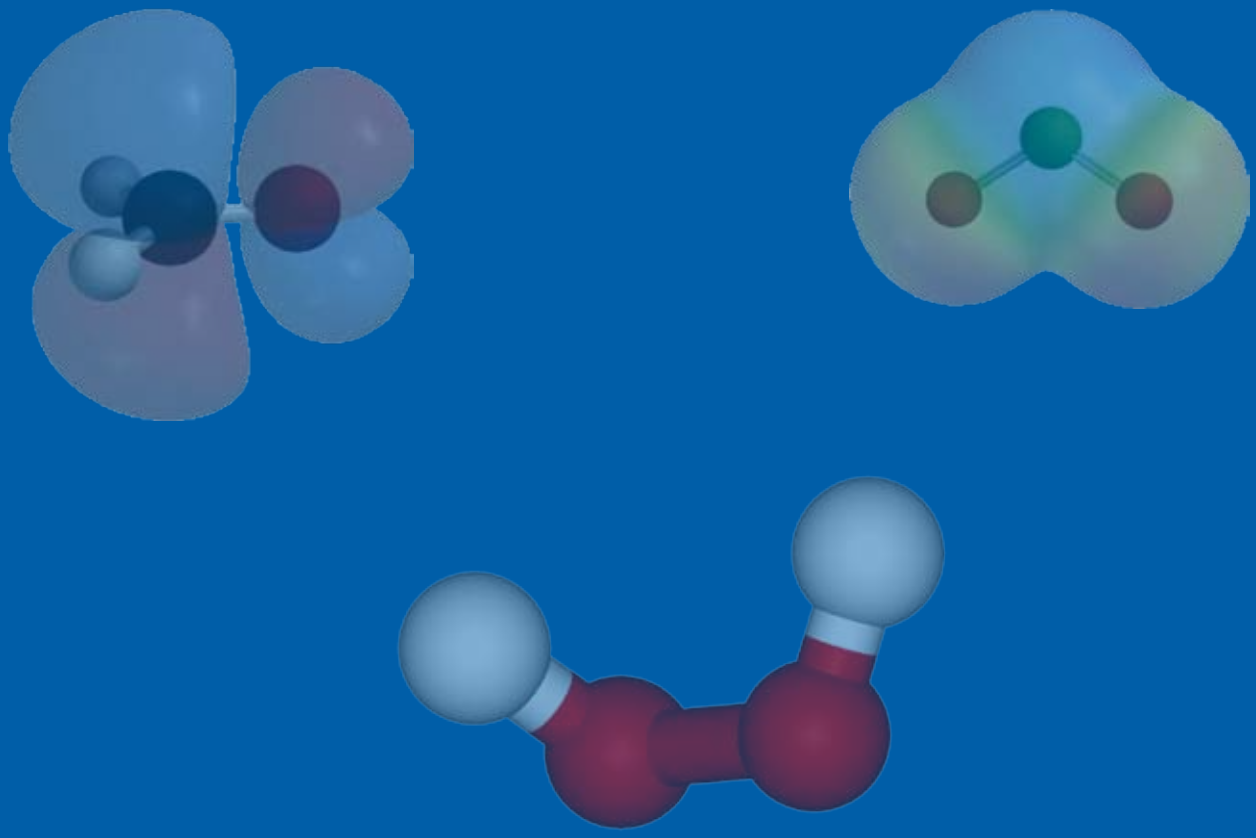
EPIZONE

Workshop
Formaldehyde replacement

Lelystad, 11-12 January 2011



Abstracts, Conclusions and Recommendations

CENTRAL VETERINARY INSTITUTE
WAGENINGEN UR

Proceedings workshop formaldehyde replacement

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Workshop
Formaldehyde replacement

Executive summary

1. Introduction

EPIZONE is the European Union (EU) funded Network of Excellence for Epizootic Disease Diagnosis and Control. EPIZONE aims to improve research on preparedness, prevention, detection, and control of epizootic diseases such as foot-and-mouth disease, classical swine fever, avian influenza, and other relevant epizootic diseases, within Europe. In order to study these diseases, several EPIZONE partner institutes have laboratories to perform in vitro studies and animal facilities to perform infection studies in animals. In order to prevent the escape of infectious agents to the environment and cross-contamination, all materials and equipment that have been in contact with these infectious agents must be either disposed of or must be decontaminated. Furthermore, all rooms which are – or may potentially be – contaminated by infectious agents must also be disinfected.

Gaseous formaldehyde[1] is widely used as a disinfectant for decontamination of animal facilities and laboratories, a process known as fumigation. Formaldehyde is a broad spectrum disinfectant against biological agents and its mechanism of action is thought to involve the production of protein-protein and protein-nucleic acid cross links. For this reason formaldehyde is generally recommended in literature and legislation. However, the use of formaldehyde for fumigation is not without drawbacks. Formaldehyde vapour is classified as extremely flammable and the vapour mixes well with air resulting in an explosive mixture between 7-70%. Due to limited penetration and slow biocidal action time, at least 12 hours exposure is recommended for formaldehyde decontamination. Furthermore, it typically takes over 24 hours to completely ventilate formaldehyde vapour. Therefore, any area that is decontaminated is out of action for at least 36 hours. Formaldehyde is known to react violently with strong oxidants (like hydrogen peroxide), and toxic vapours and gasses may be released. Under certain conditions formaldehyde can react with hydrochloric acid and chlorine-containing disinfectants such as hypochlorites to form bis(chloromethyl) ether, a potent carcinogen. In 2009 the maximal accepted concentration for formaldehyde was decreased from 1.5 mg/m^3 to 0.15 mg/m^3 by several countries. Thus, to diminish the environmental and health burden, the use of formaldehyde has to be minimized or avoided.

The use of formaldehyde as a disinfectant is not forbidden, but formaldehyde is not a registered compound in annex 1 of the Biocidal Products Directive 98/8. Therefore, it cannot be sold as disinfectant, but has to be purchased as a general chemical. Its use as a biocide is however subject to the new EU chemicals legislation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) which came into force June 1st 2007. Authorisation for the use of listed chemicals will be granted if the risk is under adequate control. If adequate control is not possible, authorisation may be granted on socio-economic grounds if there is no safer alternative. However, companies are required to make efforts to find safer substitutes that deliver lower overall risks and be technically and economically feasible. Although formaldehyde has been registered, it has not yet been indicated for authorisation. Therefore, the timeframe for evaluation is unknown.

The disadvantages of formaldehyde, which are primarily related to human safety and environmental impact, have been the motivation to strive for a reduction of the use of formaldehyde or the replacement by a safer alternative.

In order to replace formaldehyde by more safe substitutes, different institutes - including EPIZONE partner institutes - have already investigated alternative decontamination processes. Unfortunately, the majority of this information has not been published and is therefore not publicly available. Since it is in the interest of not only the EPIZONE partner institutes, but also of many other veterinary and human healthcare institutes,

1 Formaldehyde (CH2O) generally refers to the gaseous form of the chemical. Formalin or formol refer to the aqueous solution, whereas the solid form is known as paraformaldehyde.

representatives from several EPIZONE partner institutes (see Annex I) took the initiative to organize an international workshop on the replacement of formaldehyde by alternative disinfectants (EPIZONE Internal Call 2.5). The workshop was held at the Central Veterinary Institute of Wageningen UR in Lelystad, The Netherlands on January 11+12, 2011 (for programme see Annex II).

2. Replacement of formaldehyde

2.1. Fumigation

Formaldehyde fumigation has been used since the late 1880s (Lach, 1989[2]) and is generally considered a reliable, economic and easy to apply method to disinfect surfaces. There are two good reasons to restrict its use to particular situations. The first reason is the inherently limited effectiveness of formaldehyde fumigation under non-optimal conditions. The second reason is the toxicity and suspected carcinogenicity of formaldehyde in combination with the resulting technical problems and legal restrictions for its use.

The effectiveness of disinfection by gaseous formaldehyde depends on a number of factors, in particular the matrix in which the pathogens are enclosed and the surface they are attached to, the relative humidity, the temperature and the formaldehyde concentration (Lach, 1989, Munro et al. 1990[3]). The limited penetration capacity of formaldehyde means that microorganisms may be protected from inactivation by the matrix, e.g. "dirt" on the surfaces to be disinfected. It is generally assumed that the amount of formaldehyde and water needed to achieve a particular concentration and relative humidity can be calculated from the room volume. However, there are a number of processes and factors which may lead to lower than expected values. It is possible that the gaseous formaldehyde does not reach all parts of room with a complicated structure in sufficient concentrations. Under certain conditions, formaldehyde may be absorbed on surfaces or polymerize into relatively inactive paraformaldehyde. Furthermore, depending on the temperature and temperature distribution in the room, also uneven condensation can occur and as formaldehyde vapour is very soluble in water, this may reduce the vapour level.

Formaldehyde can irritate the eyes and mucous membranes and can be toxic, allergenic and carcinogenic. This led to restrictions on its use for fumigation. For example, in Germany, the respective technical regulation (TRGS 522[4]) requires that the fumigation is carried out by a qualified person (licence holder) who has to demonstrate relevant experience, has to attend a recognised training course and has to pass an official examination at the end of a theoretical and practical training course, an exam. This person must provide a telephone number for emergencies and be able to reach the site of fumigation within two hours in case of problems, e.g. a leakage and has to be supported by a helper who also knows the rules on formaldehyde fumigation. Furthermore, the wearing of personal protective equipment including a special gas mask and the use of special measuring equipment is prescribed and staff involved in fumigations is subject to occupational health screening. The regulation on formaldehyde fumigation, in most cases, also prescribes to restrict access to areas adjacent to the fumigated room to the staff carrying out the fumigation and the notification of each fumigation process to the authorities at least a week in advance. After the fumigation, it may be difficult to achieve the permissible level of residual formaldehyde in the air that allows declaring the room "safe" again.

Is fumigation always needed – or rather a "method of last resort"?

At the Friedrich Loeffler Institute (FLI) in Riems, Germany, formaldehyde fumigation has been restricted to special cases, e.g. to get sensitive (electronic) equipment out of the

2 Lach, V.H.; A study of conventional formalin fumigation methods. Journal of Applied Bacteriology (1990) 68:471-477.
3 Munro K, Lanser J, Flower R. A Comparative study of methods to validate formaldehyde decontamination of biological safety cabinets. Applied and Environmental Microbiology (1999) 65: 873-876.
4 Anonymous. Technische Regeln für Gefahrstoffe, Raumdesinfektion mit Formaldehyd, TRGS 522, vom 05. März 1992 (BArbBl. Nr. 6/1992 S. 35), zuletzt geändert am 01. August 2001 (BArbBl. Nr. 9/2001 S. 86)

containment or during the replacement process for HEPA filters. The disinfection concept of the FLI is based on a few basic rules:

- Use autoclaving where possible.
- Use liquid disinfectants (mostly organic acids and NaOH) if autoclaving is not suitable.

The stables used for Foot-and-Mouth disease virus (FMDV) experiments have been cleaned and disinfected with a commercial product based on formic acid and applied as a "sticky" foam ("Venno Vet 1 super") for many years without a subsequent fumigation step. The treatment is always carried out twice. No accidental infections due to insufficient disinfection have been observed although more than 50 FMDV cattle experiments have been carried out since the early 1990s.

- Use formaldehyde fumigation if neither autoclaving nor disinfection by liquid chemicals is suitable.
- If formaldehyde fumigation is used, make sure that surfaces are as clean as possible and consider formaldehyde fumigation only as an „additional process" to disinfect surfaces which cannot be reached or treated with liquid disinfectants and which, in all likelihood, are not severely contaminated.

2.2. Formaldehyde: the gold standard?

Over the years formaldehyde fumigation has been routinely used and has a broad base of acceptance as an effective decontamination procedure (Munro et al., 1999). However, assuming that formaldehyde could be considered as "the gold standard" for fumigation processes is difficult to state due to a lack of pathogen specific validation data and the lack of a standardized process. In order to put forward formaldehyde as gold standard, a scientific basis is required through a well-documented standardization and validation procedure of the formaldehyde fumigation process.

As is true for all other fumigation processes, the formaldehyde process is highly dependent on a complex interaction between parameters such as (1) concentration, (2) humidity, (3) temperature, and (4) the substrate to be decontaminated (Munro et al., 1999; see also 2.1 above). Optimization, standardization and strict control of these parameters should be considered when developing a robust protocol to be applied in validation studies. Taking into consideration this lack of data, a simultaneous approach applying formaldehyde fumigation in parallel with alternative methods should be used to validate alternative fumigants such as Vaporised Hydrogen Peroxide (VHP). During this validation, performed in parallel with a standardized formaldehyde fumigation process, formaldehyde fumigation could serve as a "gold standard".

Validation studies proving the efficacy of the standardized protocol should be well documented using biological indicators (real agents or surrogates) in representative conditions for high containment areas/rooms and stables (clean vs dirty conditions, spill situations, organic soiled status, etc.). When applying surrogates for validation, correlation with real agents has to be proven and should be reproducible (see also 2.6). If complete inactivation cannot be demonstrated (inefficient inactivation or non-interpretable results due to, for example, cytotoxic effects), the question should be raised what the required minimal acceptable reduction of titre is.

Another factor to be considered is the possible degradation of disinfectant compounds. Formaldehyde is a stable compound that will remain active as residue on substrates in clean and dirty conditions. VHP however, is quickly decomposed into its neutral compounds (H_2O and O_2) and has a fast degradation rate when in contact with organic materials (e.g. faecal material, cellulose) hence demonstrating less or no inactivating activity in dirty conditions. Thus validated inactivation cycles may be invalidated by traces of blood and body fluids.

When comparing different methods, an important factor to be considered in a formaldehyde fumigation process is the long ventilation cycle needed to reduce formaldehyde

concentrations beneath maximum allowable exposure levels (residue formation and long out-gassing phases of decontaminated materials). This long ventilation and absorption time, allows the prolonged exposure of the infectious agents to the inactivating product which could have an additional impact on its biological efficacy. Alternative processes such as VHP propose shorter ventilation cycles (less residues, rapid decomposition and less off-gassing). In this context, if the results with the proposed shorter ventilation process is not satisfactory it is recommended to evaluate in parallel the efficacy for alternative fumigation methods applied for longer cycle and ventilation times.

In conclusion, formaldehyde fumigation can be used as gold standard when applied in parallel and under similar conditions as the new alternative methods. The evaluations need to be performed under standardized conditions and considering the specificities and limitations of each product. If the formaldehyde and the alternative method used in parallel do not inactivate the agent completely but give a similar reduction, the conclusions will be more trusted than when compared to the actually available data for formaldehyde fumigation.

2.3. Alternatives

Due to its potential carcinogenic effects on human health and environmental concerns, formaldehyde had to be replaced in France for fumigation purposes. Also in other countries, restrictions on its use have to be expected. A number of alternatives are being studied, but it is complicated to find alternative candidates that have a similar activity profile on microorganisms as formaldehyde. In the search for alternatives to formaldehyde, we can turn our attention to five active substances with broad spectrum of activity: glutaraldehyde, chlorine, peracetic acid, hydrogen peroxide and chlorine dioxide (see Table below). For each active substance, it is necessary to analyse more accurately the advantages and disadvantages, taking into account mainly the different forms (liquid, gas or vapour), the optimal conditions in terms of temperatures, relative humidity and pH, the relative importance of reactivity with soiling, the corrosiveness and human safety.

Table 1. Comparison of properties of gaseous decontamination agents: (+) property adequate for disinfection purposes; (+/-) mostly adequate but some limitations; (-) limitation or problematic property; nd: not determined.

Disinfectant	Biocidal spectrum	Activity in the presence of organic matter	Speed of action	Chemical hazard	Chemical compatibility	Environmental concern
Formaldehyde	+	+/-	+/-	+	+	+/-
Glutaraldehyde	+	+/-	+	+	+	+/-
Peracetic acid	+/-	+/-	+	nd	+/-	nd
Hydrogen peroxide	+/-	-	nd	+	+/-	-
Chlorine	+	+	+	+	+/-	+
Chlorine dioxide	+	+	+	+	+/-	+

The biocidal spectrum, reactivity with organic matter, speed of action, chemical biohazard, corrosivity and environmental concerns must be taken into account when choosing the best candidate. One of the most promising substitutes is hydrogen peroxide, which can be used as a liquid or in a vapour form (known as Vaporised Hydrogen Peroxide, or VHP). The

vapour form has a much greater activity than the liquid form. VHP has higher MAC values than formaldehyde[5] and tends to have fewer problems with residues due to its instability.

2.4. Vaporised Hydrogen Peroxide (H_2O_2) as a promising alternative

Hydrogen peroxide reacts to form reactive oxygen species, which are highly reactive with organic matter, including DNA, proteins and lipids. Hydrogen peroxide is not classified as a carcinogenic agent and decomposes to oxygen and water vapour so leaves no problematic residues. However, according to one report oral administration of hydrogen peroxide in mice has caused adenomas and carcinomas of the duodenum[6] and H_2O_2 must therefore also be treated with great caution. Vaporised hydrogen peroxide has slightly higher permissible work place concentrations, but it is highly toxic in the concentrations used for decontamination. However, it is odourless which makes it in some way more dangerous than formaldehyde, which is smelled at low concentrations of less than 1 ppm. This means that monitoring of H_2O_2 must be taken very seriously as people have no sense to detect H_2O_2. Nevertheless, decontamination with VHP is much safer and more environmental friendly than formaldehyde fumigation, especially when the decontaminant vapour is vented into the atmosphere via a cat convertor or in very diluted form. The lower levels of toxicity exhibited by VHP, together with the lower concentrations within the target area and the 'lazy' (non-diffusive) nature of the vapour, alter the risk profile significantly. This was demonstrated practically during the SARS crisis in Singapore, where hospital room decontaminations were performed in rooms adjacent to occupied rooms (although the efficacy on SARS virus under these field conditions has not been demonstrated).

A distinct difference of VHP to formaldehyde is the boiling temperature. While formaldehyde is a true gas at ambient temperature, H_2O_2 only boils at 110°C and therefore does not behave like a gas at ambient temperatures. This means the fine vapour does not penetrate materials and does not effectively diffuse into spaces. Within rooms this can be overcome with fans and ventilation systems.

VHP has been used successfully at a range of room temperatures and humidity levels. The decontamination effectiveness of gaseous fumigants is heavily dependent on the temperature, humidity and dimension of the target area and the prescribed physical and chemical parameters have to be maintained strictly to ensure that the inactivation process works correctly. In the case of VHP, some combinations of physical and chemical parameters and process time have been used successfully which would not have worked with other fumigants, and in this respect may also offer a greater flexibility for the design of protocols.

Another issue arising from the use of formaldehyde and chlorine dioxide comes from the time it takes to complete these processes. Routine fumigation with formaldehyde takes at least 12 hours to work properly. Depending on the fumigation system and efficiency of the ventilation system venting can take another 24 hours to eliminate the toxic vapour, and more time still to clean off the residues before the room or building is safe to use again.

Whilst the fumigation process is faster with chlorine dioxide, the time it takes to prepare the room or building makes it a lengthy process. Before fumigation can begin, room temperatures and humidity must be raised to 21°C and 70% respectively. Due to its temperamental nature, if these conditions cannot be achieved or maintained during fumigation, the process may have to be stopped and restarted. With chlorine dioxide, it is also essential that adjacent rooms are evacuated before the process begins, since the occupational exposure limit for chlorine dioxide is ten times less than for H_2O_2.

5 MAC value of formaldehyde : 0,15 mg/m3 (over 8 hours)
MAC value of VHP (90 %): 1,4 mg/m3 (over 8 hours)
6 IARC [1985]. IARC monographs on the evaluation of carcinogenic risk of chemicals to man. Volume 36. Lyon, France: World Health Organization, International Agency for Research on Cancer.

As VHP decomposes over a short period it allows shorter aeration time. Typically, rooms can be completely decontaminated in less than 90 minutes, and whole buildings can be decontaminated within a day. After completion, it takes typically only 2 hours before the rooms are clean, safe and ready to be used again.

Overall, the benefits of hydrogen peroxide vapour in decontamination make it the best choice for almost all applications, from small medical or laboratory equipment, to cleanrooms, wards, laboratories and buildings. It outperforms both chlorine dioxide and formaldehyde with respect to the speed of application, health and safety. However, it requires significant financial investment in purchasing equipment, cycle development and continuing costs of servicing and this may not be justifiable for many facilities. There are also anecdotal reports of damage to laboratory materials caused by hydrogen peroxide systems.

2.5. Current experiences with VHP

Several independent tests show that the method seems to be working well with the standard parameters tested (indicator spore) and in clean areas. Like most fumigants, VHP seems to work especially well in areas that can be cleaned to a high level, such as biosafety cabinets. When materials are soiled by faeces or other organic substances general cycles adapted for clean areas are not suitable or valid. Research and validation is needed to prove the VHP efficacy, where body fluids and excretions are encountered. Tests also show that areas that have been carefully cleaned are easier decontaminated using VHP than soiled areas. VHP fumigation on a concrete surface was not successful (no killing at all). However, in these studies the performance of formaldehyde fumigation on this surface was not examined.

The VHP method needs to be further evaluated and the effectiveness of VHP to inactivate certain agents must be validated in each case using conditions similar to the different decontamination scenarios. Independent multicentre research is also required to ensure efficacy in animal laboratories working with high hazard pathogens. It is also important when performing tests that the choice of indicator should represent sector requirements (also see 2.6 below).

The choice of the appropriate process - wet (less dependent of room humidity) or dry (dehumidification of room humidity) - has to be considered in function of the set-up and dimensions of the decontamination rooms and enclosures. The distribution of the gas is very important for the result (as with other gaseous methods). Practical aspects such as odours, residues on surface, impact on different material etc., must be considered. It seems that VHP decontamination is more suitable from a work environment perspective compared to formaldehyde, but proper measurement equipment for VHP needs to be purchased to measure eventual residual VHP levels after decontamination. When the initial parameters have been set, VHP decontamination is also rather straightforward and easy to perform as a method. It is also a faster method than formaldehyde fumigation.

As most VHP generators are heavy, they are inconvenient to move between different facilities that need to be decontaminated. Gassing ports to connect the equipment on the outside of rooms/facilities would be recommendable. Different solutions integrating the VHP decontamination process with the facility and its systems should be further investigated. Evaluation studies comparing ‘dry’ and ‘wet’ generator systems have been carried out. Some tests conclude that the ‘wet’ generator is more flexible to use since it doesn’t require the same conditions as the ‘dry’ system (temperature >18°C and humidity <50%; conditions that can be difficult to meet during certain seasons).

2.6. Biological indicators (surrogate or the real agent?)

The real agent is defined as the agent(s) manipulated in the containment facilities and for which an effective decontamination has to be demonstrated. Surrogates are biological agents, often commercially available and harmless, that serve as representative organisms for the real agent and can be inactivated in decontamination processes. The use of a surrogate as biological indicators has the advantage that it is easy to apply for validation of decontamination processes such as steam or fumigation. The advantage of real agents is that they mimic better the actual conditions and risks to be contained. However they are usually more hazardous than the commercial surrogates and less standardised, resulting in a higher variability of the results. However, even with commercial biological indicators inter and even intra batch variability is an issue of concern.

Steam sterilisation is always performed in standardized equipment. Here surrogate indicators are described and standardized for use as a universal biological indicator. The most common Biological Indicator (BI) contains 10^6 spores of *Geobacillus stearothermophilus*, and has proven in many validation studies to be the most resistant organism. They are continuously applied for validation of the daily process and are commercially available.
Fumigation processes (e.g. Formaldehyde, VHP, ClO_2) are mainly applied for decontaminating rooms, equipment in rooms or laboratories, and animal facilities or even farm buildings. Therefore, they are more influenced by environmental factors (e.g. humidity, temperature, room dimensions) and used in a less standardized manner (see also 2.2). In addition it is much easier to ensure the penetration of heat to be uniform over the load of an incubator than the homogeneous penetration and distribution of a chemical.

For formaldehyde fumigation and alternatives, such as VHP, ClO_2 and O_3 the commercial biological indicators *G. stearothermophilis* and *G. atropheus* are applied in validation studies. However, as stated above, these processes are less standardized and hence efficacy varies with conditions, as was shown by results presented during the "formaldehyde replacement workshop" showing varying data for the different processes (formaldehyde, ClO_2, VHP, ozone, etc.), different surrogates (*G. stearothermophilis, B. atropheus, C. difficiles*) under various conditions (clean, dirty, spill, etc.). In several VHP fumigation processes spores of *G. stearothermophilis* and *B. atropheus* were successfully inactivated with a 6 $\log_{10}$ reduction and presented as an interesting surrogate for these processes. However, when real agents and surrogates were tested in parallel, real agents were not always completely inactivated (e.g. FMDV) in contrast to the surrogate, raising the discussion on the use of the surrogate in order to validate a fumigation process.

Taking into consideration the above mentioned limitations, it is necessary to further standardise the biological indicators (based on surrogates or real agents) in parallel with the fumigation process (see also 2.2) in view of providing a universal biological indicator or at least test appropriate biological indicators. The same standardized protocol under repetitive conditions has to be demonstrated in order to "validate" the fumigation process for real agent and/or surrogates.

A good approach when validating the fumigation process is always to perform a case-to-case evaluation, by evaluating the real agents and surrogates in parallel. When similar results for decontamination are obtained, the validated surrogate could be used, facilitating the validation of the standardized fumigation process.

In conclusion, more research is needed for the standardization of the indicator as well as the process in which they can be used. The results of these studies can provide useful data to decide when a surrogate or when a real agent can be used. Due to the variable conditions in which fumigation is used a universal indicator as used for steam sterilisation is less evident to prove and provide.

3. Conclusions

Formaldehyde is historically considered as the gold standard for gaseous decontamination of laboratories, animal facilities and animal farms. It has been used for decades without reported consequential failures in terms of pathogen inactivation. However, formaldehyde represents health hazards for humans. Therefore, European and some National regulations tend to limit its usage. The use of formaldehyde is not forbidden but its usage is more and more restricted owing to these safety requirements. The replacement of formaldehyde is therefore highly encouraged.

For the time being, controlled procedures preventing exposure of staff and the environment to formaldehyde must be followed. Alternatives exist, like VHP which is one of the most studied at this time. However, there is a lack of harmonisation between (1) the protocols used in different laboratories to compare the efficacy of formaldehyde with alternatives, (2) the parameters that have to be monitored during validation studies in one or more locations (fumigant level, temperature, humidity), and (3) the biological indicators which are insufficiently standardised and for which more care needs to be exercised to account for batch to batch variability. Further variables in the test conditions need to be recorded in a standardised format, such as types of surfaces, degree of soiling (dirty/clean surfaces), duration, volume of space, etc.

When a new molecule is tested in view of formaldehyde replacement, the comparison with formaldehyde should be done in parallel since formaldehyde is not 100% efficacious in every situation. Requesting a full efficacy of a new molecule or compound in the absence of a control to measure the formaldehyde performances under the same experimental conditions can lead to unfair conclusions.

Formaldehyde has the advantage to be active in its gaseous form and can reach areas that are inaccessible for liquid or vapour disinfectants. This has to be considered for instance when HEPA filters have to be decontaminated. In addition, the safety issue of the alternative should be also considered since complete safety of decontaminants cannot be reasonably expected. A risk/benefit assessment of such alternatives should be carried out before the final decision of replacement is made.

4. Recommendations

In view of the disadvantages - primarily relating to human safety and environmental impact - connected to the use of formaldehyde, it will have to be used in a much more controlled fashion in the future. There is a small possibility that it will have to be replaced with another disinfectant in the near future. Based on the results presented in this workshop we think that, despite concerns on its efficacy, reliability and material compatibility, VHP is so far the most promising alternative. VHP is much safer and environmentally friendly while having similar biocidal activities compared to formaldehyde for most of the infectious agents tested. Therefore, we recommend switching from formaldehyde to VHP for room fumigation as soon as it has been properly validated for the specific agents and conditions, especially if formaldehyde fumigation cannot be controlled to prevent exposure. However, it must be kept in mind that the equipment for the use of formaldehyde is much cheaper than for the use of VHP which requires special and relatively expensive equipment and support but which bring the advantages of a better controlled and easier to validate process. Thus, the decision to switch from formaldehyde to VHP may not always be that simple and may in some instances be dictated by the costs.

During this workshop the importance of cleaning before proceeding to fumigation has been stressed over and over again. No single disinfectant is effective against microorganisms that are embedded in organic material such as blood or faeces. This is even more true for gaseous disinfectants like formaldehyde or VHP. Therefore, thorough cleaning followed

by liquid disinfection should always precede fumigation. In this respect it may be argued whether fumigation is always needed. The experience of FLI using two cycles of cleaning and liquid (foam) disinfection shows that this may not always be the case. Indeed, this procedure in combination with room ventilation may already reduce the amount of the infectious agent to inconsequential levels, obviating the need for fumigation. We seriously recommend considering this option. However, in this case the choice of the correct disinfectant is critical and should be validated for the specific infectious agent(s) used in the experiment.

In this respect it should also be realized that a reduction of 6 $\log_{10}$ is not always required. If the room to be fumigated has been thoroughly aerated, cleaned and treated with a liquid disinfectant, the residual amount of infectious material is already very low. In that case, for most infectious agents a reduction of 3 to 4 $\log_{10}$ will be sufficient. The results of this workshop have shown that these orders of magnitude of reduction can be achieved by VHP for almost all agents tested.

In our opinion, for experiments involving category 2 microorganisms, cleaning and primary disinfection using a liquid disinfectant followed by VHP disinfection is more than sufficient when using a validated VHP fumigation process that is based on commercially available chemical and biological indicators (e.g. *G. stearothermophilis*). Although not strictly necessary for a validated process, we would still recommend including these indicators in each disinfection run.

In order to be able to compare the results of fumigation experiments between different institutes, it would be worthwhile to develop a standard protocol in which a number of critical parameters are specified such as the composition of the disinfectant, the conditions of use of the disinfectant (dirty/clean surfaces, duration, etc.), the selection of chemical and/or biological indicator(s), the targeted limit for efficacy, etc. This will ensure that results obtained by one institute will also be valid for other institutes and will thus save effort and costs. However, care has to be taken since differences in building materials may also be crucial. This was already mentioned for concrete walls, but may be the case for other materials as well.

When validating the VHP process for a certain situation or a certain infectious agent, it is recommended to include formaldehyde disinfection as a benchmark. In cases where the performance of VHP is lower than expected, this may also be the case for formaldehyde. In that case it is not the disinfectant that is directly responsible, and thus the VHP process should not be immediately dismissed.

Ideally, validation of the disinfection process for an infectious agent should be performed by using the same infectious agent as the biological indicator. We realise that this is not always possible. Therefore, the use of surrogate harmless biological indicators of which the properties closely resemble those of the target species should be encouraged.

Finally, we recommend that funds will be made available by international authorities to develop standard protocols for fumigation using a number of infectious agents that represent most classes of infectious agents that are of veterinary and zoonotic importance. The results of such projects should be made publicly available to the scientific community.

ANNEX I: ORGANIZING COMMITTEE

Name:	Institute	Nationality
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CIRAD: Centre de cooperation international en recherche agronomique pour le développement (Agricultural research for development), Montpellier, France.

SVA: Statens Veterinärmedicinska Anstalt (National Veterinary Institute), Uppsala, Sweden.

IAH: Institute for Animal Health, Pirbright, United Kingdom.

FLI: Friedrich Loeffler Institute , Riems, Germany.

VAR: Veterinary and Agrochemical Research Centre, Uccle, Belgium.

CVI: Central Veterinary Institute, part of Wageningen University and Research Centre, Lelystad, The Netherlands.

ANSES: Agence nationale de sécurité sanitaire (The French agency for food, environmental and occupational health safety), Maisons-Alfort, France.

VET-DTU: National Veterinary Institute – Technical University of Denmark, Lindholm, Denmark.

ANNEX II: PROGRAMME OF WORKSHOP

Programme EPIZONE Workshop Formaldehyde Replacement

Date: January 11 + 12, 2011, Lelystad, The Netherlands.

Day 1

13:00-13:50	Arrival and registration, coffee and sandwiches
13:50-14:00	Welcome
14:00-14:20	*Legislation and the use of formaldehyde;* **Steve Copping,** Institute for Animal Health (IAH), Pirbright, UK
14:20-14:40	*The chemistry of disinfection : compounds of major interest as compared to formaldehyde;* **Pierre Maris,** The French agency for food, environmental and occupational health safety (ANSES), FR
14:40-15:10	Coffee
15:10-16:50	*Experiences and studies with alternatives for formaldehyde;* **Epizone partners** **Henk Sloetjes**; Central Veterinary Institute of Wageningen UR (CVI), Lelystad, NL **Koen Quanten**; Veterinary and Agrochemical Research Centre, Uccle, BE **Yongjie Harvey**; Institute for Animal Health (IAH), Pirbright, UK **Hans Antehed**; National Veterinary Institute (SVA), Uppsala, SE **Nicolas Etteradossi**; The French agency for food, environmental and occupational health safety (ANSES), Ploufragan, FR
16:50-17:10	Coffee, snacks, refreshment
17:10-17:40	*A patient with Marburg Hemorrhagic Fever: patient management and disinfection;* **Gijsbert van Willigen,** Leiden University Medical Centre, Leiden, NL
17:40-18:30	Plenary discussion
20:00	Diner

Day 2

08:30-09:00	*Design, build and qualification of VHP bio-decontamination systems for research laboratories;* **Jon Nottingham,** CAPE, Controlled Aseptic Process Environments Europe Ltd., UK
09:00-09:20	*New building design and disinfection*; **Uwe Müller-Doblies,** Institute for Animal Health (IAH), Pirbright, UK
09:20-09:40	*New building design and disinfection;* **Bernd Haas,** Friedrich Loeffler Institute (FLI), Riems, DE
09:40-10:10	*Green gas, dry mists and dense vapors: HSL's experiences in the world of fumigation technology;* **Alan Beswick,** Health and Safety Laboratory, Buxton, UK
10:10-10:40	Coffee
10:40-11:10	*Non formaldehyde fumigation technologies at the National Microbiology Laboratory;* **Jay Krishnan,** National Microbiology Laboratory, Winnipeg, CA
11.10-11:40	*Assessment of the limitations of gaseous disinfectants;* **Allan Bennett,** Health Protection Agency, Porton Down, UK
11:40-12:40	Plenary discussion
12:40-13:00	Summary and conclusions

ANNEX III: RESULTS OF QUESTIONNAIRE

Summary of questionnaire regarding use of formaldehyde and alternative disinfection methods.

This questionnaire was distributed by email to participants in the Epizone formaldehyde replacement workshop, 11th-12th of January 2011 in Lelystad.
The purpose of the questionnaire was to assess the status of use of formaldehyde and alternative techniques in Europe, including patterns of change, efforts to validate any use of gaseous disinfection, and gaps in the on-going validation.
Twenty three government facilities and one private laboratory answered the questionnaire. Four of these facilities were not using formaldehyde, while twenty used formaldehyde fumigation as follows:

Large animal stables	8
Small animal stables	6
Laboratories	10
Fumigation chambers	13
HEPA-filter boxes	10
Other	6: BSC, isolators, production facilities

Twelve of the facilities neutralized with ammonia, and validation was also carried out by twelve facilities – seven used spores, two chemical indicators and two specific agents.
The laboratories had the following opinion about formaldehyde:

Satisfied	5	simple, cheap, safe if using proper PPE, robust
More or less satisfied	1	complaints over residues and smell
Satisfied but concerned about safety	8	additional concerns: off-gas from absorbent materials, difficulties finding suitable spores and difficult legislation, laboratory materials damaged
Not satisfied	3	carcinogenic, threshold lowered, residues, health and environment issues, ruins quality of clothes

Eight facilities were using other gaseous disinfectants as follows:

Large animal stables	2
Small animal stables	4
Laboratories	6
Fumigation chambers	4
HEPA-filter boxes	5
Other	3: BSC, isolators

Seven facilities working with a wide range of bacteria and viruses, including exotic and notifiable agents had concrete plans to change to hydrogen peroxide for various combinations of the given options (large animal stables, small animal stables, laboratories, fumigation chambers, HEPA-filter boxes and others). Three facilities were planning to switch during 2010-2013, while two indicated that more research and validation was required and three were working with validation.

Six facilities – also working with a wide range of bacteria and viruses – had more tentative plans to change – also for various combinations of the options given. Three of these were considering hydrogen peroxide and one chlorine dioxide. Three were working with validation, and concerns included price, weight, technical problems, validation and effect for e.g. PCV.

Non-gaseous disinfectants were indicated by 13 facilities working with a wide range of bacteria and viruses including notifiable agents. Non-gaseous disinfectants were also used for various combinations of the options given, and disinfectants included VirkonS fog, VirkonS, Venno Vet 1 super, F10, hydrogen peroxide + peracetic acid, hydrogen peroxide, alkalis and hypochlorites. Three laboratories were working with validation.

Conclusions:

- Formaldehyde is still used - users are generally satisfied, but concerned about safety
- 63% of responders were using or were moving to alternative methods – primarily hydrogen peroxide
- 25% of responders were considering moving to alternative methods
- Responders expressed their concerns regarding validation

ANNEX IV: ABSTRACTS OF PRESENTATIONS

Formaldehyde replacement inside the animal facilities at the Central Veterinary Institute of Wageningen UR.

Henk Sloetjes *(Head Department Animal Technology),* Gerdina Draaijer *(QA-Officer) Central Veterinary Institute of Wageningen UR, The Netherlands.*

Current disinfection method

After every animal experiment and before gassing, bedding, faeces and other organic material will be removed out of the facility. Then the room will be cleaned with water (high pressure), treated with a liquid disinfectant and when needed with an acid solution. On completion, disinfection will be carried out by trained personnel with an electric fumigation system. This system fumigates paraformaldehyde and after dwell, the system automatic turns over to neutralise the room with ammoniac. In cold winters and hot summers, it is difficult to create an optimal climate for paraformaldehyde (> 20°C and >70%RH) what is proven to be most effective. This is one of the reasons to replace formaldehyde disinfection, BUT more important is the human health and environmental discussion, the carcinogenicity the reduced MAC-value, the acceptance and the availability of the chemical.
CVI started with investigation/orientation on implementing VHP technology for its animal-room disinfection activities in 2007, in order to establish a valid alternative for formaldehyde.

Objectives and Requirements

In the beginning of 2009 CVI tested two systems, namely equipment of PMV BV, Woerden (Steris VHP 1000EDS) and Tecnilab-BMI (BIOQUELL-Z). The equipment demands were: being mobile, easy to handle, less preparation to start the process, no damage to facility and no extra preconditioning of the room. The objectives of the bio-decontamination validation studies with vaporised hydrogen peroxide were: 6 $\log_{10}$ reduction of biological indicators (BI) (*G. staerothermophilus*) after bio de-contamination, decontamination of all compartments (changing room, animal room, pass lock in/out) in the animal room, create at least a 4 $\log_{10}$ reduction of BI in exhaust ductwork, after exhaust HEPA-filters, determine effectiveness of the bypass-circuit (behind exhaust filters) and determine effectiveness of a recirculation system .

Study using STERIS system

Validation study with the Steris VHP-1000EDS system was carried out in an animal facility which is representative for other facilities of CVI and consists of 5 different rooms (animal holding room, personal and material locks). PMV claimed two requirements to the climatic conditions before the process could be started: temperature >18°C and humidity < 50%. Heaters and fans had to be placed to create those conditions. Twenty-four biological and chemical indicators to validate the process were used and the generator with a separate distributor was placed in the sluice of the facility. During the process (gassing, dwell, aeration) the room was contained and no assistance from HVAC system was available, only the by-pass valve has been continuously opened. By reaching 1ppm VHP the indicators were collected for analysis. All chemical indicators did reach their turning (end) point; only one biological indicator showed a positive result. Further no material damage was witnessed or recorded on any equipment or room surface exposed to hydrogen peroxide. CVI decided not to do further validation study's, although the system did get full inactivation (>6 $\log_{10}$ reduction).

The reasons for this decision were: in summer en winter it is more difficult to create conditions mentioned by PMV, use of extra heaters, positioning distributor is too labour intensive and not possible to start and end the cycle from outside the room.

Study using BIOQUELL-Z generator

Several study's, similar to the Steris system were carried out with equipment of BIOQUELL. In these studies CVI also wanted to reach a full inactivation (6 $\log_{10}$) in exhaust ductwork and after the (HEPA) filters. Study 1A: During the disinfection process (gassing, dwell, aeration) the room was contained (by-pass valve opened in dwell phase) and upon reaching aeration, the HVAC exhaust and supply valves were opened. The duration of the process was less than 4 hours. The results showed: full inactivation (>6 $\log_{10}$ reduction) within animal room, full inactivation (>6 $\log_{10}$ reduction) in material air locks, within personal air lock positive BI's due to failed distribution, exhaust duct up till absolute (HEPA) filters an inactivation with 6 $\log_{10}$ reduction and < 4 $\log_{10}$ reduction after absolute (HEPA) filters. Study 1B: During the disinfection process (gassing, dwell, aeration) the room was contained and no assistance from HVAC system, only the by-pass valve has been continuously opened. This process was carried out overnight (>16 hours), comparable with the current paraformaldehyde disinfection method. Study 1b gave as results: a full inactivation (>6 $\log_{10}$) within animal holding room, a full inactivation (>6 $\log_{10}$) within material air lock, a full inactivation (>6 $\log_{10}$) with personal air locks, an exhaust duct up till absolute (HEPA) filters inactivated with >6 $\log_{10}$ reduction and >4 $\log_{10}$ reduction after double absolute (HEPA) filters.

Based on the conducted study's and achieved results, preliminary decision was made to continue with Bioquell methodology. Further analysis was requested to continue work in progress on recirculation in holding rooms and to achieve >6 $\log_{10}$ reduction after two absolute (HEPA) filters. A fan was installed between HVAC exhaust (HEPA H13) and supply duct (pre filter F9), which switched on 30 min in dwell phase of the disinfection process. With recirculation a full inactivation (>6 $\log_{10}$) on animal holding room, personal air locks, material air lock, supply and exhaust duct, and 2 absolute & 1 pre-filters was achieved.

Conclusion

BSL2 animal-rooms at CVI can be effectively de-contaminated by VHP, using the BIOQUELL-Z generator The BIOQUELL-Z generator system in cooperation with the CVI animal rooms and the applied SOP's, are validated, also in terms of reproducibility and traceability.

Discussion/Questions

Effectiveness of VHP to inactivate FMDV must be validated in a separate route, such as EPIZONE. Effectiveness of VHP to inactivate other Cat. 3 agents (RVFV, WNV, Q-fever, etc.) should be proven by empirical research and (peer reviewed) literature

Vaporised hydrogen peroxide: a promising alternative for formaldehyde fumigation?

Koen Quanten and Frank Koenen, Veterinary and Agrochemical Research centre, Brussels, Belgium.

Decontamination of materials leaving high containment facilities is of crucial importance in avoiding accidental spread of pathogens into the environment. Although formaldehyde fumigation is a current and widely used method for decontaminating heat and water sensitive materials, it has several drawbacks in relation to health, safety and environment. A safer and more flexible alternative could be provided by vaporised hydrogen peroxide (VHP) but needs more validation data. In this context, an efficacy study was performed in a BSL3-setting at VAR institute to evaluate 2 VHP methods based on a so-called "wet process" (independent of room humidity (RH)) and a so-called "dry process" (dehumidification of RH). In several trials different types of biological indicators (BI), prepared for (highly) contagious animal/zoonotic diseases manipulated in the BSL3 facilities of VAR, were subjected to both VHP processes. Furthermore, two types of conditions were simulated by including BI's under clean conditions, representative for the most occurring contamination situation of materials leaving a lab, and dirty conditions. The latter were prepared by spiking blood or faecal material with the respective pathogens (CSFv). These conditions allow an evaluation of the impact on the inactivating capacity of VHP when surfaces are "soiled". In order to validate the processes also commercial BI's *G. Stearothermophilus* were tested in all trials.

A complete inactivation of the commercial BI (*G. Stearothermophilus*), all tested bacteria (*E. coli, E. faecalis, M. gilvum and G. stearothermophilus*) and the viruses Avian Influenza and Newcastle Disease was achieved. For a second group of viruses CSFv, BTv, CaPv complete inactivation could not be demonstrated, due to cytotoxic effects. Nevertheless, a clear log reduction of virus titer was observed for both processes. For FMDV a similar log reduction was shown in the dry VHP process. In contrast, for the wet process additional research is needed as live virus could be isolated after the VHP cycle. The fact that the commercial BI was inactivated but FMDV was not, clearly demonstrates the importance of an in situ validation. In contrast to the clean conditions, virus was isolated from the CSFv BI's under dirty conditions. This difference between both results shows the necessity of cleaning the materials before using VHP decontamination.

Conclusions

The present study demonstrates the potential of VHP methods for the decontamination of "clean" enclosures and materials leaving high containment facilities. However, a case to case approach/evaluation is required in relation to the contaminating agents. When materials are soiled by faeces or other substances, more research and validation is still needed.
The choice of the appropriate process "wet versus dry" has to be considered in function of the set-up and dimensions of decontamination rooms and enclosures.

Experience and studies on Formaldehyde fumigation at the Institute for Animal Health

Yongjie Harvey; Institute for Animal Health (IAH), Pirbright, UK

Formaldehyde has been used as the only fumigant at Institute for Animal Health to decontaminate equipment from the restricted high containment area and rooms. It has been also routinely used to decontaminate animal accommodation. Its success is dependent on complex interaction between formaldehyde levels, humidity and temperature.
Our experiences demonstrate that FMDV on non-porous surface will lose infectivity without treatment after one week at dry form in room temperature. There are no major differences between non-soiled and soiled samples. However, swine vesicular disease virus (SVDV) on non-porous surface has been demonstrated to retain infectivity after one month in dry form at room temperature. This information is helpful to assess residual risk if there is insufficient decontamination achieved.

Formaldehyde fumigation ($10g/m^3$) has been validated in our laboratory by achieving 4 $\log_{10}$ reduction against FMDV. However, complete virus inactivation is not achieved; therefore, cleaning and disinfection before fumigation are essential as part of the decontamination process.

There is good correlation between 10^6 *B. atrophaeus* spore strips with dried FMDV and bovine enterovirus (BEV) whereas the 3M attest 1294 rapid bio-indicator system shows less sensitivity. Therefore, 3M attest 1294 rapid bio-indicators are not ideal to monitor formaldehyde fumigation cycle for high risk transfers.
A similar approach as described could be used to validate an alternative fumigant such as VHP. We are keen to change to a healthy and environmentally friendly alternative. Studies on VHP efficacy against FMDV have been planned.

Key messages:
Formaldehyde fumigation ($10g/m^3$) is validated against FMDV. *B. atrophaeus* spore strips can be used as a bio-indicator at Institute for Animal Health.

VHP decontamination at the National Veterinary Institute of Sweden

Hans Antehed, Swedish Veterinary Institute (SVA), Uppsala, Sweden

The following summary describes the results of three tests with VHP decontamination using a Steris VHP 1000ED-S generator carried out at the National Veterinary Institute of Sweden, 2006 and 2010.

Test 1, 31.8.2006, Decontamination of a room in BSL-3 lab 1

Area and volume of the room: 39 m^2 and 110 m^3. Two fans were used helping spreading the gas
Spores and indicators: Live spores (*Geobacillus stearothermophilus* 2,5 x 10^6 CFU) and VHP indicators from STERIS were positioned together at different places in the room
Results: 20 out of 21 spores deceased
Lessons learned:
- Likely the recommended amount of VHP concentration (400 ppm) was not reached
- Too little VHP was loaded in the machine
- Calculation error?

Test 2, 29.9.2006, Decontamination of BSL-3 lab F271

Area and volume of the lab: 27 m^2 and 75 m^3
Spores and indicators: Live spores (*G. stearothermophilus* 2,5 x 10^6 CFU) and VHP indicators from STERIS were positioned together at different places in the lab
Results: 20 out of 20 spores deceased
Lessons learned:
- A relatively higher amount of VHP was used
- Likely the recommended concentration of VHP (400 ppm) was reached.
- Some spores were placed in ”inconvenient” spots, but deceased anyway

Test 3, 17.12.2010, Decontamination of BSL-3 lab 1

Parameters:
- Humidity in lab: 67% Rh
- Temperature: 23 °C
- Amount of VHP: approx. 1850 g
- Room area: 78,5 m^2
- Room volume: 221 m^3
- Number of fans: 7
- Number of spores: 20 (*G. stearothermophilus* 2,5 x 10^6 CFU)
- Number of chemical indicators: 20 VHP indicators from STERIS

Results:

- All 20 spores were killed
- The requested 400 ppm concentration of VHP was likely reached during the decontamination
- The gas was probably easier spread out in the lab thanks to the 7 fans

Conclusions:

- The method seems to be working well with the parameters tested (indicator spores)
- It’s an easy method to perform.
- As the VHP generator is heavy, thus inconvenient to move between different facilities needed to be decontaminated, fittings to enable plugging in the machine on the outside of the laboratories, would be recommendable (already installed in F271 which contributed facilitating the test).
- VHP is better from a work environment perspective but proper measurement equipment for VHP needs to be purchased to measure eventual VHP remainders after decontaminations.
- The decontamination process with VHP is faster than using formaldehyde.
- After 12 hours of ventilation no smell and effect on the lab itself or the equipment could be traced.
- The method needs to be further validated with more tests carried out.

Progress towards a laboratory assay to assess the virucidal activity of aerial disinfectants, using infectious bursal disease of chickens as a test virus.

Nicolas Eterradossi (1), Didier Toquin (1), Michel Amelot (2), Roland Cariolet (3)
French Agency for Food, Environmental and Occupational Health Safety (ANSES), Laboratory for Poultry, Swine and Fish Research, BP53, 22440 Ploufragan, France : (1) Avian and Rabbit Virology Immunology and Parasitology Unit (VIPAC), (2) Experimental services for Avian Pathology (SEEPA), (3) Experimental Services for Swine Pathology (SPPAE).

The French Agency for Food, Environmental and Occupational Health Safety (ANSES), Ploufragan laboratory investigates the health and welfare of poultry and swine, as well as the safety of the food products derived thereof. As such, Anses-Ploufragan maintains level 2 and level 3 contained laboratories and animal facilities and frequently performs experiments and laboratory work involving live infectious agents (bacteria, viruses, parasites). It is therefore of paramount importance that an efficient way to decontaminate the contained facilities is defined. Aerial disinfection is especially suitable as, when properly implemented, it may reach even remote areas which are not readily accessible for spraying disinfectant. Until recently, aerial disinfection was performed in Anses-Ploufragan using gaseous formaldehyde generated from heated trioxymethylene. Recent discussions about the carcinogenic potential of formaldehyde have raised the issues of i) which alternative should be used and ii) how the efficacy of aerial disinfectants should be compared. Indeed, the only standardized test to assess the virucidal activity of compounds intended for veterinary use (NF EN 14675) deals with disinfectants added into virus suspensions (liquid phase), not with aerial disinfection.

This study was therefore implemented in order to develop a quantitative laboratory assay that could be easily used to assess the virucidal activity of aerial disinfectants. Infectious bursal disease virus (IBDV) of chickens (Avibirnavirus, non-enveloped, two segments of dsRNA) was selected as a test virus because i) some apathogenic attenuated (vaccine) strains are available, ii) these strains grow to a high titre in chicken embryo fibroblasts (CEF) and iii) IBDV is extremely resistant in the environment and survives up to 6 months in contaminated premises. A high titre IBDV suspension was produced and serially diluted. Twenty µl of each dilution (16 wells per dilution) were then distributed in 96-well cell culture plates. These were dried under a microbiology safety cabinet, thus mimicking droplets of virus-contaminated biological fluids drying on environmental surfaces. The dried 96-well plates were then kept at -70°C until use. To evaluate the virucidal efficacy of aerial disinfectants, several plates were unfrozen : one was kept in the laboratory unexposed to the disinfectant (effect of unfreezing), another one was kept open in the disinfected area for the duration of the disinfection but in a sealed container (effect of ambient temperature on virus survival), several other plates were opened and exposed to aerial disinfection. At the end of the disinfection process, the possibly surviving virus in the 96-well plates was rehydrated and added onto fresh CEF culture. The infectious virus titre was calculated 5 days later from the detected cytopathogenic effect. The decrease of virus titre after exposure to aerial disinfection was considered as an indication of virucidal activity.

The virucidal activity of several compounds was compared in several preliminary disinfection assays performed in animal containment cells: gaseous formaldehyde, a commercial compound (including glutaraldehyde, quaternary ammonium and phenol) and Hydrogen peroxide achieved 2 to 3, 3 to 4 and 2 to 3.5 $\log_{10}$ reduction in virus titre, respectively. Striking differences were observed within the same disinfection run, depending on where the virus plates were positioned. The assay therefore appears suitable to assess the homogeneity of disinfection within the studied volume. Bottlenecks with this laboratory assessment method included the need for a high titre virus suspension to start with (otherwise a 4 $\log_{10}$ virus reduction cannot be demonstrated) and the occasional carry-over of disinfectant residues to the CEF culture.

The chemistry of disinfection: compounds of major interest as compared to formaldehyde.

Pierre Maris, The French agency for food, environmental and occupational health safety (ANSES), France.

In order to speak about the chemistry of the disinfection and to offer some points of discussion concerning the search for alternatives to formaldehyde, we have to know first that Biocides Directive 98/8/EC and more specifically European Regulation n° 1451/2007 provides us data about notified active substances under revision in Europe in order to be or not registered on the European Commission's positive list in the next three years.

If we take as an example the product type n°3 concerning the veterinary field, 76 active substances are currently notified. They belong to the three broad categories: the first category includes oxidizing agents which are in wide use (halogenated products, such as chlorine and iodine, the peroxigens, such as peracetic acid and hydrogen peroxide, and chlorine dioxide) – the second category producing specific interactions between macromolecules (proteins and nucleic acids); included in this group aldehydes (glutaraldehyde and formaldehyde), phenolated products and alcohol – the third category brings together a wide variety of product classes that disrupt and disorganize the structure and function of cell wall and membrane of bacteria, and the envelope of viruses (quaternary ammonium compounds, bisbiguanide, diamidine and organic acids).

We must also consider the performance of a complex product in its formulation: each active substances is quite often associated with one, two, or even three other active agents of different chemical families, and besides them a series of ingredients bring very important functions (optimizing activity, ensuring better stability, improving surface wettability, reducing corrosion, increasing or decreasing foaming properties, regulating pH, decreasing water hardness, etc.).

To make the best selection of a disinfectant, we have first to consider the nature and the level of the identified or assumed risk, and in relationship with this risk to define the level of performance. Four levels of performance can be described between the low level of performance (killing vegetative bacteria, certain fungi and some enveloped viruses) to a very high level of performance with specific requirements, such as destroying prions for example. In addition with this first criterion, five other parameters have to be considered: the chemical reactivity with organic matter, the chemical hazard, the corrosiveness to materials and the environmental risk.

Finally, in order to search the alternatives to formaldehyde, we can turn our attention to five active substances with broad spectrum of activity: glutaraldehyde, chlorine, peracetic acid, hydrogen peroxide and chlorine dioxide. For each active substance, it is necessary to analyse with more precisions advantages and disadvantages, taking into account mainly the different forms (liquid, gas or vapour), the optimal conditions in terms of temperatures, relative humidity and pH, the relative importance of reactivity with soils, the corrosiveness and human safety.

A patient with Marburg haemorrhagic fever: patient management and disinfection

Gijsbert van Willigen, Leiden University Medical Centre, Leiden, The Netherlands

Marburg haemorrhagic fever virus is the first discovered filovirus and is closely related to Ebola virus. The virus was first discovered in African grivets that were imported by a German company based in Marburg. The first symptoms of the illness resemble the common flu or malaria. In later stages the patient suffers from multi organ failure, and multiple bleedings. The virus spreads via all bodily fluids and the most likely route of infection is via small droplets through the air and direct contact with damaged or intact skin. No cure for the disease is available.

In July 2008 a patient was transferred from the Elkerliek hospital in Helmond, the Netherlands to the Leiden University Medical Center (LUMC). The patient became ill a week after returning from a holiday in Uganda. After arrival in the LUMC, the patient was immediately put in strict isolation at an intensive care united (ICU), because there was a small change that the patient suffered from a viral haemorrhagic fever. The patient was nursed according to the protocol "Very contagious diseases with risk for the personnel". This protocol was developed after the first ever patient with Lassa fever in the Netherlands who was also hospitalized in the LUMC. The protocol describes patient management, personal protection for nurses and doctors during the various stages of the disease and the setup of a crisis management team.

The patient was treated with a number of medical devices such as artificial respiration, artificial kidney and an artificial liver. During hospitalization a limited amount of diagnostic test were run in the patient room (X-rays, point-of-care equipment), in BSL3 laboratories by technicians wearing FFP2-masks, double pairs of gloves, eye-protection, coveralls etc. or outside of containment after the patient material was inactivated. For the transport of patient samples triple packaging was used comparable with the packaging instruction PI 602 (IATA) and a simple track and trace system was in place to prevent samples would get lost during transport.

The diagnosis "Marburg haemorrhagic fever" was made by the Berhard Nocht Institute in Hamburg, Germany by isolating the virus from patient materials.
For the disinfection of the isolation room and the medical devices used during hospitalization of the patient three methods were considered:

- cleaning and disinfection by hand
- fumigation using formaldehyde
- fumigation using hydrogen peroxide vapour (HPV) or vaporised hydrogen peroxide (VHP)

In the consideration there were 3 main issues:

- efficacy of the method
- the ICU should stay fully operational
- the medical devices should be decontaminated inside and not be damaged

The only method that was suitable for our situation was fumigation HPV/VHP. The complete procedure was executed by Bioquell / TechniLab BMI. Before decontamination the room was stripped of all materials that could prevent the fumigation to be fully effective. Curtains, the mattress etc. were disposed of as contaminated waste and most dirt was removed from the floor using a chlorine solution. In the room, biological indicators were placed at the most difficult spots for the HPV to reach. Two days after fumigation all spore strips were negative and the room was cleaned by hand and released to the ICU.

Assessment of the limitations of gaseous disinfectants for use in animal facilities and biological laboratories

Allan Bennett, Biosafety, Microbiology Services Division, Health Protection Agency, Porton Down, UK.

All of the existing gaseous disinfection systems have the potential of inactivating the agent of interest contained in laboratories or biological safety cabinets. Successful disinfection is normally validated using commercial biological indicators that consist of clean resistant spores of *G. stearothermophilus* or *B. atrophaeus* dried on stainless steel at loadings of 6 logs. This has proved a successful approach for the pharmaceutical industry. However, in other settings gaseous disinfection systems may have to deal with environments that are less clean, contain a wide range of different materials, with different micro-organisms within different suspending (organic) fluids. The Biosafety Unit at Porton Down has spent many years studying the use of gaseous disinfection systems for a range of different sectors including biological laboratories, hospitals wards and isolation rooms and the space industry. In this presentation our experience in determining the limitations of gaseous decontamination systems will be discussed and recommendations will be made that will help to ensure a successfully validated gaseous disinfection.

New building design and disinfection at the Friedrich-Loeffler Institute

Bernd Haas, Friedrich Loeffler Institute (FLI), Riems, Germany

Friedrich Loeffler, Professor of Hygiene in Greifswald, was the first scientist who realized that the pathogen causing foot-and-mouth disease (FMD) represented a new type of infectious agents – a virus (1898). After October 1910, he performed his FMD experiments on the Isle of Riems in the Baltic Sea near Greifswald in order to prevent accidental infections of the German livestock population. Today, the Isle of Riems is the Headquarters of the Friedrich-Loeffler Institute (FLI). The main focus of the work of the FLI is the health and wellbeing of farm animals and the protection of humans from zoonoses, i.e. from infections which can be transmitted from animals to humans. The FLI conducts basic and applied research covering different scientific fields, including physiology, ethology, epidemiology, immunology, virology, bacteriology, parasitology, and related sciences. As federal research institute and independent higher federal authority the FLI belongs to the portfolio of the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV). Currently, the Friedrich-Loeffler-Institut has 850 employees in eleven institutes at seven sites. Its official tasks according to German legislation include the diagnosis of many animal diseases. In particular for the work on foreign animal diseases like FMD, the FLI has a high security building (BSL3 and BSL3+) on the Isle of Riems: Currently, the new laboratory and stable building consisting of BSL2, BSL3**, BSL3, BSL3+ and BSL4 sections is nearing completion. The sections are strictly separated from each other. Within the sections, the stables are strictly divided into "red" and "green" corridor areas. The German Federal Government is spending about 260 Mio. € on 89 laboratories and 163 stables. An overview on the layout of the new building and of the biorisk management system was given.

The disinfection concept is based on a few basic rules:

- Use autoclaving where possible.
- Use liquid disinfectants (mostly organic acids and NaOH) if autoclaving is not suitable.

We have cleaned the stables used for FMD experiments with a commercial product based on formic acid and applied as foam („Venno Vet 1 super") for many years without fumigating the stables afterwards. We never observed an accidental infection due to insufficient disinfection.

- Use formaldehyde fumigation if neither autoclaving nor disinfection by liquid chemicals is suitable.
- If formaldehyde fumigation is used, make sure that surfaces are as clean possible and consider formaldehyde fumigation only as an „additional process".

We mainly use formaldehyde fumigation to get sensitive e.g. electronic, equipment out of the containment and during the replacement process for HEPA filters. It is applied by a few specially trained staff members, usually over the weekend when the building is mostly empty.

New building design and disinfection

Uwe Müller-Doblies, Institute for Animal Health (IAH), Pirbright, UK

The capability to decontaminate CL3 and CL5 laboratory and animal facilities with gaseous agents is a regulatory requirement. While the regulatory requirement is limited to the sealability of the containment spaces a lot more considerations have to go into the facility design process.

In order to achieve process validation key environmental parameters have to be maintained throughout the fumigation process (temperature, humidity and fumigant concentration). Depending on the fumigant air circulation in the space is critical during the fumigation process to reach all surfaces with the fumigant (particularly a problem with VHP) and after the process for the venting of fumigants, where these persist on surfaces and in air such as formaldehyde. Most containment facilities are predominantly heated with air.

During the fumigation process the ventilation is turned off and external walls can cool down quite rapidly. Avoiding temperature gradients is important, but can be quite difficult and may limit the ability to fumigate spaces at certain times of the year.

Ductwork mounted HEPA filters come with long stretches of contaminated duct work and need special planning to permit gaseous decontamination using closed loop fumigation systems, but the bio-burden in these ducts must be minimized with room mounted pre-filters.

Freezer farms pose their own challenges as they (a) cannot be switched off during fumigation, (b) can easily overheat while the ventilation is shut off and (c) come with significant temperature gradients, which make them very difficult to fumigate.

Process validation has to consider the biological challenge to be encountered in real life. In the case of HEPA filters this means that validation on clean HEPA filters is not sufficient. Other challenges include heat sources that prevent local condensation, voids that cannot be reached by gas diffusion and pressure cascades that could draw fumigant into neighboring occupied spaces.

Green gas, dry mists and dense vapours: HSL's experiences in the world of fumigation technology.

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The HSE-funded lab fumigation study, and other related work we have undertaken for the healthcare sector, has highlighted considerable differences in efficacy between the standard fumigation technologies on the market, and that efficacy against different organisms can be variable, even for individual systems. In view of these variations we would suggest the following:

- Individual laboratories need to find appropriate surrogates (test challenges) if considering replacing formaldehyde with an alternative fumigation system, or if commissioning a new facility with an alternative fumigation system in place. We would very much recommend conducting a test with the chosen system wherever possible and before any major financial commitments, because we have found room to room variations with individual systems, as well as between different VHP systems. There is no doubt that, subject to the types of bugs being handled and the geometry of rooms, certain systems will be suited to some laboratories but not to others;
- In view of the above, as much supportive data as possible should be requested from the manufacturer on the efficacy of the fumigation system, with particular attention to the type of microbial challenges likely to be faced by the end user. If these data have been independently generated, then all the better!
- Consider the logistics and ease of use of fumigation equipment as well as outright efficacy – look at more than one available system if at all possible, to allow comparison. If looking at mobile systems then consider size and weight. Some of these systems are very bulky;
- Have a good look at the user interface for the system you are interested in and, if at all possible, compare this to other similar systems. We have seen some very easy-to-use systems, with nice user controls, but other systems have been awful. Does the system log cycle detail/completion for possible future audits? Is there the potential for future software improvements without excessive cost to you?
- Any equipment purchases should include the views of appropriate partners (e.g. occupational health and safety advisors; risk management staff; fire officers - some systems will set off certain types of fire alarm - as we have discovered);
- When considering the cost of new fumigation equipment, ask for information on service provision or hire costs as well as outright purchase (if this option exists for you), and ensure that consumable costs for on-going use are acceptable as a long-term commitment. The total costs for such equipment are considerable and final decisions need to be well informed;

Health and safety is of paramount importance when using fumigation equipment, and comprehensive information and advice should be provided by the system supplier prior to installation and use of their system. This should include:

- Ensuring that end users receive comprehensive training if the systems are to be purchased outright or hired by an institution and operated by its own personnel - ask the system supplier to outline their plans in this respect, if you don't already have this information from them;
- Ensuring that any handling of chemicals associated with the machine, e.g. bulk 37% hydrogen peroxide solution is done with full awareness of their potential for harm, and in line with your local/national chemical regulations for transportation, handling and bulk storage. Local risk assessments would play an important part in this;

- The ability to check for residual levels of fumigant after use of the machine – this is usually possible with some form of hand held monitoring device, in order to avoid re-entering a room that exceeds the Workplace Exposure Limit (WEL) for the fumigant. This must be discussed with the supplier prior to use of their system, i.e. is detection of the fumigant likely to be a problem and what safeguards are available to ensure that staff are not put at risk by residual fumigant that exceeds the WEL? The ventilation system in Class-3 labs would be able to safely remove any residual vapour, but even this may take time and may be incomplete again something we have experienced directly during our studies.

As a final comment – longer-term studies are probably needed to assess levels of corrosion of equipment and of the fabric of the room, following repeated fumigation treatments. Damage to various pieces of equipment as a result of installing a VHP system is not unknown, and chlorine dioxide related pitting of certain surfaces has also been described by some (though not observed in our studies). Any reassurance, or insurance, you can get to cover this potential problem would be worth asking about.

Non Formaldehyde Fumigation Technologies at the Canadian Science Centre for Human and Animal Health

Jay Krishnan, Senior Biosafety Officer, National Microbiology Laboratory, Canadian Science Centre for Human & Animal Health, Winnipeg, MB, Canada.

The Canadian Science Centre for Human and Animal Health has been using formaldehyde fumigation for the decontamination of its high containment laboratories, animal cubicles, biosafety cabinets and HEPA housings. Because of the health and environmental impacts of formaldehyde, we have been validating safer technologies for area/space decontaminations.

Vaporised hydrogen peroxide (VHP), Gaseous chlorine dioxide (GCD) and Dry fogging system (DFS) have been validated. VHP generators were purchased from Steris (dry VHP), Bioquell (wet VHP), GCD generator from Clordysis and DFS from Ikeuchi (portable fogger) and Mar Cor Purification (mini fogger). In order to present the hardiest inactivation process challenge, a preparation of Bacillus atrophaeus spores was used as the test microbial agent. A standardized disinfectant testing protocol named Quantitative Carrier Test (QCT) was used for the validation experiments. QCT is a standard at the American Society of Testing and Materials (ASTM) international.

All three fumigation technologies did inactivate 106 bacterial spores that had been deposited and dried on stainless steel coupons; however, their microbiocidal efficacy was greatly reduced when the spores were mixed with a standard tripartite protein soil load (BSA, tryptone, and mucin). VHP was unable to completely inactivate the spores in protein soil, whereas GCD and DFS were able to inactivate them completely, given a significantly longer exposure time.

In conclusion, this study shows the potential use of VHP, GCD and DFS for the decontamination of laboratory spaces and other areas. VHP requires lower humidity in the area to achieve a higher vapour concentration, while GCD requires approximately 75% relative humidity (RH) for effective microbial killing. Dry fogging process elevates the RH in the space being decontaminated; it shouldn't be allowed to increase beyond 75% as this could affect material compatibility. DFS, unlike VHP and GCD, is extremely portable thus having the added advantage of being easily deployed in the field for decontamination of mobile/temporary laboratories and equipment during disease outbreak and BT incident response.

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Mission of EPIZONE

EPIZONE is an EU funded Network of Excellence for Epizootic Disease Diagnosis and Control to improve research on preparedness, prevention, detection, and control of epizootic diseases within Europe to reduce the economic and social impact of future outbreaks of Foot-and-mouth disease, Classical swine fever, Avian influenza, and other relevant epizootic diseases like Bluetongue and African swine fever, through increased excellence by collaboration.