question posed by AFSSA in its letter:

"Are data available in EU MS demonstrating scientifically that biocides notified in Annex II of EC 2032/2003 have the same efficacy as sodium hydroxide and sodium carbonate when used in farms for which are infected by viruses such as FMDV, CSFV…?"

This Report consists in a preliminary literature search and a summary of the current knowledge in scientific literature on the effectiveness of the biocides notified in Annex II of Regulation (EC) No 2032/2003 and their comparison to sodium hydroxide and sodium carbonate when used in farms for which animals are infected by viruses of highly contagious diseases.

EVALUATION

1. Introduction

Virucidal biocides can be grouped into ten main categories, such as alkalis (sodium hydroxide and sodium carbonate), acids (organic acids – formic citric, lactic, mallic, glutaric, propionic – and inorganic acids – nitric, hydrochloric, sulphuric, phosphoric, sulphamic) chlorine and chlorine compounds (in both liquid – sodium hypochlorite – and solid forms – calcium hypochlorite), oxidizing agents (hydrogen peroxide), aldehydes (glutaraldehyde, formaldehyde and formalin), phenol (carbolic acid) and phenol compounds, quaternary ammonium compounds (cationic compounds containing -NH4+), alcohols (ethanol, propan-1-ol, …), iodophoros (povidone-iodine) and soaps and detergents (i.e. soapy combinations of QACs and phenols) (De Benedictis et al., 2007, Bruins and Dyer, 1995).

When a major notifiable epizootic disease outbreak occurs (i. e., Foot and Mouth disease, Classical Swine Fever, African Swine Fever, Avian Influenza, Swine Vesicular Disease, Aujeszky's disease and Prion diseases), it is of paramount importance that all the causal pathogens are eliminated (Owen, 1995). If the disease can be transmitted through contact with contaminated housing, feed or water, disinfection is an essential element of control. High on-farm virucidal efficacy of the various biocides is therefore fundamental for an optimum standard of disinfection at all housing levels (Fotheringham, 1995).

2. Objectives

The objective of the present document consists in a preliminary literature review of the virucidal efficacy under field conditions of the major biocides listed in Annex II of Regulation (EC) No 2032/2003 against some selected highly pathogenic viral diseases and of their possible comparison to the efficacy of sodium hydroxide and sodium carbonate.

The first part of the literature review is therefore focussed on how to assess virucidal efficacy of environmental surface disinfectants (identification of criteria for virucidal activity tests) and on the factors to be considered for a correct evaluation of disinfection procedures under field conditions.

The second part of the search is directed to the evaluation, based on the data collected, of the efficacy of the listed biocides and of their comparison to sodium hydroxide and sodium carbonate. Particularly, the research is restricted to the major classes of biocides listed in Regulation (EC) 2032/2003 and cited by AFSSA2 and to their effect against some selected high pathogenic viral diseases present in list A of OIE.

3. MATERIALS AND METHODS

3.1. Biocides evaluated

Due to the large amount of biocides present in Annex II of Regulation (EC) 2032/2003, the literature search was limited to some representatives of the major classes of the notified biocides that, as reported by the French Competent Authority[4], are considered ineffective by the UK Department of

[4] See French Essential Use Application Form for Biocides submitted to the EC DG Env. Available at: <http://circa.europa.eu/Public/irc/env/bio_reports/library?l=/review_programme/essential_use/hydroxide_290706/comments_290706&vm=detailed&sb=Title>. Accessed 01/04/2008.

Health Guidance from the Advisory Committee of Dangerous Pathogens and the Spongiform Encephalopathy Advisory Committee (15/12/2003), such as:

- Hydrogen peroxide.
- Iodophoros.
- Peracetic acid.
- Phenolics.
- Alcohols.
- Aldehydes (formaldehyde).
- Ethylene oxide (gas).

During the literature search, other listed biocides (*i.e.* sodium hypochlorite and quaternary ammonium compounds) were recognised as often used and were therefore included in the review.

3.2. Selected viruses

The review was restricted to some selected viral diseases present in list A of OIE, according to AFSSA concerns with regard to their possible inactivation by biocides other than sodium hydroxide:

- Foot and Mouth Disease virus (FMDV). Family: *Picornaviridae*. Genus: *Aphthovirus*.
- Classical Swine Fever virus (CSFV). Family: *Flaviviridae*. Genus: *Pestivirus*.
- African Swine Fever virus (ASFV). Family *Asfarviridae*. Genus: *Asfivirus*.
- Avian Influenza virus (AI). Family: *Orthomyxoviridae*. Genus: *Influenzavirus A*.
- Swine Vesicular Disease virus (SDV). Family: *Picornaviridae*. Genus: *Enterovirus*.
- Aujeszky's Disease virus (ADV). Family: *Herpesviridae*. Genus: *Varicellovirus*.
- Prion diseases (Transmissible Spongiform Encephalopathies - TSEs: Scrapie of sheep, Bovine Spongiform Encephalopathy - BSE).

3.3. Criteria for keywords identification

The keywords were identified taking into account the biocides to be assessed and their effect on the above indicated viruses. Pairwise combinations of virus and biocide term were used.
In addition to this, some general words related to biocides activity ("biocide efficacy", "viral disinfection", "inactivation", "viral biocide", …) and sodium hydroxide/carbonate efficacy were considered.

3.4. Databases and Scientific Journals

The databases used were Pubmed/Medline and ISI Web of Knowledge (including Web of Science®, CAB Abstracts®, Current Contents Connect®, Food Science and Technology AbstractsTM). Some Scientific Journals, such as The Journal of Infectious Diseases and The Veterinary Record, were also screened.

3.5. Criteria for articles eligibility

The papers were selected for evaluation when complying with the following criteria:

- Issue:

  - based on virucidal efficacy evaluation (criteria for virucidal efficacy assessment).
  - based on tests for virucidal efficacy of the biocides listed above.
- Date: up to 15 years (older when considered relevant).
- Language: English/Spanish/Italian.

3.6. Criteria for articles evaluation

The papers were evaluated taking into account the following factors:

- Test viruses.
- For *in vitro* tests: extent of reproduction of field conditions (including, but not restricted to, the types of substrates used for testing).
- Consistency of virucidal activity tests (according to standardised criteria).
- Possible comparison to sodium hydroxide or sodium carbonate.
- Some reviews were also taken into account.
- Biocides characteristics other than virucidal efficacy/activity were not considered (such as corrosiveness, toxicity to handlers, practical use, cost…).

4. RESULTS OF THE PRELIMINARY LITERATURE SEARCH

Fifty-nine articles were selected. Twenty-eight papers were based on the efficacy assessment of the selected biocides against the requested viruses (Table 1 in Appendix A). In Twenty-six the test viruses were not those under concern (Table 2 in Appendix A). Only five articles contained both the efficacy evaluation of some biocides listed in Annex II of Regulation (EC) 2032/2003 and the sodium hydroxide (two were reviews) and, among these, one included also sodium carbonate.

5. RESULTS ON HOW TO ASSESS VIRUCIDAL EFFICACY OF BIOCIDES UNDER FIELD CONDITIONS

It was noticed that for a consistent assessment of virucidal activity of environmental surface disinfectants, standardized tests based on uniform protocols (Sattar and Springthorpe, 2001a, b), reproducing as much as possible the field conditions, should be used.

5.1. Characteristics of standardised laboratory tests

Performance and design criteria for biocides' virucidal activity tests *in vitro*, described for all major classes of chemical disinfectants and for several types of human and animal pathogenic viruses, were identified (Sattar *et al.*, 2003; Springthorpe and Sattar, 2005):

1. Test virus/es: should be selected taking into account their safety to laboratory workers, ease of handling, ability to grow to titres sufficiently high for testing, relative resistance to chemical germicides as well as their potential for spread on environmental surfaces.
2. Substrate materials: suitable substrate materials which mimic the properties of surfaces in disinfection practice are necessary. Besides suspension tests without and with organic load, virus carrier tests, to simulate conditions of porous surfaces and reflect the activity of chemical biocides under field conditions should be used (*i. e.* poplar wood with organic load, Yilmaz et al., 2004).

3. Product performance criterion: in test with viruses, it is usual to aim for a 2-4 $\log_{10}$ reduction in infectivity titre after exposure to a test germicide in a proper carrier test (Hartnack et al., 2008, Sattar et al., 2003).
4. Nature and level of soil loading: the "organic or soil load" in which the viruses are usually shed in field conditions can interfere with the activity of the biocides (by either interacting with them or reducing their effective concentration or by preventing their access to the target virus through physical protection). Therefore, it must be reproduced using suitable and tested organic matrices.
5. Diluent: the use of tap water as germicide diluent is not recommended in a standard test protocol because of its geographically and temporally heterogeneous characteristics. Distilled water does not reflects practical conditions. In view of this, water with a standard level of hardness in it (*e.g.* 200/400 ppm $CaCO_3$) is a more desirable diluent in tests for virucidal activity.
6. Contact between virus and biocide: the test protocol must incorporate contact times to reflect the field conditions. Too long contact times could produce an overestimation of the test product activity. To determine the required contact time of a disinfectant reaction, times of 15, 30, 60 and 120 min in different concentrations of the disinfectant at room temperature have been suggested for the tests (Yilmaz et al., 2004). However, in some instances short-time disinfection for example on vertical smooth surfaces or in spray wash stations for vehicles is needed.
7. Temperature: Unless a product is designed for use only in a specific apparatus, or under specified conditions, biocides are required to demonstrate potency at 20C.
8. Neutralization of virucidal activity: the biocide virucidal activity must be interrupted instantaneously after the end of the contact time to prevent overestimations of the germicidal efficacy. A validation of the effectiveness of the neutralization method adopted must be carried out before the test results can be accepted.
9. Procedure for elimination of cytotoxicity: any prospective biocide cytotoxicity for the cell culture system must be removed immediately at the end of the contact time to avoid interference with the results.
10. Product lots: at least two lots of the test formulation should be evaluated for more reliable results.
11. Additional controls: in addition to the usual cell culture controls, additional controls (cytotoxicity and control for interference with virus infectivity) must be included to determine that germicide residues in the eluates do not have a negative or positive effect on the infectivity of the test virus.

5.2. Evaluating biocides efficacy under field conditions

It was observed that field efficacy of a disinfectant depends on a variety of factors, including, but not limited to, cleanability and other properties of the surface, water quality (hardness, pH, inorganic ions), presence of organic material (feed, excreta, secreta), temperature, pH, short contact times (De Benedictis et al., 2007, Amass, 2004; Tamasi, 1995; Fotheringham, 1995).

Test settings must be suitable for evaluating the efficacy of biocides in animal husbandry and the methods for testing virucides activity should consider factors like organic soiling and surface porosity, which in the veterinary field may considerably hamper the inactivating potency of chemical substances (Yilmaz et al., 2004).

Furthermore, disinfection of slurry requires a different approach than disinfection of surfaces and this also has to be considered when evaluating the efficacy of a virucide during an outbreak.
The timing of sample collection is another important factor. Samples should not be taken from wet surfaces since the end-point of disinfection could be misinterpreted (due to the possible persistence of the biocide activity on a wet surface) (Tamasi, 1995).

The extent of the survival of the test viruses after disinfection can be used as a measure of the efficacy of a biocide under field conditions. Whereas this value was reported for laboratory tests (reduction rate 2-4 $\log_{10}$, Hartnack et al., 2008, Sattar et al., 2003), the same precise indication was not found for virucidal efficacy tests under field conditions.

5.3. Biocides mode of action in relation to virus structure

Virucide mode of action is another important factor to be considered when assessing virucidal efficacy of a biocide. It was noticed that two major factors influence the mechanism of action of a biocide against viruses (Maris, 1995):

- Presence of lipids in the viruses.
- Size of the viruses.

On the basis of their resistance to chemical agents, Noll and Youngner (1959) classified the viruses in three groups:

- Group A: lipid-containing viruses (*e.g.* Avian Influenza virus, Aujeszky's Disease virus).
- Group B: small (20-30nm), non-lipid viruses (*e.g.* Swine Vesicular Disease virus, Foot and Mouth Disease virus, Classical Swine Fever virus).
- Group C: other non-lipid viruses (*e.g. Adenoviridae*, *Reoviridae*, *Papovaviridae*).

The presence of lipid in a virus is associated with a high degree of susceptibility to all disinfectants; the absence of lipid and small size are associated with resistance to lipophilic chemical agents.

6. ASSESSMENT OF BIOCIDES EFFICACY, ON THE BASIS OF THE PAPERS COLLECTED

6.1. Results on scientific information consistency

The papers based on the notified biocides efficacy against the selected viruses (Table 1 in Appendix A) were evaluated considering the major above mentioned criteria for test protocols and the extent of their reproduction of the field conditions.

The following observations apply to most of the studies reported in Table 1 in Appendix A (and discussed in the following section):

1. A hierarchy of susceptibility to the notified chemicals as related to virus size and structure is not established. In addition to this, surrogate organisms for claims of activity against highly pathogenic viruses are not specified and testing should be required against each virus type. Nevertheless, a small amount of studies on the virucidal activity of the listed disinfectants against highly pathogenic diseases (such as FMD, CSF, ASF, AI and TSEs) under field conditions was identified.
2. Substrates materials used for assessing virucides activity are various and few specifications are given on their capacity to absorb a virus suspension, effect on the infectivity titre of the absorbed virus, availability, consistent quality, possibility to sterilize. Moreover, often they do not reflect the characteristics of field substrates like litters, liquid manures, grounds which represent the environmental surfaces in case of on farm disinfection during an outbreak of a highly pathogenic disease.

3. The virucidal efficacy of the various listed compounds is not homogenously expressed in the different studies. In addition, the true relationship between the biocide activity of a product evaluated in laboratory conditions and its ability to prevent the spread of infections in the field is difficult to determine.
4. Field conditions concerning to the use of appropriate organic matrices for test viruses are not always reproduced in laboratory tests and this makes the results scarcely applicable to outbreaks situations.
5. Reported contact times between the test viruses and virucides, as well as the temperature applied during the trials, often are not in a range that it is appropriate for the practical use of the product, not reflecting field circumstances.
6. Specifications on neutralization of virucidal activity procedures, cytotoxicity elimination practices, as well as specific controls during testing are not always given.

Furthermore, most of the biocide activity tests collected were *in vitro* assays with only one test undertaken under field conditions (Suarez et al., 2003). However, in that study very few and heterogeneous details were provided concerning the protocols used (test methodology, timing of sample, test surfaces, etc).

6.2. Results on scientific information availability on notified biocides efficacy and on their comparison to sodium hydroxide and carbonate

This preliminary literature search led to the following considerations on nine main categories of virucidal biocides (alkalis such as sodium hydroxide and sodium carbonate were not considered), as regards to their virucidal efficacy against Foot and Mouth disease, Classical Swine Fever, African Swine Fever, Avian Influenza, Swine Vesicular Disease, Aujeszky's disease virus and Prion diseases and their possible comparison to sodium hydroxide and sodium carbonate (Table 1 in Appendix A).

6.2.1. Acids

Hydrochloric acid and citric acid were considered effective against AIV in disinfecting floors and body respectively, with the best effect at an optimum of 20C (De Benedictis et al., 2007). Yilmaz et al. (2004) showed that a biocide containing 55% formic acid and 7% glyoxylic acid was significantly effective against AIV (H7N1) at 20C (with decreased efficacy when organic load increased ant T decreased) in both suspension and carrier tests (reaction time 10 min). Short-time disinfection (5 min) was not successful on poplar carriers at 4C. It this study a laboratory test was undertaken, but various factors were actually considered to reflect field conditions, such as different T, presence/absence of organic load, different contact times.

In the case of slurry disinfection from viruses such as FMDV, CSFV, and ASFV, Haas et al. (1995) reported that organic acids have a reduced inactivation effect due to the proteins present in the substrate. The same authors illustrated that oxidising inorganic acids (peracetic acid) are rarely used for slurry disinfection because of their corrosive effect.

In another study (Poulin and Christianson, 2006), inorganic and organic acids were used for the first two phases of a 3-steps disinfection procedure against FMDV on a sow farm (ceilings, walls, floors and feeders, buildings) which was concluded with fumigation with formaldehyde and potassium permanganate. The virucidal efficacy of the biocides, as well as the disinfection protocol, were not specified, but the overall programme of eradication (including vaccination) was reported as

successful. Propionic and citric acid were also shown as ineffective for FMDV inactivation in skimmed milk (Sonder et al., 1990).

The use of a disinfectant containing 0.2 % peracetic acid and low concentrations of sodium hydroxide was described by Lemmer et al. (2004) as a potent prions (PrPSc/PrP27-30) decontaminant of steel surfaces (surgical instruments).

6.2.2. Chlorine and chlorine compounds

According to Rice et al. (2007), Highly Pathogenic Avian Influenza (HPAI) subtype H5N1 is readily inactivated by chlorination. In this study, the maintenance of a free chlorine residual in water (0.52–1.08 mg/L) was sufficient to inactivate the virus by >3 orders of magnitude within an exposure time of 1 minute. The authors reported that Ct (the chlorine concentration, C [mg/L], multiplied by the exposure time, t [min]) values of 6 and 8 mg-min/L would be more than sufficient to inactivate HPAI (H5N1) in the water environment. In another laboratory study on AI virus (H5N9, H7N3) inactivation (Suarez et al., 2003), sodium hypochlorite was reported as effective for both inactivating virus and preventing amplification by reverse transcriptase-polymerase chain reaction (evaluation of efficacy for inactivating influenza as well as disrupting the RNA so that it could not be detected by the RRT-PCR test). Calcium hypochlorite and sodium hypochlorite against AIV were indicated as efficacious for floors, clothes and equipment, but with the potential to be inhibited by organic materials and basic values of Ph (De Benedictis et al., 2007). Davison et al. (1999) showed a decreased inactivation power of sodium hypochlorite against AIV when mixed with antifreeze, posing some doubts on the use of this compound in low temperature situations.
Sodium hypoclorite was reported as a good virucide against four enveloped viruses, included African Swine Fever virus, but not against Swine Vesicular Disease virus (Shirai et al., 2000). However, this study did not take into account other factors influencing disinfection (i.e. T, pH, etc.), such as under field conditions. Classical Swine Fewer virus was indicated as sensitive to chlorine-based disinfectants by Edwards (2000).
The effectiveness of sodium hypoclorite against Prions was clearly demonstrated by Yao et al. (2005). The results of this study showed that sodium hypoclorite (2 mol/l 2. NaOCl for 1 h) was completely effective for inactivation of scrapie (*in vitro*, scrapie 263K infected homogenates). Furthermore, concerning the protease (PK) resistance, the study indicated that sodium hypoclorite solutions annihilate both PK resistance of PrP^{Sc} and PrP at the same concentrations (whereas sodium hydroxide necessitated a higher concentration for removing PK resistance of the protein itself). Therefore, it was concluded that sodium hypoclorite is an effective biocide for inactivating infectivity of scrapie 263K and for annihilating the protease resistance of its PrP^{Sc}.

6.2.3. Oxidizing agents (hydrogen peroxide)

For disinfection procedures against AIV, hydrogen peroxide seems to decrease its efficacy in presence of organic compounds and it is used more as a virucide for laboratory equipment (De Benedictis et al., 2007). This biocide showed reduced infectivity but not complete inactivation power against AIV in an experiment undertaken by Neighbor et al. (1994).
Heckert et al. (1997) estimated the virucidal efficacy of vapor-phase hydrogen peroxide (VPHP) against various viruses (Avian Influenza virus, African Swine Fever virus, Bluetongue virus, Hog Cholera virus, Newcastle Disease virus, Pseudorabies virus, Swine Vesicular Disease virus, Vesicular Exanthema virus, Vesicular Stomatitis virus), with positive results (reduction of the titres to 0 ELD_{50} for avian viruses or less than 10 $TCID_{50}$ for mammalian viruses), except for Hog Cholera virus suspended in blood. However, although virus inactivation by VPHP was assessed both with viruses

suspended in liquid and dried onto a solid support, materials such as rubber, cloth, plastic, were not considered in the tests (only laboratory equipment and inanimate material such as glass and steel).
Hydrogen peroxide did not show virucidal efficaciousness against Foot and Mouth virus in skimmed milk (Sonder et al., 1990).
Vaporised hydrogen peroxide (VHP) was tested both in combination with an enzymatic cleaner and alone for disinfection of medical and surgical instruments (stainless steel wires) contaminated with Prions, with good results (4.5 log reduction in infectivity for VHP alone) (Fichet et al., 2004). In the same study, the effectiveness of sodium hydroxide for the equivalent disinfection purposes was also demonstrated. However, both experiments did not take into consideration practical/outbreaks situations. The virucidal efficacy of a formulation containing hydrogen peroxide in combination with copper metal ions against Prions was evaluated by Solassol et al. (2006). In this study, an *in vitro* assay, confirmed by an animal assay (reduction in Prion infectivity) showed a considerable reduction of the level of prion protein, demonstrating that Cu_2+-H_2O_2 formulation can be a suitable method for disinfection of sensitive medical equipment and instruments. For Prion inactivation, Suyama et al. (2007) tested a formulation of iron ions combined with hydrogen peroxide, with good results (significant decrease in PrPSc levels), indicating that iron ions in presence of high concentrations of hydrogen peroxide can be applied in decontamination of fragile instruments susceptible to Prion contamination.

6.2.4. Aldehydes

Formaldehyde showed virucidal efficaciousness against Foot and Mouth Disease (type A, 0, C and Asia-1) in a laboratory study undertaken by Dekker (1998). In the same study the efficacy of ethylene oxide against FMDV is also reported. The virus was inactivated to levels below the detection limit in laboratory conditions (air-dried virus on coverslips). Barteling and Cassim (2004) reported that a combination of binary ethyleneimine (not listed in Annex II of Regulation EC 2032/2003) and formaldehyde is effective against Foot and Mouth Disease virus cultures (inactivation rate 2.5-3.5 $logs_{10}$ per hour). However, the study was based on vaccine production and did not reproduce on farm outbreaks situations. The latter applies also to a study undertaken by Twomey et al. (1995), in which the efficacy of formaldehyde against Foot and Mouth disease virus in vaccine production was demonstrated.
Edwards (2000) indicated that Classical Swine Fever virus is sensitive to formaldehyde and glutaraldehyde. In a review on the inactivation of viruses in liquid manure, Haas et al. (1995) stated the high effectiveness of formaldehyde for disinfection of slurry from Foot and Mouth Disease, Classical Swine Fever and African Swine Fever. Alkalis such as sodium hydroxide were also indicate as effective. Nevertheless, no figures on the efficacy of the different compounds were reported. Fumigation with formaldehyde and potassium permanganate was successful as a third disinfection step (after pressure washing and soaking with organic acid and disinfection with inorganic acid) against Foot and Mouth disease on a sow farm (Poulin and Christianson, 2006).
In a study (Weissmann et al., 2002) on the inactivation of Prion-coated steel wires under experimental conditions, formaldehyde (10%, 1 h, 25C; incubation days [±DS] 92±8) was insufficient to sterilize infectious wires (No. sick/total = 6/6), whereas sodium hydroxide (1 M, 1 h, 25C; incubation days [±DS] >260) showed a very high inactivation activity (No. sick/total = 0/6).
According to de Benedictis et al. (2007), aldehydes (formalin, glutaraldehyde and formaldehyde) are effective against AIV, although decrease their efficacy in presence of organic compound. Yilmaz et al. (2004) indicated 20% formaldehyde and 12% oligomer pentaerythritose condensate as an effective disinfectant against AIV (at 20C for 10 min). However, short-time disinfection (5 min) was not successful on poplar carriers at 4 C and 10C, even at 2% concentrations of the virucides. This was indicated as a possible cause of severe problems in the undercarriage and tire disinfection of vehicles entering and leaving farms through spray wash stations in the cold seasons of the year.

6.2.5. Phenol (carbolic acid) and phenol compounds

Suarez *et al.* (2003) showed that phenolic disinfectants have good inactivation activity against AI virus (H5N9 and H7N3) under laboratory conditions, although AIV RNA could still be detected by reverse transcriptase-polymerase chain reaction (RRT-PCR). In addition to this *in vitro* evaluation of the phenolic virucidal activity, this study included a field assessment of the efficacy of phenolic based disinfectants. Three randomly selected live-bird markets that had been previously identified as having birds infected with AI H7N2 were depopulated of birds through normal commerce and the premises were thoroughly cleaned and disinfected using a phenolic biocide. The outcomes reflected the laboratory test, with good viral inactivation effect of the phenolic compound but negative results on RRT-PCR. However, no details were provided on the field test protocol used (*i. e.* timing of sample collection, test surfaces, etc.).
The virucidal efficacy of phenols (cresolic acid, synthetic phenols, phenol crystal) against Avian Influenza virus, also in presence of organic material, was reported by De Benedictis et al. (2007) and was demonstrated in another *in vitro* test by Muhmmad et al. (2001). Davison et al. (1999), showed that phenols are effective against AIV also in presence on antifreeze products (such as ethylene glycol/propylene glycol or methyl alcohol), suggesting the use of these biocides also in low temperature conditions
Edwards (2000) indicated that Classical Swine Fever virus is also sensitive to phenolics.

6.2.6. Quaternary ammonium compounds (QACs)

According to Shirai *et al.* (1997), QACs have a limited spectrum of activity and are ineffective against most viruses, mycobacteria and bacterial spores. Among viruses, QACs are effective only against enveloped viruses (group A). However, this study demonstrated that QACs plus a small amount of sodium hydroxide inactivated Swine Vesicular Disease virus (belonging to the enteroviruses - group B, small non-enveloped viruses), indicating that the activity of the QACs was enhanced by 0.1% NaOH. These findings were confirmed by a successive study (Shirai et al., 2000), in which quaternary ammonium compounds (didecyldimethylammonium chloride) showed high effectiveness at low concentration (0.003%) against four enveloped viruses (Vesicular stomatitis virus, African Swine Fever virus, Equine Viral Arteritis virus, and Porcine Reproductive/Respiratory Syndrome virus) and against African Horse Sickness virus (non-enveloped) but had virucidal activity against Swine Vesicular disease virus only with 0.05% NaOH. However, this study did not reproduce field conditions with regard to factors such as temperature, pH, etc.
A quaternary ammonium compound was indicated as effective against AI virus (H5N9, H7N3) in an *in vitro* evaluation undertaken by Suarez et al. (2003). In this study, the QAC was efficacious for inactivating Avian Influenza, but did not show good RNA virus disruption (as detected by the RRT-PCR test). De Benedictis et al. (2007) reported QACs as efficacious virucides against AIv also in presence of anti-freeze compounds.
The efficacy of these biocides against Classical Swine Fever is reported by Edwards (2000).

6.2.7. Alcohols

Three alcohol-based hand rubs (based on propan-2-ol, propan-1-ol, or ethanol) were indicated as good virucides against various enveloped viruses such as Avian Influenza A virus (H3N8). The test was based on virus suspensions that allowed a reduction of at least 4 $\log_{10}$-steps, with different types of organic loads (Kampf et al., 2007). In this study, the alcohols activity was assessed *in vitro* and no experiment was undertaken under practical conditions (although it has to be considered that *in vivo* assessments can be difficult for biosafety reasons). These compounds were also indicated as effective for clothes disinfection, but not for plastic (De Benedictis et al., 2007).

6.2.8. Iodophoros

Ito *et al.* (2006) described the inactivation effect of six povidone-iodine products against Avian Influenza virus (H5N1, H5N3, H7N7, H9N2). In this *in vitro* study on embryonated hen's eggs, viral infectious titres were reduced to levels below the detection limits by incubation for only 10 Sec. The study did not reproduce field conditions. Iodine (potassium tetraglicine triiodide, at concentrations of 0.015% to 0.0075%) was also indicated as an effective virucide against African Swine Fever virus and other enveloped and non-enveloped viruses (such as Swine Vesicular Disease virus) by Shirai et al. (2000).

6.2.9. Soaps and detergents

Soapy combinations of phenols and quaternary ammonium compounds were described as efficacious against Avian Influenza virus mainly for cleaning procedures (De Benedictis et al., 2007), while saponin was reported to be effective in inactivating enveloped viruses such as Classical Swine Fever virus (Edwards, 2000). Both the cited articles are review and do not report testing protocols for virucide efficacy evaluation.

7. DISCUSSION

7.1. Data availability and consistency

Based on the information collected in this preliminary literature review it is concluded that virucide activity tests are various and heterogeneous while standardised trials on biocides' efficacy, with specific and detailed protocols, are rarely in place. As a consequence, a reliable comparison between the various disinfectants' efficacy is rather complex. Furthermore, there is lack of studies assessing the effectiveness under field conditions (*i.e.* outbreaks) of the biocides listed in Annex II of Regulation EC 2032/2003 against high pathogenic viruses (FMD, CSF, ASF, AI…). When field studies are present, few and heterogeneous details are given concerning the protocols used for virucide testing. In addition to this, a small amount of comparative efficacy studies between the listed disinfectants and sodium hydroxide or sodium carbonate was identified.

7.2. Listed biocides efficacy

Apparently biocides with lipophilic properties (QACs, phenols) are active against group A (enveloped) viruses and not against group B and C (small non-enveloped and other non-enveloped viruses), whereas chlorine and iodine compounds, oxidising agents, some aldehydes and strong acidic agents seem to have an effect against most viruses. However, few standardized studies, which properly reproduce field outbreaks situations, have been found to substantiate the efficacy of these biocides against high pathogenic viral diseases.

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APPENDICES

APPENDIX A

Table 1. Data on notified biocides efficacy against Foot and Mouth disease, Classical Swine Fever, African Swine Fever, Avian Influenza, Swine Vesicular Disease, Aujeszky's disease virus and Prion diseases and on their comparison to sodium hydroxide and sodium carbonate.

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
Barteling and Cassim, 2004	1. Binary ethyleneimine (BEI) 2. Binary ethyleneimine + Formaldehyde (BEI-FA)	FMDv	Virus cultures	BEI: inactivation rate 0.4-1.0 $\log_{10}$ per hour. BEI-FA: more effective (2.5-3.5 $\log s_{10}$ per hour)	/	Vaccine production. No field conditions. Binary ethyleneimine is not listed in Annex II
Davison et al., 1999	Four biocides mixed with 50% ethylene glycol/propylene glycol or 70% methyl alcohol (antifreeze): phenol-based product 1: sodium o-phenylphenate, sodium o-bezylp-chlorophenate, sodium p-tertiary-amylphenate. phenol-based product 2: o-phenilphenol, p-tertiary amyphenol, quaternary ammonium. a combination biocide: 2-(hydroxymethyl)-2-nitro-1,3-propanediol, formaldehyde, alky; dimethy; benzyl ammonium chloride. Sodium hypochlorite detergent	AIV (H7N2)	Allantoic fluid inoculated	The addition of antifreeze (ethylene glycol/propylene glycol or methyl alcohol) as an additive to the use dilution of disinfectants to prevent freezing did not decrease the efficacy of phenol and quaternary ammonium compounds. The combination product and the sodium hypochlorite had decreased efficacy	/	Phenols and quaternary ammonium compounds effective against AIV also in presence of antifreeze. No comparison to NaOH
De Benedictis et al., 2007 (review)	1. Soapy combinations of phenols or QACs. 2. Alkalis: calcium hydroxide. 3. Acids: Hydrochloric	AIVs	/ (Review - No trials)	Most active at an optimum of T>20C. ALL EFFICACIOUS. 1. Soaps and detergents: for cleaning procedures	Alkalis in general: not efficacious at room T (the activity	AIV very sensitive to a large choice of chemical agents (9 major groups included NaOH and Na_2CO_3)

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
	acid/Citric Acid. 4. Chlorine and chlorine compounds: Calcium hypochlorite/ Sodium hypochlorite. 5. Oxidizing agents: Hydrogen peroxide. 6. Aldehydes. 7. Phenol compounds. 8. QACs; 9. Alcohols: ethanol			2. Alkalis : calcium hydroxide; not efficacious at room T. Efficacious for walls, floors. 3. Acids: Hydrochloric acid: for floors. Citric Acid: Clothing and body. 4. Chlorine compounds. inhibited by organic materials and by basic values of Ph (floors, clothes and equipment). 5. Hydrogen peroxide: decreasing efficacy in presence of organic compounds. Laboratory equipment. 6. Aldehydes: decreasing efficacy in presence of organic compounds. 7. Phenol compounds: floors. Efficacious in presence of organic materials 8. QACs: personal use. Efficacious in presence of anti-freeze compounds. 9. Alcohols: Clothes and equipment. Not for plastic. Ethanol in association with other compounds in hand disinfectants	increases at high T). NaOH: Recommended for floors and cloths. Not in presence of Aluminium and derived alloys. Na_2CO_3: efficacious in presence of high concentration of organic material. Thermo labile and light-sensitive	
Dekker,	Formaldehyde; ethylene	FMDV type A, 0, C and	Virus air-dried	Both efficacious: virus	/	Laboratory conditions. No

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
1998	oxide	Asia-1	on coverslips	inactivated to levels below the detection limit		field conditions. No comparison to NaOH
Edwards, 2000 (review)	Organic solvents (i. e. chloroform) and detergents (deoxycholate) or saponin. Chlorine-based disinfectants, detergents, phenolics, quaternary ammonium compounds, and aldehydes (formaldehyde, glutaraldehyde)	CSFV	/ (Review, no trials reported)	As an enveloped virus, swine fever is inactivated by organic solvents, detergents or saponin. CSFV is sensitive to all the chemicals cited	/	Review, no trials reported. No comparison to NaOH
Fichet et al., 2004	1. NaOH 1N, NaOCl 20000 ppm; 2. autoclaving in water at 134 degrees C; 3. autoclaving without immersion; 4. phenolic disinfectant; 5. alkaline cleaner; 6. combination of an enzymatic cleaner and vaporised hydrogen peroxide (VHP); 7. VHP alone	Prions	Medical and surgical instruments (stainless steel wires) contaminated with prions to the hamster-adapted scrapie strain 263K)	• 1 and 2: effective (reduction of infectivity by >5.6 log10 lethal doses); • 3: less effective than 1 and 2 (4-4.5 log reduction). • 4-5-6: effective. • 7: 4.5 log reduction in infectivity (like 3)	Both effective	Disinfection of surgical instruments. No practical/outbreaksconditions
Gao et al., 2006	Guanidine (Gdn)	Prions (PrP(Sc))	Prions: proteinase K (PK) resistance in vitro and infectivity of scrapie strain 263K	Effective at reducing or even destroying the infectivity, but the infectivity of PrP(Sc) inactivated by denaturation could be partially restored by renaturation. Gdn enhanced PK-sensitivity in	/	A complete loss of PK-resistance of PrP(Sc) may not necessarily mean its full non-infectivity. No comparison with NaOH

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
				a dose-dependent manner. The PK-resistance in vitro of PrP(Sc) denatured with lower concentrations of Gdn (<2.5 mol/l) could partially resume by renaturation		
Haas et al., 1995 (review)	1. Aldehydes (formaldehyde). 2. Organic acids. 3. Oxidising acids (peracetic acid). 4. Alkalis (calcium hydroxide and sodium hydroxide)	FMDV, CSFV, ADV, ASFV, Swine influenza virus, Porcine paramixovirus, bovine virus diarrhoea, transmissible gastroenteritis of pigs virus	Slurry	1. Formaldehyde: very effective. 2. Organic acids: reduced inactivation effect due to the proteins contained in the slurry. 3. Inorganic acids: rarely used (corrosive effect). 4. Oxidising agents: limited practical use due to strong foaming (peracetic acid recommended for small volumes)	Alcalis: effective	No figures on the different efficacies (review)
Heckert et al., 1997	Vapor-phase hydrogen peroxide (VPHP)	Avian influenza virus; African swine fever virus; Bluetongue virus; Hog cholera virus; Newcastle disease virus; Pseudorabies virus; Swine vesicular disease virus; Vesicular exanthema virus; Vesicular stomatitis virus	Virus inactivation by VPHP evaluated both with viruses suspended in liquid or dried onto a solid support. Laboratory equipment and inanimate materials (glass	Efficacious (except for hog cholera virus suspended in blood): reduction of the titres to 0 ELD50 for avian viruses or less than 10 TCID50 for mammalian viruses	/	Not tested on farm conditions. Not tested for materials such as rubber, cloth, plastic, etc. Not effective when a virus was present in blood. No comparison to NaOH

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
			and steel) tested			
Ito et al., 2006	Povidone-iodine (6 PVP-I products: 2% PVP-I solution, 0.5% PVP-I scrub, 0.25% PVP-I palm, 0.23% PVP-I gargle, 0.23% PVP-I throat spray and 2% PVP-I solution for animals)	AIV (H5N1, H5N3, H7N7, H9N2)	*In vitro*: embryonated hen's eggs	Effective: Viral infectious titers reduced to levels below the detection limits by incubation for only 10 Sec	/	No field conditions. No comparison to NaOH
Kampf et al., 2007	Three commonly-used alcohol-based hand rubs: 1. A: based on 45% propan-2-ol, 30% propan-1-ol and 0.2% mecetronium etilsulfate. 2. B: based on 80% ethanol. 3. C: based on 95% ethanol	Vaccinia virus and bovine viral diarrhea virus (BVDV) (test viruses in a quantitative suspension test to determine the activity of a disinfectant against all enveloped viruses). Herpes simplex virus (HSV). Human and avian influenza A virus/duck/Ukraine/1/63 (H3N8)	Virus suspensions that allowed a reduction of at least 4 log10-steps, with different types of organic loads	All three reduced the infectivity of vaccinia virus and BVDV by ≥ 4 $\log_{10}$-steps within 15 s, irrespective of the type of organic load. Similar reductions of infectivity were seen against the other four enveloped viruses within 15 s with or without organic load	/	Hand rubs (for medical settings/healthcare facilities) tested in vitro. No experiment under practical conditions (but difficult to test in vivo for biosafety reasons). Non-enveloped viruses not considered
Lemmer et al., 2004	• 0.2 % SDS (sodium dodecyl sulphate)/0.3 % NaOH (pH 12.8); • a commercially available alkaline cleaner (pH 11.9-12.2); • a disinfectant containing 0.2 % peracetic acid and low concentrations of NaOH (pH 8.9) or 5 %	Prions PrPSc/PrP27-30	Steel surfaces (surgical instruments) (in vitro carrier assay)	Potent decontaminating activities	NaOH included	Addition of NaOH. No comparison with other biocides

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
	SDS (pH 7.1)					
Muhmmad et al., 2001	Formalin, phenol crystal	AIV (H7N3)	Chicken embryos	Formalin: at concentration of 0.06 and 0.12% resulted in inactivation of the AIV within six hours. Phenol solution (0.2%) inactivated the virus	/	*In vitro* test. No comparison to NaOH
Neighbor et al., 1994	Hydrogen Peroxide (5% or 10%)	Newcastle disease virus, Avian influenza virus	Viruses dried on glass Petri dishes	Reduced infectivity but not complete inactivation.	/	*In vitro* test. No comparison with NaOH
Poulin and Christianson, 2006	3-steps disinfection: 1. pressure washing and soaking in organic acid; 2. final disinfection with organic acid; 3. fumigation with formaldehyde and potassium permanganate	FMDV on a sow farm.	Ceilings, walls, floors and feeders, buildings	Not specified, but the overall programme of eradication (including vaccination) was successful	/	Pigs are relatively resistant to infection by airborne FMDV (compared to ruminants). No specifications on the disinfection protocol. No comparison to NaOH
Rice et al., 2007	Chlorine	Highly pathogenic avian influenza (HPAI) subtype H5N1	Infective amnioallantoic fluid.	Readily inactivation (by >3 orders of magnitude within an exposure time of 1 minute)	/	Chlorine effective against AIV. No comparison with NaOH
Shirai et al., 2000	1. chlorine (sodium hypochlorite). 2. iodine (potassium tetraglicine triiodide). 3. quaternary ammonium compound (didecyldimethylammonium chloride)	Four enveloped viruses: 1. Vesicular stomatitis virus. 2. African swine fever virus. 3. Equine viral arteritis virus 4. Porcine reproductive/ respiratory syndrome virus. Two non-enveloped	Cell cultures. Each virus sample directly used and mixed with equal volume of each diluted disinfectant and the mixtures were incubated at	1. Chlorine: effective against all viruses except SVDV at concentrations of 0.03% to 0.0075% (dose response). 2. Iodine: very effective against all viruses at concentrations of 0.015% to 0.0075% (dose response not observed). 3. Quaternary ammonium	/	No field conditions. QAC effective against SVDV only with NaOH. Other effects for disinfection (i.e. T, pH, etc.) not examined

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
		viruses: 1. swine vesicular disease virus (SVDV). 2. African horse sickness virus (AHSV)	room T for 30 min.	compound: very effective in low concentration of 0.003% against four enveloped viruses and AHSV. Effective against SVDV only with 0.05% NaOH		
Shirai et al., 1997	Quaternary ammonium compounds (Didecyldimethylammonium chloride-DDAC) with 0.1 % NaOH	Swine vesicular disease virus (SVDV)	IBRS-2 cell cultures	Didecyldimethylammonium chloride without NaOH: no effect, even at high concentration. Didecyldimethylammonium chloride with 0.1% NaOH (at 40C, pH around 11.0, 1 min): very effective	NaOH included	The disinfection procedure includes NaOH. No field conditions
Solassol et al., 2006	Formulation of copper metal ions in combination with hydrogen peroxide	Prions (PrP(Sc))	In vitro assay (homogenates of samples from prion-infected brains), confirmed by an animal bioassay(mice)	Considerable reduction of the level of prion protein. The animal bioassay confirmed the reduction in prion infectivity	/	Great potential for prion sanitization indicated by the animal bioassay. Effective for sensitive medical equipment and instruments. No comparison with NaOH
Sonder et al., 1990	Propionic acid, citric acid and hydrogen peroxide	FMDV	Skimmed milk	Neither acidification nor hydrogen peroxide effective for the inactivation of FMDV in skimmed milk	/	Skimmed milk. No outbreaks conditions
Suarez et al., 2003	1. phenolic disinfectants. 2. a quaternary ammonium compound. 3. a peroxygen compound. 4. sodium hypochlorite	AI virus (H5N9, H7N3)	Field study: evaluation of infected markets (for testing the	All five disinfectants were effective for inactivating AIV at the recommended concentrations (evaluation of efficacy for inactivating	/	No comparison with NaOH. Laboratory conditions

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
			phenolic compound). In vitro evaluation (infectious virus in allantoic fluid) (incubation for 10 min or 1 h at room T)	influenza as well as disrupt the RNA so that it could not be detected by the RRT-PCR test). 1 and 2 inactivated samples: AIV RNA could still be detected by RRT-PCR. 3 and 4 effective for both inactivating virus and preventing amplification by RRT-PCR		
Suyama et al., 2007	Formulation of iron ions combined with hydrogen peroxide	Prion	Scrapie-infected brain homogenates	Effective at reducing infectivity (significant decrease in PrPSc levels)	/	Effective for sensitive medical equipment. No comparison with NaOH
Twomey et al., 1995	Formaldehyde	FMDV	Vaccine production	Effective: formaldehyde-inactivated vaccine stable below pH 7 (and the RNA could not be released)	/	Vaccine production. No field conditions
Weissmann et al., 2002	Formaldehyde (10%, 1 h, 25C; incubation days [±DS] 92±8)	Prion protein (scrapie)	Prion-coated steel wires (experimental condition)	Insufficient to sterilize infectious wires (No. sick/total = 6/6)	NaOH (1 M, 1 h, 25C; incubation days [±DS] >260): efficacious (No. sick/total = 0/6)	NaOH more efficacious than Formaldehyde
Yao et al., 2005	Several disinfection methods: 1. NaOH; 2. NaOCl (sodium hypochlorite); 3. heating or autoclaving at 80, 121 and 134C in the solutions with or without	PrPSc, to test protease resistant activity in vitro and infectivity in vivo of scrapie strain 263	Protease resistance of PrPSc: protease K (PK) digesting Western blot. Infectivity of PrPSc:	For infectivity: NaOCl effective at 2%, 1-h exposure). Mixing with NaOH (2 mol/l) or NaOCl 2%, autoclaving at 134, as well as heating at 100C or autoclaving at 121C in the	For infectivity: NaOH completely effective at 2 mol/l. For protease resistance: NaOH effective	NaOCl can be used as an alternative to NaOH

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
	sodium dodecyl sulphate (SDS)		intracerebral inoculation into experimental hamsters	solutions with 3% SDS: completely effective. For protease resistance: 1. see right column 2. NaOCl: effective at ≥0.1%; autoclaving: effective at >121C. Heating: effective >80C (with 3% SDS)	at >0.05 mol/l	
Yilmaz et al., 2004	1. 20% (w/w) formaldehyde and 12% (w/w) oligomer pentaerythritose condensate. 2. 55% (w/w) formic acid and 7% (w/w) glyoxylic acid	AIV (H7N1)	Suspension (with and without protein load) and carrier tests (poplar wood). Tests at 20, 10, 4 degrees. Reaction times: 5 and 10 min	Both disinfectants significantly effective at 20C (with decreased efficacy when organic load increased ant T decreased) in both suspension and carrier tests. Short-time disinfection (5 min) not successful on poplar carriers at 4 C and with biocide 1 also at 10C	/	Laboratory test, but various factors considered such as different T, presence/absence of organic load, different contact times. No comparison with NaOH

Table 2. Data on notified biocides efficacy against viruses other than Foot and Mouth disease, Classical Swine Fever, African Swine Fever, Avian Influenza, Swine Vesicular Disease, Aujeszky's disease virus and Prion diseases and on their comparison to sodium hydroxide and sodium carbonate.

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
Eleraky et al., 2002	1. chlorine dioxide. 2. potassium peroxymonosulfate. 3. a quaternary ammonium compound. 4. citricidal (grapefruit extract)	Feline herpesvirus, feline calicivirus, and feline parvovirus	/	1 and 2: completely inactivated the three viruses. 3 and 4: not effective	/	Not the selected viruses
Fages et al., 1998	Supercritical CO2 alone COMPARED TO 4-steps processing: Supercritical CO2+hydrogen peroxide, sodium hidroxide, ethanol	Human immunodeficiency virus type 1 (HIV-1), Sindbis virus, polio Sabin type I virus, Pseudorabies virus (PRV)	Human bone tissue	Effective in inactivating viruses in human femoral heads (measured as mean cumulated reduction factors [log10] for the four viruses)	The level of inactivation of supercritical CO2 alone is similar to that obtained by the 4-steps process (which includes NaOH)	Not the selected viruses. No field conditions
Hartnack et al., 2008	1. formic acid 550 g/l, glyoxylic acid 88 g/l). 2. acetic acid 172.5 g/l, peracetic acid 172.5 g/l, hydrogen peroxide 241.5 g/l). 3. (glutaraldehyde 249 g/l, formaldehyde 184 g/l. 4. sulfamic acid 150 g/l, sodium chlorine cyanurate 50 g/l, potassium persulphate 231 g/l)	Modified vaccinia virus Ankara (MVA) and vaccinia virus strain Elstree (VACV)	Suspension tests (examination under conditions with and without protein load) and carrier tests (autoclaved poplar wood)	All four disinfectants were similarly virucidal for the two viruses, in terms of disinfectant concentrations and reaction times (15, 30, 60 and 120 min), with a maximum titre reduction of at least 4 log10 TCID50/ml	/	Not the selected viruses. Not all the compounds tested are listed in EC/2032/2003. No field conditions. No comparison to NaOH
Herńndez et al., 2000	Peroxygenic acid	Poliovirus	Dilution suspension test	Efficacious at concentration of 1 %	/	Not the selected viruses. Compounds not in the list

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
Hodde and Hiles, 2002	Peracetic acid 0.18%/aqueous 4.8%	Porcine parvovirus (PPV), porcine reovirus, murine leukemia retrovirus (LRV), porcine pseudorabies (herpes) virus (PRV)	Porcine small intestine, small intestinal submucosa biomaterial, (contact time ranging from 5 min to 2 h)	Viral titers reduced by more than 14.0 log10 PPV, 21.0 log(10) reovirus, 40.0 log10 PRV, and 27.0 log10 LRV, Enveloped viruses more easily inactivated than non-enveloped viruses, but material processed for 30 minutes or longer inactivated all of the viruses	/	Not the selected viruses. Test on biomaterial for transplantation into humans
Jannat et al., 2005	NaOH and CIP-100 (Formulated Alkaline Cleaner)	Adenovirus type 5	Different sample matrices and adenovirus constructs	> 6log reduction in the potency of adenovirus type 5	/	The efficacy of NaOH is demonstrated. Not the selected viruses. No comparison with other biocides is made
Kampf et al., 2005	3 ethanol-based hand rubs (95% ethanol; 80% ethanol; 75.1% ethanol), controlled with 70% ethanol and 70% propan-1-ol	Feline calicivirus (FCV)	2-steps procedure: • three different organic loads. • with 5% faecal suspension	Hand rub based on 95% ethanol more effective than those based on 70% ethanol (mean log10 reduction factor: 2.17 vs. 1.56; P=0.17) and 70% propan-1-ol (mean RF: 1.63 vs. 0.95; P=0.0003). Hand rub based on 80% ethanol more effective than those based on 70% ethanol (mean RF: 1.25 vs. 1.03: P=0.20) and 70% propan-1-ol (mean RF: 1.43 vs. 1.09; P=0.03). Hand rub based on 75.1% ethanol less effective than those based on 70% ethanol (mean RF: 1.07 vs. 1.27;	/	Ethanol has superior efficacy against FCV than propan-1-ol. Not the selected viruses

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
				P=0.47) and 70% propan-1-ol (mean RF: 0.78 vs. 0.97; P=0.35)		
Kramer et al., 2006	Formula with reduced ethanol content (55%) in combination with 10% propan-1-ol, 5.9% propan-1.2-diol, 5.7% butan-1.3-diol and 0.7% phosphoric acid	Seven enveloped (influenza A and B, herpes simplex 1 and 2, bovine corona, respiratory syncytial, vaccinia, hepatitis B, bovine viral diarrhoea) and four non-enveloped (hepatitis A, polio, rota, feline calicivirus)	Quantitative suspension tests, with and without protein load.	Efficacious: reduction of infectivity of both enveloped and non-enveloped viruses (>10(3)-fold within 30s)	/	Not the selected viruses
Maes et al., 2007	Ethanol, peracetic acid, sodium hypochloride and peroxigenic acid	Puumala virus	/	Inactivation of Puumala virus effective after 10min with all products except ethanol. Inactivation with absolute ethanol effective only after 30min	/	Not the selected viruses
Malik et al., 2006	Phenolic compound	Calicivirus	FCV dried on fabrics and carpets, followed by treatment with a given disinfectant for a defined contact time of 1, 5 or 10 min	Not uniformly effective (effectiveness increasing with increasing of exposure time). A disinfectant was considered to be effective if it inactivated at least 99% of the applied virus.		Not the selected viruses
Royer et al., 2001	Ethanol, polyalkylenelycol-iodine complex, two phenolic compounds, two quaternary ammonium	Porcine circovirus type 2	Cells cultures	Several biocides were effective. Chlorhexidine, formaldehyde, iodine and ethanol not significantly	No comparison made	Not the selected virus

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
	compounds, a formaldehyde and QAC, chlorhexidine, sodium hydroxide and a mixture of potassium peroxymonosulfate and sodium chloride			decrease PCV		
Sauerbrei et al., 2006	Peracetic acid (PAA), povidone-iodine (PVPI) and formaldehyde.	Duck hepatitis B virus	Obtained from congenitally infected ducks or prepared from the transfected hepatoma D2 cell line	Inactivation achieved with lo when compared to the negative controlwer concentrations of the biocides and within shorter exposure time intervals	/	Not the selected virus
van Engelenburg et al., 2002	High concentration alcohol mixture (80% ethanol and 5% isopropanol)	ENVELOPED: Human immunodeficiency virus, bovine viral diarrhoea virus, a specific model virus for hepatitis C virus, pseudorabies virus, vaccinia virus. NON-ENVELOPED: viruses hepatitis A virus, canine parvovirus, reovirus type 3	/	High virucidal potential in particular for the blood-borne enveloped-viruses reduction by a factor of >106 after 60 sec)	/	Not the selected viruses (but BVDV in list A OIE)
Wutzler and Sauerbrei, 2000	0.2% peracetic acid and 80% (v/v) ethanol (PAA-ethanol)	Enveloped vaccinia virus and papova virus SV 40 and non-enveloped adenovirus type 2 and poliovirus type 1	/	All test viruses inactivated by PAA-ethanol within an exposure time of 1minute, as measured by a log(10)reduction of 4 in virus titres.	/	No the selected viruses

Reference	Biocide/ disinfection procedure	Test viruses	Type of carrier/ substrate	Outcomes		
				Virucidal efficacy	Comparison to NaOH/Na2CO3	Comment
Yilmaz and Kaleta, 2003a	1. Formic Acid. 2. Disinfectant containing aldehydes and alcohols. 3. Disinfectant containing aldehydes. 4. Disinfectant containing peroxiacetic acid	Three non-enveloped viruses: • bovine enterovirus type 1 (ECBO virus). • mammalian orthoreovirus type 1. • bovine adenovirus type 1 (BAV1)	Suspension tests and in carrier tests (poplar wood virus carriers)	1. Formic acid: • effective against ECBO at concentration 1% and 20C or 2% 10C (60 min reaction time). • Ineffective against reovirus and bovine adenovirus. 2. Effective against the three viruses at room T (without protein load for reovirus inactivation). 3. Effective at room T but reduced effect in the presence of organic matter. 4. Effective against all test viruses at a concentration of 0.5% within 15 min independent of T and protein load	/	Not the selected viruses . No comparison with NaOH
Zoni et al., 2007	Chlorine dioxide	Feline calicivirus (F9 strain); Coxsakie B5 virus; Hepatitis A virus (strain HM-175)	Experimental conditions: CRFK (feline kidney) cultures; RC-37 (monkey kidney); FRhK4 (monkey kidney embryonic) cultures	Feline calicivirus and Hepatitis present strong resistance since inactivated at disinfectant concentrations greater than 0.6 mg/l. Coxsackie B5 shows great sensitivity at all concentrations assayed	/	Not the selected viruses. No comparison with NaOH

ABBREVIATIONS

AFSSA Agence Française de Sécurité Sanitaire des Alimentes

AHAW Animal Health and Welfare

EFSA European Food Safety Authority

OIE Office International des Epizooties