## Section A5.3 Efficacy Data
## Annex Point IIA5.3 Surgical hand disinfection

| | | | Official use only |
|---|---|---|---|
| | | **1 REFERENCE** | |
| **1.1** | **Reference** | Kampf, G., Ostermeyer, C. 2005. Efficacy of two distinct ethanol-based hand rubs for surgical hand disinfection – a controlled trial according to prEN 12791. BMC Infect. Dis. 5:17-21. | |
| **1.2** | **Data protection** | Not applicable | |
| 1.2.1 | Data owner | | |
| 1.2.2 | Criteria for data protection | | |
| **1.3** | **Guideline study** | Yes, prEN 12791. | |
| **1.4** | **Deviations** | yes, see 2.3.4 | |
| | | **2 METHOD** | |
| **2.1** | **Test Substance (Biocidal Product)** | Propan-1-ol | |
| 2.1.1 | Trade name/ proposed trade name | Not applicable | |
| 2.1.2 | Composition of Product tested | Aqueous solution of 60% (v/v) propan-1-ol | |
| 2.1.3 | Physical state and nature | Liquid disinfectant | |
| 2.1.4 | Monitoring of active substance concentration | Not applicable. | |
| 2.1.5 | Method of analysis | Not applicable | |
| **2.2** | **Reference substance** | Two biocidal products were tested in parallel:<br>Sterillium rub (containing 80% (w/w) ethanol) and Avaguard (containing 61% (w/w) ethanol and 1% chlorhexidine gluconate) | |
| 2.2.1 | Method of analysis for reference substance | Not applicable | |
| **2.3** | **Testing procedure** | | |
| 2.3.1 | Test population / inoculum / test organism | The resident hand flora of 20 subjects for each of two experiments served as bacterial test population. Hands were pre-washed with soap for 1 min. The bacterial prevalue of the hands prior to biocide treatment was obtained by rubbing finger tips in tryptic soy broth (TSB) for 1 min. | |
| 2.3.2 | Test system | Determination of the efficacy of a surgical hand disinfection product in a controlled cross-over trial simulating practical conditions (prEN 12791). | |
| 2.3.3 | Application of TS | As prescribed by guideline | |
| 2.3.4 | Test conditions | Hands of volunteers were pre-washed with soap for 1 min and the bacterial load present was established by rubbing finger tips in tryptic soy broth (TSB) for 1 min. Each volunteer treated the hands with either 60% propan-1-ol (applied in several 3 ml portions over 3 min to keep the skin moist) or one of the 2 biocidal products (applied as specified | |

| | | |
|---|---|---|
| | | for propan-1-ol). The bacterial load after biocide application (immediate effect) was established by rubbing finger tips of one hand in TSB with added neutralizer (3% Tween 80, 3% lecithin, 0.1% histidine, 0.1% cysteine) for 1 min. The other hand was gloved for 3 h. The 3 h sustained effect value was determined by removing gloves and rubbing finger tips in TSB for 1 min. The bacterial load in TSB samples was determined by serial dilution and surface culture. |
| 2.3.5 | Duration of the test / Exposure time | 3 min exposure to biocide with immediate and 3h post treatment evaluation |
| 2.3.6 | Number of replicates performed | as prescribed by guideline |
| 2.3.7 | Controls | as prescribed by guideline |
| **2.4** | **Examination** | |
| 2.4.1 | Effect investigated | In vivo efficacy for surgical hand disinfection under practical conditions tested according to prEN 12791 (phase 2, step 2). |
| 2.4.2 | Method for recording / scoring of the effect | The log 10 reduction factor for the resident microbial flora present on the finger tips of volunteers was determined by comparison of the pre treatment to the post treatment log 10 values. |
| 2.4.3 | Intervals of examination | Reduction in microbial load present on finger tips of volunteers was determined directly and with a 3 h delay after exposure to the test substance. |
| 2.4.4 | Statistics | Differences of log10 pre- and post treatment values were calculated individually for each volunteer and the means were analyzed with the Wilcoxon matched-pairs signed-ranks test. |
| 2.4.5 | Post monitoring of the test organism | Not applicable. |
| | | **3 RESULTS** |
| **3.1** | **Efficacy** | Propan-1-ol was able (immediate RF value = 2.58 (±1.16) and 2.98 (±0.9) and 3 h sustained RF value = 1.67 (±0.96) and 2.56 (±1.17)) to significantly reduce the microbial load present on the finger tips of volunteers at a concentration of 60%. |
| 3.1.1 | Dose/Efficacy curve | Not applicable |
| 3.1.2 | Begin and duration of effects | Effect was reported immediately and 3 hours after exposure to the product. |
| 3.1.3 | Observed effects in the post monitoring phase | Not applicable |
| **3.2** | **Effects against organisms or objects to be protected** | None reported |
| **3.3** | **Other effects** | None reported. |
| **3.4** | **Efficay of the reference substance** | The two biocidal products tested in parallel were equally (Sterillium rub) and less effective (Avagard). |

**3.5 Tabular and/or graphical presentation of the summarised results**

Table 3.5.1 Table 1: Reduction in bacterial load (mean log RF) present on the finger tips of volunteers after exposure to aqueous propan-1-ol (60%).

| Tested organisms | Exposure time (min) | Log microbial load prior to exposure | Mean log RF - immediate (0h) effect value | Mean log RF - sustained (3h) effect value |
|---|---|---|---|---|
| Resident bacterial flora Test 1 | 3 | 4.44 (± 0.90) | 2.58 (± 1.16) | 1.67 (± 0.96) |
| Resident bacterial flora Test 2 | 3 | 4.38 (± 0.66) | 2.98 (± 0.90) | 2.56 (± 1.17) |

**3.6 Efficacy limiting factors**

3.6.1 Occurrences of resistances: none reported

3.6.2 Other limiting factors: none reported

## 4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS

**4.1 Reasons for laboratory testing**

Using the guideline method pr EN 12791, the in vivo efficacy for surgical hand disinfection under practical conditions was tested for propan-1-ol at a concentration of 60% with 3 min exposure. The results obtained in this study are relevant for the intended use of the test substance.

**4.2 Intended actual scale of biocide application**

not stated

**4.3 Relevance compared to field conditions**

4.3.1 Application method: The test conditions of the test method simulating practical conditions are representative for the actual conditions in the main field of use of the test substance.

4.3.2 Test organism: The resident hand flora of 20 volunteers for each of two experiments served as bacterial test population and can be considered an ideal representative for the target organisms in the intended area of use of the biocide.

4.3.3 Observed effect: The obtained efficacy result of the test substance is relevant for determining the disinfectant properties of the product in the intended area of use.

**4.4 Relevance for**

**Section A5.3 Efficacy Data**
**Annex Point IIA5.3** Surgical hand disinfection

| | | | |
|---|---|---|---|
| | **read-across** | | |
| | | **5 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **5.1** | **Materials and methods** | A hand disinfection test was carried out in accordance with the prEN 12791 guideline method for testing the in vivo effectiveness of surgical hand disinfectants under practical conditions (phase 2, step 2). The test substance was propan-1-ol at a concentration of 60% and the effect it has on the resident microbial flora present on the finger tips of volunteers was determined. | x |
| **5.2** | **Reliability** | Reliability factor1. (Guideline study) | |
| **5.3** | **Assessment of efficacy, data analysis and interpretation** | Propan-1-ol was effectively removing the resident microbial flora present on the finger tips of volunteers at 60% and at an exposure time of 3 min. | |
| **5.4** | **Conclusion** | The guideline test used in this study is a useful method for detecting the in vivo disinfection properties of biocidal products under practical conditions. | |
| **5.5** | **Proposed efficacy specification** | | |

| **Evaluation by Competent Authorities** | |
|---|---|
| | *Use separate "evaluation boxes" to provide transparency as to the comments and views submitted* |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 2008/10/30 |
| **Materials and methods** | 5.1: Propan-1-ol was not the test substance, but reference substance. |
| **Conclusion** | Applicant's version is adopted |
| **Reliability** | 1 |
| **Acceptability** | acceptable |
| **Remarks** | In the study report only mean values are given, no raw data as required according to the guideline. Therefore a validation of the data is not possible. Nevertheless, the results obtained show a sufficient reduction of the resident hand flora. Additionally, 60% propan-1-ol is the reference substance of the guideline, which implies the effectiveness of the substance under given conditions. |
| | **COMMENTS FROM ...** |
| **Date** | *Give date of comments submitted* |
| **Results and discussion** | *Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.*<br>*Discuss if deviating from view of rapporteur member state* |
| **Conclusion** | *Discuss if deviating from view of rapporteur member state* |
| **Reliability** | *Discuss if deviating from view of rapporteur member state* |
| **Acceptability** | *Discuss if deviating from view of rapporteur member state* |

## Section A5.3 Efficacy Data
## Annex Point IIA5.3 Surgical hand disinfection

**Remarks**

**Section A5.3** **Efficacy Data**
**Annex Point IIA5.3** Microbicidal activity – bacteria and fungi in the presence of organic load

| | | | Official use only |
|---|---|---|---|
| | | **1 REFERENCE** | |
| **1.1** | **Reference** | Pullen, W., & van Klingeren, B. 1991. Vergelijkend onderzoek naar de desinfekterende werking van alcoholen in de Europese suspensie test. Rapport Nr. 358704003. RVVM, Bilthoven. | |
| **1.2** | **Data protection** | Not stated | |
| 1.2.1 | Data owner | | |
| 1.2.2 | Criteria for data protection | Not stated | |
| **1.3** | **Guideline study** | Yes, Test methods for the antimicrobial activity of disinfectants in food hygiene, European council, 1987. | |
| **1.4** | **Deviations** | Yes, see 2.3.4 | |
| | | **2 METHOD** | |
| **2.1** | **Test Substance (Biocidal Product)** | | |
| 2.1.1 | Trade name/ proposed trade name | Not applicable | |
| 2.1.2 | Composition of Product tested | Propan-1-ol (p.a, Merck 997) at different concentrations in water | |
| 2.1.3 | Physical state and nature | Liquid disinfectant | |
| 2.1.4 | Monitoring of active substance concentration | Not reported | |
| 2.1.5 | Method of analysis | Not applicable | |
| **2.2** | **Reference substance** | Propan-2-ol and ethanol tested in parallel. | |
| 2.2.1 | Method of analysis for reference substance | Not applicable | x |
| **2.3** | **Testing procedure** | | |
| 2.3.1 | Test population / inoculum / test organism | Table 2.3.1.1 Bacterial and fungal strains employed to test the biocidal efficacy of propan-1-ol | |

| Species/strain | Source/origin | Representative for |
|---|---|---|
| *Pseudomonas aeruginosa* | ATCC 15442 | Gram negative bacteria |
| *Staphylococcus aureus* | ATCC 6538 | Gram positive bacteria |
| *Enterococcus faecium* | DVG 8582 | Gram positive bacteria |
| *Proteus mirabilis* | ATCC 14153 | Gram negative bacteria |

| | | |
|---|---|---|
| *Mycobacterium terrae* | ATCC 15755 | Gram positive bacteria |
| *Candida albicans* | ATTC 10231 | Yeasts |
| *Aspergillus niger* | ATCC 16404 | Moulds |

The bacterial suspensions contained ca. 10E8 CFUs/ml, the yeast suspension ca. 10E7 CFUs/ml, the conidial suspension ca. 10E7 CFUs/ml. Stock cultures of all strains but *A. niger* and *M. terrae* were kept on tryptone soy agar. *M. terrae* was kept on Middlebrook 7H10 Agar with 10% OADC whilst *A. niger* was kept on malt extract agar. Working cultures (2 subsequent times 24h growth on TSA at 32°C) were used to prepare suspensions for all bacterial strains (exception *M. terrae*) and the yeast by using glass beads and glass wool filtration. *M. terrae* suspensions were obtained from 7d stock cultures using glass beads and subsequent filtration with glass wool. *A. niger* conidia were harvested from 4d stock cultures using 0.6% Tergitol 7, harvested by centrifugation (20 min @ 2000 g). All suspensions were prepared in saline with 0.1% peptone.

| | | |
|---|---|---|
| 2.3.2 | Test system | Quantitative suspension test under conditions representative of practical use (e.g. CEN - Phase 2, Step1). |
| 2.3.3 | Application of TS | As prescribed by test method, diluted in water of standard hardness (WSH). |
| 2.3.4 | Test conditions | Concentrations tested (20 up to 80% propan-1-ol (v/v)), dilution in sterile hard water, bovine serum albumin at 0.03% served as organic load, test was run at 20°C+/-1°C, dilution in neutralizer solution used to stop the effect of the biocide. Neutralizer/inactivation medium used contained 3% Tween 80, 3% Saponin, 0.1% Histidin, and 0.1% Cystein. |
| 2.3.5 | Duration of the test / Exposure time | 2 and 5 min |
| 2.3.6 | Number of replicates performed | As prescribed by guideline |
| 2.3.7 | Controls | As prescribed by guideline |
| **2.4** | **Examination** | |
| 2.4.1 | Effect investigated | Reduction in viability of test organisms using a quantitative suspension test (phase 2/step1) as prescribed by the guideline method employed. |
| 2.4.2 | Method for recording / scoring of the effect | Determination of CFUs/ml of the respective test organism in the test suspension before and after exposure to the test product. |
| 2.4.3 | Intervals of examination | CFUs determined once after termination of exposure. |
| 2.4.4 | Statistics | |
| 2.4.5 | Post monitoring of the test organism | Not applicable |

**3 RESULTS**

| | | |
|---|---|---|
| **3.1** | **Efficacy** | Propan-1-ol exhibited at 30% and >= 2 min exposure time sufficient microbicidal activity (i.e. log RF >5) for all bacterial strains and the |

yeast strain tested. A log RF of almost 5 was achieved for *Aspergillus niger* at a concentration of 80% and an exposure time of 5 min.

3.1.1 Dose/Efficacy curve — Not applicable.

3.1.2 Begin and duration of effects — Effect was only reported for the given exposure times.

3.1.3 Observed effects in the post monitoring phase — Not applicable.

**3.2 Effects against organisms or objects to be protected** — None reported

**3.3 Other effects** — None reported

**3.4 Efficay of the reference substance** — Propan-2-ol was less effective than propan-1-ol but more effective than ethanol (exception *A. niger*).

**3.5 Tabular and/or graphical presentation of the summarised results**

Table 3.5.1 Reduction of CFUs/ml after exposure to aqueous propan-1-ol solution

| Species/strain | Exposure time (min) | Concentration of test product (%, v/v) | Viability reduction (log RF CFUs/ml) |
|---|---|---|---|
| *Pseudomonas aeruginosa* | 2 | 20 | >=5 |
| | | 30 | >=5 |
| | 5 | 20 | >=5 |
| | | 30 | >=5 |
| *Staphylococcus aureus* | 2 | 20 | >=5 |
| | | 30 | >=5 |
| | | 40 | >=5 |
| | 5 | 20 | >=5 |
| | | 30 | >=5 |
| | | 40 | >=5 |
| *Enterococcus faecium* | 2 | 20 | 3 |
| | | 30 | >=5 |
| | | 40 | >=5 |
| | 5 | 20 | 4.6 |
| | | 30 | >=5 |
| | | 40 | >=5 |
| *Proteus mirabilis* | 2 | 20 | >=5 |
| | | 30 | >=5 |

| | | | |
|---|---|---|---|
| | 5 | 20 | >=5 |
| | | 30 | >=5 |
| *Mycobacterium terrae* | 2 | 30 | >=5 |
| | | 40 | >=5 |
| | | 50 | >=5 |
| | 5 | 30 | >=5 |
| | | 40 | >=5 |
| | | 50 | >=5 |
| *Candida albicans* | 2 | 20 | >=5 |
| | | 30 | >=5 |
| | | 40 | >=5 |
| | 5 | 20 | >=5 |
| | | 30 | >=5 |
| | | 40 | >=5 |
| *Aspergillus niger* | 2 | 40 | 1.6 |
| | | 50 | 1.7 |
| | | 60 | 2.3 |
| | | 70 | 3 |
| | | 80 | 3.8 |
| | 5 | 40 | 2.4 |
| | | 50 | 3.2 |
| | | 60 | 3.5 |
| | | 70 | 4.3 |
| | | 80 | 4.7 |

**3.6 Efficacy limiting factors**

3.6.1 Occurrences of resistances: none reported

3.6.2 Other limiting factors: none reported

## 4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS

**4.1 Reasons for laboratory testing**

The microbicidal activity of the product was tested using three Gram positive (*Staphylococcus aureus, Mycobacterium terrae* and *Enterococcus faecium)* and two Gram negative bacterial species (*Pseudomonas aeruginosa* and *Proteus mirabilis)* as well as two fungal species *(Candida albicans* and *Aspergillus niger)*. The data obtained in this study are relevant for the intended field of use.

**4.2 Intended actual scale of biocide**

| | | | |
|---|---|---|---|
| | **application** | | |
| **4.3** | **Relevance compared to field conditions** | | |
| 4.3.1 | Application method | The test conditions of the quantitative suspension test (phase 2/step1) in the presence of organic load are representative for the actual conditions during practical use of the product. | |
| 4.3.2 | Test organism | The test organisms used in this study representing both gram-positive and gram-negative bacterial as well as fungal species are appropriate representatives for the target organisms in the intended field of use. | |
| 4.3.3 | Observed effect | The obtained efficacy result of the test product in this study using 5 different bacteria and 2 fungi under simulated use conditions in the presence of organic load is important for evaluating the biocidal activity of the product in the intended field of use. | |
| **4.4** | **Relevance for read-across** | | |
| | | **5 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **5.1** | **Materials and methods** | The microbicidal activity of propan-1-ol in water was evaluated using a generally accepted suspension test (phase 2/step1). Three gram positive (*S. aureus, E. faecium, M. terrae*), two gram negative bacterial species (*P. aeruginosa, P. mirabilis*) and two fungi (*A. niger, C. albicans*) were used as test organisms. The suspension test was carried out in the presence of organic load (0.03% bovine serum albumin) to simulate practical conditions. The test was carried out at 20°C for an exposure time of 2 and 5 min at various concentrations (20 - 80%). The reduction in viability was determined via CFU/ml counts. | x |
| **5.2** | **Reliability** | Reliability factor 1 (guideline study). Study was conducted according to an internationally accepted guideline test method. | |
| **5.3** | **Assessment of efficacy, data analysis and interpretation** | The results of this study show that 30% propan-1-ol in water tested in the presence of organic load (0.03% bovine serum albumin) and at an exposure time of >=2 min was effective (i.e. log RF >=5) against all the bacterial and the yeast species tested in the study. However, the study showed that the product was only effective against *Aspergillus niger* conidia at a higher concentration of 80% and an exposure time of 5 min thereby achieving almost a log 5 reduction. | |
| **5.4** | **Conclusion** | The tested bacterial and fungal species can be regarded as representatives for gram negative and gram positive facultative pathogenic bacteria and pathogenic fungi that could be encountered in the intended area of use of the product Using a quantitative suspension test the effectiveness of the product against such pathogenic bacteria and a yeast species was demonstrated. | x |
| **5.5** | **Proposed efficacy specification** | | |

| Evaluation by Competent Authorities | |
|---|---|
| | *Use separate "evaluation boxes" to provide transparency as to the comments and views submitted* |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 2008/10/30 |
| **Materials and methods** | The study was performed according to an old European guideline of a suspension test in the field of food hygiene. The test bacteria used include not all the bacterial test organisms prescribed in the most relevant DIN EN norm 1276. |
| **Conclusion** | Applicant's version is adopted with the exception that the test shows that the active substance propan-1-ol is effective against the organisms tested (not the product). |
| **Reliability** | 1 |
| **Acceptability** | acceptable |
| **Remarks** | The study is not suitable to proof efficacy of a hand disinfectant. Nevertheless, general conclusions concerning effectiveness of propan-1-ol against relevant target organisms in the field of use can be drawn from the study. |
| | **COMMENTS FROM ...** |
| **Date** | *Give date of comments submitted* |
| **Results and discussion** | *Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.*<br>*Discuss if deviating from view of rapporteur member state* |
| **Conclusion** | *Discuss if deviating from view of rapporteur member state* |
| **Reliability** | *Discuss if deviating from view of rapporteur member state* |
| **Acceptability** | *Discuss if deviating from view of rapporteur member state* |
| **Remarks** | |

## Section A5.3 Efficacy Data

**Annex Point IIA5.3** Microbicidal activity – bacteria and fungi in the presence of organic load

## Section A5.3 Efficacy Data
## Annex Point IIA5.3 Bactericidal activity against MSSA and MRSA

Official use only

### 1 REFERENCE

| | | |
|---|---|---|
| **1.1** | **Reference** | Kampf, G., Jarosch, R., Rüden, H. 1997. Wirksamkeit alkoholischer Händedesinfektionsmittel gegenüber Methicillin-resistenten *Staphylococcus aureus* (MRSA). Der Chirurg. 68:264-270. |
| **1.2** | **Data protection** | Not applicable |
| 1.2.1 | Data owner | |
| 1.2.2 | Criteria for data protection | |
| **1.3** | **Guideline study** | Yes, DGHM (German Society for Hygiene and Microbiology, Zbl. Bakt. Hyg. 1982, Orig. B. 172:534) guideline for the testing and evaluation of chemical disinfection methods. |
| **1.4** | **Deviations** | Yes, 2.3.4 |

### 2 METHOD

| | | |
|---|---|---|
| **2.1** | **Test Substance (Biocidal Product)** | Propan-1-ol |
| 2.1.1 | Trade name/ proposed trade name | Not applicable |
| 2.1.2 | Composition of Product tested | Propan-1-ol diluted with water of standardized hardness to 30, 40 and 60%. |
| 2.1.3 | Physical state and nature | Liquid disinfectant |
| 2.1.4 | Monitoring of active substance concentration | Not applicable. |
| 2.1.5 | Method of analysis | Not applicable |
| **2.2** | **Reference substance** | Two biocidal preparations (Sterillium and Spitaderm) were tested in parallel. |
| 2.2.1 | Method of analysis for reference substance | Not applicable |
| **2.3** | **Testing procedure** | |
| 2.3.1 | Test population / inoculum / test organism | Table 2.3.1.1 Bacterial strains employed to test the virucidal efficacy of propan-1-ol. |

| Species | Source/origin | Representative for |
|---|---|---|
| MSSA, Oxacillin sensitive strains (n=3) | | |
| *Staphylococcus aureus* | ATTC6538 | Gram positive bacteria |
| *Staphylococcus aureus* | Clinical isolate | Gram positive bacteria |
| *Staphylococcus aureus* | Clinical isolate | Gram positive bacteria |

**Section A5.3 Efficacy Data**
**Annex Point IIA5.3** Bactericidal activity against MSSA and MRSA

| Species | Source/origin | Representative for |
|---|---|---|
| MRSA, Oxacillin resistant strains (n=3) | | |
| *Staphylococcus aureus* | ATTC43300 | Gram positive bacteria |
| *Staphylococcus aureus* | Clinical isolate | Gram positive bacteria |
| *Staphylococcus aureus* | Clinical isolate | Gram positive bacteria |

MSSA strains = all 3 MecA negative

MRSA strains = all 3 MecA positive

(0.1 ml of a 24 hour culture was used for experiments)

| | | |
|---|---|---|
| 2.3.2 | Test system | Quantitative suspension test for the basic activity of the product (e.g. CEN - Phase 1) |
| 2.3.3 | Application of TS | As prescribed by guideline |
| 2.3.4 | Test conditions | 0.1ml of a 24 hour culture was added to 9.9 ml of disinfectant solution and tested according to the guideline. No organic load was used in the study and experiments were performed at room temperature. At some concentrations and exposure times in addition to the guideline procedure an additional filtration step was included (i.e. after inactivation of individual exposure assays by dilution a cellulose-nitrate filter with a pore size of 0.45µm was used and applied to a TS-plate after filtration of the inactivated suspension and incubated for 48h at 37°C). Reactions were stopped by 1:10 dilutions of samples in NaCl-Trypton solution (this was shown sufficient as 6% propan-1-ol was unable to reduce the CFUs significantly even after 1 h incubation as compared to the water only control, n=12, p=0.875, T-test). After exposure 1 ml samples were taken and diluted via 4 dilution steps, each of these 4 dilutions was used to produce duplicate TS-agar plates.<br>For the 2 biocidal preparations tested in parallel an inactivation medium consisting of Tween 80(3%), Cystein (0.1%), Histidin (0.1%9 and Saponin (3%) was used instead. |
| 2.3.5 | Duration of the test / Exposure time | 15, 30 and 60 sec |
| 2.3.6 | Number of replicates performed | as prescribed by guideline |
| 2.3.7 | Controls | Exposure assays using sterile water of standardized hardness were employed as controls. |
| **2.4** | **Examination** | |
| 2.4.1 | Effect investigated | The reduction in viable cells (MSSA strains, n=3 and MRSA strains, n=3) after exposure to propan-1-ol was investigated. |
| 2.4.2 | Method for recording / scoring of the effect | The CFUs after exposure were quantified after incubation for 48h at 37°C on TS-agar and the difference to water only controls was calculated for establishing the log RF. |

| | | |
|---|---|---|
| 2.4.3 | Intervals of examination | Reduction in viable cells was determined only once after exposure to the test substance. |
| 2.4.4 | Statistics | The T-test was applied, a *p* value of <0.05 was considered as significant. |
| 2.4.5 | Post monitoring of the test organism | Not applicable. |

**3 RESULTS**

| | | |
|---|---|---|
| **3.1** | **Efficacy** | Propan-1-ol at a concentration of 60% was most effective against both the 3 MSSA as well as the 3 MRSA strains tested. |
| 3.1.1 | Dose/Efficacy curve | Not applicable |
| 3.1.2 | Begin and duration of effects | Effects reported only for the given exposure times |
| 3.1.3 | Observed effects in the post monitoring phase | Not applicable |
| **3.2** | **Effects against organisms or objects to be protected** | None reported |
| **3.3** | **Other effects** | None reported. |
| **3.4** | **Efficay of the reference substance** | The two biocidal products tested in parallel were equally effective. |
| **3.5** | **Tabular and/or graphical presentation of the summarised results** | Table 3.5.1 Table 1: Reduction in cfu (mean log RF) after exposure to aqueous propan-1-ol (60%). |

| Species/strain | Exposure time (sec) | Concentration of test substance (%) | Log RF (mean value of 3 strains tested) |
|---|---|---|---|
| MSSA (n=3) | 15 | 30 | >4 |
| | | 40 | >5 |
| | | 60 | >6 |
| | 30 | 30 | >4 |
| | | 40 | >5 |
| | | 60 | >6 |
| | 60 | 30 | >6 |
| | | 40 | >6 |
| | | 60 | >6 |
| MRSA (n=3) | 15 | 30 | >=4 |
| | | 40 | >=4 |
| | | 60 | >5 |
| | 30 | 30 | >4 |

| | | | |
|---|---|---|---|
| | | 40 | >5 |
| | | 60 | >5 |
| | 60 | 30 | >5 |
| | | 40 | >=6 |
| | | 60 | >6 |

The MRSA type strain (ATCC43300) was less sensitive than the 2 clinical MRSA isolates and the 3 MSSA strains tested. This strain showed at 15 sec exposure at 60% a log RF of >4 as opposed to the other strains with a log RF of >5.

**3.6 Efficacy limiting factors**

3.6.1 Occurrences of resistances: 3 of the strains tested (MRSA) were resistant against methicillin /oxacillin.

3.6.2 Other limiting factors: none reported

## 4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS

**4.1 Reasons for laboratory testing**: Using the quantitative suspension test method in accordance with the DGHM guidelines the efficacy of propan-1-ol in various concentrations and with different exposure times against MSSA- and MRSA strains was evaluated. The results obtained in this study are relevant for the intended use of the test substance.

**4.2 Intended actual scale of biocide application**: not stated

**4.3 Relevance compared to field conditions**

4.3.1 Application method: The test conditions of the in-vitro suspension test method are representative for the actual conditions in the main field of use of the test substance.

4.3.2 Test organism: The test organisms employed, 3 MSSA- and 3 MRSA strains, can be considered appropriate representatives for the target organisms in the intended area of use of the biocide.

4.3.3 Observed effect: The obtained efficacy result of the test substance is relevant for determining the bactericidal activity of the product in the intended area of use.

**4.4 Relevance for read-across**

## 5 APPLICANT'S SUMMARY AND CONCLUSION

**5.1 Materials and methods**: A suspension test was carried out in accordance with the DGHM guidelines for testing the effectiveness of chemical disinfection methods. The test substance was propan-1-ol in various dilutions and its effectiveness upon the viability of *Staphylococcus aureus* strains of clinical relevance. 6 Strains were exposed to the alcohol at 3 different concentrations for 15, 30 and 60sec. At the end of the exposure time,

**Section A5.3 Efficacy Data**
**Annex Point IIA5.3** Bactericidal activity against MSSA and MRSA

| | | | |
|---|---|---|---|
| | | the action of the alcohol in an aliquot of the test mixture was stopped by serial dilutions. 0.1ml of each dilution was transferred in duplicate to TS-agar plates. After incubation the CFUs were estimated. The RF is calculated by subtracting the log cfu of inactivated bacterial suspensions from that of the control. | |
| **5.2** | **Reliability** | Reliability factor 1. (Guideline study) | |
| **5.3** | **Assessment of efficacy, data analysis and interpretation** | Propan-1-ol was most effective (average log RF > 5) against all the 6 tested strains of *Staphylococcus aureus* at 60% and an exposure time of 60 sec. | |
| **5.4** | **Conclusion** | At a concentration of 60 % propan-1-ol was sufficiently effective (RF>=5) against all the 6 tested strains at >=30 sec exposure time. The quantitative suspension test used in this study is a sufficient method for detecting the basic bactericidal activities of disinfectants. | x |
| **5.5** | **Proposed efficacy specification** | | |

| **Evaluation by Competent Authorities** | |
|---|---|
| | *Use separate "evaluation boxes" to provide transparency as to the comments and views submitted* |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 2008/10/31 |
| **Materials and methods** | - |
| **Conclusion** | This conclusion can not be drawn from the data because only mean reduction factors of all 3 MSSA or MRSA strains, respectively, are given for the exposure time 30 sec. The data of the single strains are only given for the time 15 s. Therefore two conclusions can be drawn from the study: At a concentration of 60 % propan-1-ol was sufficiently effective (RF>=5) against 5 of 6 tested strains (except for ATCC 43300) at > 15 sec exposure time. It also can be concluded that, on the basis of the mean values for the MSSA and MRSA strains, at a concentration of 60 % propan-1-ol was sufficiently effective (RF>=5) in the test at >=30 sec exposure time.<br>A general bactericidal activity can not be concluded from this study because only *Staphylococcus aureus* strains were tested. |
| **Reliability** | - |
| **Acceptability** | acceptable |
| **Remarks** | - The tests were not performed under the conditions of organic load as required by the guideline.<br>- In the study report, only mean values are given, no raw data as prescribed in the guideline. |
| | **COMMENTS FROM ...** |
| **Date** | *Give date of comments submitted* |

## Section A5.3 Efficacy Data
## Annex Point IIA5.3
Bactericidal activity against MSSA and MRSA

| | |
|---|---|
| **Results and discussion** | *Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.*<br>*Discuss if deviating from view of rapporteur member state* |
| **Conclusion** | *Discuss if deviating from view of rapporteur member state* |
| **Reliability** | *Discuss if deviating from view of rapporteur member state* |
| **Acceptability** | *Discuss if deviating from view of rapporteur member state* |
| **Remarks** | |

## Section A5.3 Efficacy Data
## Annex Point IIA5.3 Non enveloped virus

Official use only

### 1 REFERENCE

| | | |
|---|---|---|
| **1.1** | **Reference** | Gehrke C., Steinmann, J., Goroncy-Bermes, P. 2004. Inactivation of Feline Calicivirus, a surrogate of norovirus (formerly Norwalk-like viruses), by different types of alcohol in vitro and in vivo. Journal of Hospital Infection 56:49-55. |
| **1.2** | **Data protection** | Not stated |
| 1.2.1 | Data owner | |
| 1.2.2 | Criteria for data protection | |
| **1.3** | **Guideline study** | Yes, Guidelines of the German Federal Health Office and the German Association for the Control of Virus Diseases for testing the effectiveness of chemical disinfectants against viruses. Zbl. Hyg. 1990, 189:554-562. |
| **1.4** | **Deviations** | yes, see 2.3.4 |

### 2 METHOD

| | | |
|---|---|---|
| **2.1** | **Test Substance (Biocidal Product)** | Propan-1-ol |
| 2.1.1 | Trade name/ proposed trade name | Not applicable |
| 2.1.2 | Composition of Product tested | Propan-1-ol diluted with double-distilled water to 50, 70 and 80%. |
| 2.1.3 | Physical state and nature | Liquid disinfectant |
| 2.1.4 | Monitoring of active substance concentration | Not applicable. |
| 2.1.5 | Method of analysis | Not applicable |
| **2.2** | **Reference substance** | Ethanol and propan-2-ol were tested in parallel at similar concentrations. |
| 2.2.1 | Method of analysis for reference substance | Not applicable |
| **2.3** | **Testing procedure** | |
| 2.3.1 | Test population / inoculum / test organism | Table 2.3.1.1 Virus strain employed to test the virucidal efficacy of propan-1-ol. |

| Species | Source/origin | Representative for |
|---|---|---|
| Feline Calicivirus strain F9 | Prof. H. Schirrmeier, Bundesforschungsanstalt für Viruskrankheiten der Tiere, Germany | Non enveloped virus |

The virus strain was cultivated in KE-R-cells, a fibroblastoid cell line derived from a whole cat embryo. The KE-R cells were grown with Eagle`s minimum essential medium and 10% fetal calf serum. After a

| | | |
|---|---|---|
| | | cytopathic effect had developed in the cell culture, the virus was harvested by freeze-thawing three times followed by centrifugation to remove cell debris. |
| 2.3.2 | Test system | Quantitative suspension test for the basic activity of the product (e.g. CEN - Phase 1) |
| 2.3.3 | Application of TS | As prescribed by guideline (concentrations tested: 50, 70 and 80%) |
| 2.3.4 | Test conditions | As prescribed by guideline but FCV was used as virus strain in the study and no organic load was used in the test. Test performed at Room temperature, exposure stopped by serial dilution in EMEM Media, KE-R cells to detect cytopathic effect incubated at 37°C |
| 2.3.5 | Duration of the test / Exposure time | 30s, 1, 3 and 5min |
| 2.3.6 | Number of replicates performed | as prescribed by guideline |
| 2.3.7 | Controls | as prescribed by guideline |
| **2.4** | **Examination** | |
| 2.4.1 | Effect investigated | The reduction in virus titre of Feline calicivirus strain F9 after exposure to propan-1-ol at 3 concentrations was investigated. |
| 2.4.2 | Method for recording / scoring of the effect | The viral cytopathic effect on KE-R cells was examined using an inverted microscope |
| 2.4.3 | Intervals of examination | Reduction in viral infectivity was determined only once after exposure to the test substance |
| 2.4.4 | Statistics | as prescribed by guideline |
| 2.4.5 | Post monitoring of the test organism | Not applicable. |
| | | **3 RESULTS** |
| **3.1** | **Efficacy** | Propan-1-ol was effective (RF>=4) at a concentration of 50 and 70% at an exposure time of >= 0.5 min. |
| 3.1.1 | Dose/Efficacy curve | Not applicable |
| 3.1.2 | Begin and duration of effects | Effect was only reported for the given exposure times |
| 3.1.3 | Observed effects in the post monitoring phase | Not applicable |
| **3.2** | **Effects against organisms or objects to be protected** | None reported |
| **3.3** | **Other effects** | None reported. |
| **3.4** | **Efficay of the reference substance** | Propan-2-ol was effective (RF>=4) at a concentration of 50% at an exposure time of >= 3 min. |

**Section A5.3 Efficacy Data**
**Annex Point IIA5.3** Non enveloped virus

**3.5 Tabular and/or graphical presentation of the summarised results**

Table 3.5.1 Reduction in virus titre (RF log ID50) after exposure to aqueous propan-1-ol solutions.

| Species/ strain | Propan-1-ol (%) | Exposure time (min) | Reduction of virus titre (log ID50) |
|---|---|---|---|
| Feline Calicivirus F9 | 50 | 0.5 | >=4.13 |
| | | 1 | >=4.31 |
| | | 3 | >=5.13 |
| | | 5 | >=4.73 |
| | 70 | 0.5 | >=4.06 |
| | | 1 | >=4.06 |
| | | 3 | >=4.13 |
| | | 5 | >=4.13 |
| | 80 | 0.5 | 1.9 |
| | | 1 | >=3.58 |
| | | 3 | >=4.13 |
| | | 5 | >=3.98 |

**3.6 Efficacy limiting factors**

3.6.1 Occurrences of resistances: none reported

3.6.2 Other limiting factors: none reported

## 4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS

**4.1 Reasons for laboratory testing**: Using the suspension test method in accordance with the guidelines issued by the German Federal Health Office and the German Association for The Control of Virus Diseases, the efficacy of propan-1-ol in various concentrations against Feline calicivirus, a surrogate for norovirus could be tested. The results obtained in this study are relevant for the intended use of the test substance. x

**4.2 Intended actual scale of biocide application**: not stated

**4.3 Relevance compared to field conditions**

4.3.1 Application method: The test conditions of the in-vitro suspension test method are representative for the actual conditions in the main field of use of the test substance.

4.3.2 Test organism: The test organism, Feline calicivirus is a surrogate for norovirus and can be considered an ideal representative for the target organisms in the x

| | | | |
|---|---|---|---|
| | | intended area of use of the biocide. | |
| 4.3.3 | Observed effect | The obtained efficacy result of the test substance is relevant for determining the virucidal activity of the product in the intended area of use. | |
| **4.4** | **Relevance for read-across** | | |
| | | **5 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **5.1** | **Materials and methods** | A suspension test was carried out in accordance with the guidelines of the German Federal Health Office and the German Association for the Control of Virus Diseases for testing the effectiveness of chemical disinfectants against viruses. The test substance was propan-1-ol in various dilutions and the effect it has on feline calicivirus (a non enveloped virus) was determined. The test was carried out in the absence of organic load and thereby deviating from the guideline. The virus was exposed to the alcohol for 0.5, 1, 3 and 5 min. At the end of the exposure, the action of the alcohol in an aliquot of the test mixture was stopped by serial dilutions (1:10) in EMEM. 0.1ml of each dilution was transferred into wells of a microtitre plate containing a confluent monolayer of KE-R cells. After incubation the viral cytopathic effect was examined using an inverted microscope. The titre reduction is calculated by subtracting the logarithmic titres of the inactivated virus suspension from that of the virus control. | |
| **5.2** | **Reliability** | Reliability factor:1. (Guideline study) | |
| **5.3** | **Assessment of efficacy, data analysis and interpretation** | Propan-1-ol was effective against the tested virus strain at 50% and 70% at an exposure time of >= 0.5 min by achieving a log10 reduction of > 4 in virus titre. | |
| **5.4** | **Conclusion** | At a concentration of 50 and 70% propan-1-ol was effective (RF>=4) against the tested virus strain at >= 0.5 min exposure time. The quantitative suspension test used in this study is a sufficient method for detecting the virucidal activities of disinfectants. | x |
| **5.5** | **Proposed efficacy specification** | | |

| Evaluation by Competent Authorities | |
|---|---|
| | *Use separate "evaluation boxes" to provide transparency as to the comments and views submitted* |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 2008/09/24 |
| **Materials and methods** | Additional to the in vitro study described in the study summary, an in vivo study was performed on artificially FCV-contaminated fingertips of adult panellists according to the standard test method ASTM E-1838-96. |
| **Conclusion** | At a concentration of 50 and 70% propan-1-ol was effective (RF>=4) against the tested virus strain FCV at >= 0.5 min exposure time. A general virucidal activity of propan-1-ol can not be deduced from the study. In order to obtain the general label claim "virucidal", at least the non enveloped viruses poliovirus and adenovirus have to be tested. FCV is not recommended by the guideline used and also not in the appropriate European guidelines. Additionally, it is much more sensitive than the recommended test viruses and therefore not a suitable representative for the determination of the general virucidal activities of disinfectants. Therefore, the study could not be used to support a label claim "virucidal", only "activity against feline calicivirus". |
| **Reliability** | 2 |
| **Acceptability** | acceptable with restrictions (see remarks) |
| **Remarks** | 4.3.2: Feline calicivirus is not considered as surrogate virus for noroviruses. Additionally, they are not as stable as the test viruses according to the recommended guidelines. Therefore, it could not be considered as a suitable representative for the target organisms in the intended area of use of the biocide.<br>The test was not performed under "dirty conditions" (no organic load) as prescribed in the guideline.<br>In the in vivo experiment, a $\log_{10}$ reduction of 3.58 was observed for 70% 1-propanol after an exposure time of 30s on fingertips (number of fingertips: 16). |
| | **COMMENTS FROM ...** |
| **Date** | *Give date of comments submitted* |
| **Results and discussion** | *Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.*<br>*Discuss if deviating from view of rapporteur member state* |
| **Conclusion** | *Discuss if deviating from view of rapporteur member state* |
| **Reliability** | *Discuss if deviating from view of rapporteur member state* |
| **Acceptability** | *Discuss if deviating from view of rapporteur member state* |
| **Remarks** | |

## Section A5.3 Efficacy Data
## Annex Point IIA5.3 3 different non enveloped viruses

Official use only

### 1 REFERENCE

| | | |
|---|---|---|
| **1.1** | **Reference** | Kurtz J.B, Lee, T.W., Parsons, A.J. 1980. The action of alcohol on rotavirus, astrovirus and enterovirus. J. Hosp. Inf. 1:321-325. |
| **1.2** | **Data protection** | Not applicable |
| 1.2.1 | Data owner | |
| 1.2.2 | Criteria for data protection | |
| **1.3** | **Guideline study** | No. |
| **1.4** | **Deviations** | Not applicable |

### 2 METHOD

| | | |
|---|---|---|
| **2.1** | **Test Substance (Biocidal Product)** | Propan-1-ol |
| 2.1.1 | Trade name/ proposed trade name | Not applicable |
| 2.1.2 | Composition of Product tested | Propan-1-ol diluted to various concentrations (20-90%). |
| 2.1.3 | Physical state and nature | Liquid disinfectant |
| 2.1.4 | Monitoring of active substance concentration | Not applicable. |
| 2.1.5 | Method of analysis | Not applicable |
| **2.2** | **Reference substance** | Ethanol, methanol, propan-2-ol and butan-2-ol were tested in parallel at similar concentrations. |
| 2.2.1 | Method of analysis for reference substance | Not applicable |
| **2.3** | **Testing procedure** | |
| 2.3.1 | Test population / inoculum / test organism | Table 2.3.1.1 Virus strains employed to test the virucidal efficacy of propan-1-ol. |

| Species | Source/origin | Representative for |
|---|---|---|
| Bovine Rota virus | Dr. Bridger, ARC, Compton, Berkshire, UK | Non enveloped virus |
| Astrovirus | Human faecal extract (10%), not further specified | Non enveloped virus |
| Echovirus 11 | Not specified | Non enveloped virus |

Rotavirus=The virus strain was cultivated in LLCMK2-cells and stored at -70°C.

**Section A5.3 Annex Point IIA5.3** **Efficacy Data**
3 different non enveloped viruses

| | | | |
|---|---|---|---|
| | | Astrovirus= The human faecal extract 810%) was used directly as inoculum.<br>Echovirus= The virus strain was cultivated in human embryo lung fibroblasts and stored at -70°C. | x |
| 2.3.2 | Test system | Quantitative suspension test under conditions representative of practical use (e.g. CEN – Phase 2, Step 1) | |
| 2.3.3 | Application of TS | The test product was applied in aqueous solution | |
| 2.3.4 | Test conditions | Rotavirus<br>The test virus was exposed to the biocide for 1min (0.3 vol virus suspension plus 0.7 vol of biocide dilution to give the appropriate final concentrations, as control 0.7 vol of PBS was added instead) and the virucidal effect of the alcohol was stopped after exposure by addition of 2ml of serum free 199T medium followed by an immediate additional 10 fold dilution in 199T (in controls the 199T used for dilution contained 2.5% of alcohol to account for residual amounts present at this stage). Virus infectivity was estimated using LLCMK2 cells by centrifugation of samples of above dilutions added to cover slips, addition of 199T medium, incubation at 37°C for 18-24h, fixation with acetone, treatment of cover slips with bovine anti rota virus serum followed by addition of fluorescein labelled rabbit anti bovine globulin and analysis of these samples by fluorescence microscopy. The impact of organic load was tested by using equal volumes of virus and sterilized faeces.<br>Astrovirus<br>The test virus was exposed to the biocide for 1min (0.1 vol human faecal extract (10%) plus 0.9 vol of biocide dilution to give the appropriate final concentrations, as control similar vol of PBS was added instead) and the virucidal effect of the alcohol was stopped after exposure by addition of 19 ml of 199S medium followed by an immediate additional 10 fold dilution in 199S (in controls the 199S used for dilution contained 4.5% of alcohol to account for residual amounts present at this stage). Virus infectivity was estimated using 1 ml of dilutions added to monolayers of human embryo kidney cells on cover slips, which was removed and replaced after 1 h by 199S, incubation at 37°C for 18-24h, fixation with acetone, treatment of cover slips with human anti astrovirus serum followed by addition of fluorescein labelled rabbit anti human globulin and analysis of these samples by fluorescence microscopy. The impact of organic load was tested by using equal volumes of virus and sterilized faeces.<br>Echovirus<br>0.05 ml virus suspension and 0.05 calf serum added to 0.4ml of biocide dilution (alcohol concentration in the reaction mixtures was 4/5 of the initial concentration) followed after 1 min by addition of 4.5 ml skim milk (17.5g/100ml) to stop exposure followed by immediate additional 2 and 10 fold dilutions in Eagles MEM (with 2% calf serum added). Virus infectivity was estimated using 1 ml of these dilutions inoculated into monolayers of HEL cells which were checked for up to 5 day for cytopathic effects. Controls used 0.1 virus –serum mixture were added to 0.4 ml PBS and skim milk (containing 9.5% of the alcohol) added after 1 min. | |
| 2.3.5 | Duration of the test / Exposure time | 1 min | |
| 2.3.6 | Number of | 4 experiments | |

| | | |
|---|---|---|
| | replicates performed | |
| 2.3.7 | Controls | Viruses were exposed to phosphate buffered saline (PBS) instead of the test substance. |
| **2.4** | **Examination** | |
| 2.4.1 | Effect investigated | The reduction in infective virus units/ml of 3 tested viruses after exposure to propan-1-ol at different concentrations was investigated. |
| 2.4.2 | Method for recording / scoring of the effect | The presence of viral units or appearance of viral cytopathic effect on host cells was examined by microscopy and compared to controls. |
| 2.4.3 | Intervals of examination | Reduction in viral infectivity was determined only once after exposure to the test substance |
| 2.4.4 | Statistics | Reported data are the means of 4 experiments |
| 2.4.5 | Post monitoring of the test organism | Not applicable. |

**3 RESULTS**

| | | |
|---|---|---|
| **3.1** | **Efficacy** | Propan-1-ol was able to reduce the amount of infective units/ml in the case of the bovine rota virus but failed to inactivate the astro and echo virus at 1 min exposure. |
| 3.1.1 | Dose/Efficacy curve | Not applicable |
| 3.1.2 | Begin and duration of effects | Effect was only reported for the given exposure time |
| 3.1.3 | Observed effects in the post monitoring phase | Not applicable |
| **3.2** | **Effects against organisms or objects to be protected** | None reported |
| **3.3** | **Other effects** | None reported. |
| **3.4** | **Efficay of the reference substance** | Ethanol at high concentrations was effective against astro and echo virus. |
| **3.5** | **Tabular and/or graphical presentation of the summarised results** | Table 3.5.1 Log infective virus units before and after 1 min exposure to aqueous propan-1-ol solutions. |

| Species/ strain | Propan-1-ol (%) | Organic load present | Virus titre in control (log infective units/ml) | Virus titre after 1 min exposure (log infective units/ml) |
|---|---|---|---|---|
| Bovine rota virus | 20 | No | 5.9 | 2.9 |
| | 30 | No | 5.9 | 2.2 |
| | | Yes | 5.2 | 1.9 |

| | | | | |
|---|---|---|---|---|
| | 40 | No | 5.9 | <1.9 |
| | 50 | Yes | 5.2 | <1.9 |
| Astro virus | 90 | Yes | 5.3 | 5.3 |
| Echo virus | 60 | Yes | 6.3 | 6.3 |
| | 76 | Yes | 6.3 | 6.3 |

x

**3.6 Efficacy limiting factors**

3.6.1 Occurrences of resistances: none reported

3.6.2 Other limiting factors: none reported

## 4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS

**4.1 Reasons for laboratory testing**: Using a quantitative suspension test method the efficacy of propan-1-ol in various concentrations against 3different non enveloped viruses could be tested. The results obtained in this study are relevant for the intended use of the test substance.

**4.2 Intended actual scale of biocide application**: not stated

**4.3 Relevance compared to field conditions**

4.3.1 Application method: The test conditions of the quantiative suspension test method in the presence of organic load are representative for the actual conditions in the main field of use of the test substance.

4.3.2 Test organism: The test organisms can be considered appropriate representatives for the target organisms in the intended area of use of the biocide.

4.3.3 Observed effect: The obtained efficacy results for the test substance are relevant for determining the virucidal activity of the product in the intended area of use.

**4.4 Relevance for read-across**

## 5 APPLICANT'S SUMMARY AND CONCLUSION

**5.1 Materials and methods**: A suspension test was carried out in accordance with established lab procedures for testing the effectiveness of chemical disinfectants against viruses. The test substance was propan-1-ol in various dilutions and the effect it has on 3 non enveloped viruses was determined. The test was carried out in the presence of organic load and the viruses were exposed to the alcohol for 1 min. After incubation the viral infective units/ml were quantified and compared to those of controls.

**5.2 Reliability**: Reliability factor 2. Study meets generally accepted scientific principles.

**5.3 Assessment of**: Propan-1-ol was effective against 1 (bovine rota virus) of the 3 viruses

**Section A5.3 Efficacy Data**
**Annex Point IIA5.3** 3 different non enveloped viruses

| | | | |
|---|---|---|---|
| | **efficacy, data analysis and interpretation** | tested at >=30% at an exposure time of 1 min by achieving a log10 reduction in infective units/ml of > 3 even in the presence of organic load. Neither the astro nor the echo virus were inactivated in the presence of organic load. | x |
| **5.4** | **Conclusion** | At a concentration of >=30 propan-1-ol could inactivate (RF>3) the tested bovine rota virus strain at 1 min exposure. However, propan-1-ol was unable to inactivate the other 2 viruses tested. The quantitative suspension test used in this study is a useful method for detecting the virucidal activities of disinfectants by simulating practical conditions. | x |
| **5.5** | **Proposed efficacy specification** | | |

| **Evaluation by Competent Authorities** | |
|---|---|
| | *Use separate "evaluation boxes" to provide transparency as to the comments and views submitted* |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 2008/10/07 |
| **Materials and methods** | 2.3.1: Astrovirus= The human faecal extract (10%) was used directly as inoculum. |
| **Conclusion** | 5.4 Applicant's version is adopted with the exception that propan-1-ol was effective against bovine rota virus at **>=40% not at >=30%** at an exposure time of 1 min.<br>Since bovine rota virus is not recommended by guidelines to test the virucidal effectiveness it is not representative for detecting the general virucidal activities of disinfectants under practical conditions. A general virucidal activity of propan-1-ol can not be deduced from the study. In order to obtain the general label claim "virucidal", at least the non enveloped viruses poliovirus and adenovirus have to be tested. Bovine rotavirus is not recommended by the guideline used and also not in the appropriate European guidelines. Additionally, propan-1-ol was not effective against the other two tested viruses. Therefore, the study could not be used to support a label claim "virucidal", only "activity against bovine rotavirus". |
| **Reliability** | 2 |
| **Acceptability** | acceptable |
| **Remarks** | 3.5: In the case of astrovirus and echovirus no organic load was added during testing of propan-1-ol.<br>5.3: Propan-1-ol was effective against 1 (bovine rotavirus) of the 3 viruses tested at **>=40% not at >=30%** at an exposure time of 1 min because a reduction factor >=4 is required to prove effectiveness.<br>The study is not suitable to proof efficacy of a hand disinfectant. Nevertheless, general conclusions concerning effectiveness of propan-1-ol against relevant target organisms in the field of use can be drawn from the study. |
| | **COMMENTS FROM ...** |
| **Date** | *Give date of comments submitted* |
| **Results and discussion** | *Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*<br>*Discuss if deviating from view of rapporteur member state* |

## Section A5.3 Efficacy Data
## Annex Point IIA5.3
3 different non enveloped viruses

| | |
|---|---|
| **Conclusion** | *Discuss if deviating from view of rapporteur member state* |
| **Reliability** | *Discuss if deviating from view of rapporteur member state* |
| **Acceptability** | *Discuss if deviating from view of rapporteur member state* |
| **Remarks** | |