Competent Authority Report

Programme for Inclusion of Active Substances in Annex I to Council Directive 98/8/EC

Amines, N-C10–C16-alkyltrimethylenedi-, reaction products with chloroacetic acid; Ampholyt (PT 2, 3, 4)

CAS-No. 139734-65-9

DOCUMENT IIIA (A5)

Evaluation Report

Rapporteur: Ireland

April 2015

Ampholyt (PT2, 3, 4)

Document A5

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Section A5 Effectiveness against target organisms and intended uses

Annex Point IIA5

		Official use only
Function (IIA5.1)	**Reference A5.1/01:** Anonymous (2000): Ampholyt 20 – Function (Benefit/Usefullness) Goldschmidt GmbH, Essen, February, 28th, 2000 (unpublished). Disinfectant: PT 2, 3, 4 bactericide, fungicide, limited virucide	
Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)		
Organism(s) to be controlled	Bacteria, fungi, viruses contaminating surfaces, floors, walls or other working areas.	
Products, organisms or objects to be protected	Humans and animals at risk of exposure to germs (communicable diseases) in public areas (e.g. hospitals, spas, swimming baths, etc.; product type 2), animal housings or transport means (product type 3), and in the food processing industry (product type 4). The treated objects are thus pre-cleaned walls, floors, work surfaces, conveyors, pipelines and equipment in the above areas, as appropriate.	
Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)		
Effects on target organisms	Ampholyt 20 is an amphotheric surfactant, reducing the number of viable bacterial or fungal cells and reproductive viruses.	
Likely concentrations at which the A.S. will be used	Aqueous solutions of 0.5–1% Ampholyt 20 (=TEGO 2000), corresponding to 0.1–0.2 % a.i. final concentration.	

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Mode of action (including time delay) (IIA5.4)	**Reference A5.4/01:** Anonymous (undated): Mechanism of Killing Microorganisms. Goldschmidt GmbH, Essen, undated (unpublished).
Mode of action	Ampholyt 20 is an amphotheric surfactant, reducing the number of viable bacterial or fungal cells and reproductive viruses by alteration of the electrochemical charge while integrating/penetrating into the cell/ viral envelope. This results in changes in permeability and irreversible alteration of the structure of the cellular membrane or viral envelope, respectively. The reference elucidates the mode of action of amphoterics in general with an amphoteric disinfectant termed TEGO 51. The adverse effects of amphoterics described in the study, relay on the active amphoteric substances, which justifies the read-across of effects from TEGO 51 to Ampholyt 20. Further, the composition of the amphoteric disinfectant product TEGO 51 is a mixture of Ampholyt 20, plus additional other components, being similar in molecular structure.
Time delay	No time delay, since the microbicidal effect is immediate. However, a certain residence time needs to be allowed for, in order to ensure sufficient reduction of germ numbers. Residence times are given as 15 to 30 min and up to 2 h depending on kind of contamination (target) and level of pollution of the area. The residence time for bacteria and fungi (1% TEGO 2000) is determined to be 30 minutes for dirty conditions at room temperature, 60 minutes at 10 °C. The mandatory residence time for the requested efficacy against viruses depends as well on the target viruses, for example for HBV, 1% of TEGO 2000 requires 60 min, for the reduction of Herpes simplex virus contamination 15 minutes with 0.75% Ampholyt 20 is sufficient. For more details please refer to Section B5.10.2.
Field of use envisaged (IIA5.5)	
MG01: Disinfectants, general biocidal products	PT 02: Private area and public health area disinfectants and other biocidal products PT 03: Veterinary hygiene biocidal products PT 04: Food and feed area disinfectants
MG02: Preservatives	–
MG03: Pest control	–
MG04: Other biocidal products	–
Further specifications	–
User (IIA5.6)	Users of the disinfectant are (i) professional cleaners and (ii) professionals of medical health, foodstuff manufacturing industry, or other industry that needs disinfection of surfaces.

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Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA5.7)		
Development of resistance	Specific resistance to Ampholyt 20 has not become known to date. Resistance to amphoteric disinfectants is not expected due to the relatively unspecific mode of action of amphoterics, which is at least partly based on surface activity. Amphoteric surfactants integrate into the cell wall of bacteria (or the envelope of viruses) and thereby cause leakage of intracellular components in bacteria. Furthermore, the intended uses, including performance of mechanical cleaning procedures hinder the formation of biofilms, thereby additionally reducing the likelihood of development of resistance. Having said that, bacteria or other micro-organisms may generally have an intrinsic or natural capacity of developing resistance to basically any antimicrobial agent. Resistance may in principle also be acquired by adaptation. Resistance may be mediated by resistance genes, which insert in specific sequences or by acquisition of plasmids or transposons, encoding a mechanism to disable a specific antimicrobial action. Microbial resistance to antimicrobial agents – or more generally biocides – is favoured by frequent use of sublethal concentrations and misuse of the agents which imposes a selective pressure. However, since the mode of action of amphoteric surfactants is relatively unspecific, including (as the name implies) surface activity, selection for specific resistance genes is hardly conceivable. Instead, because of the multiply charged character of the molecule, amphotheric agents effectively bind to cellular or viral surfaces, and disrupt the barrier that ensures impermeability. The interaction with membrane components may further disorganise signalling. These effects lead to the very effective decrease in cell viability and viral infectivity. In conclusion, it is therefore considered unlikely that bacteria or viruses develop resistance against Ampholyt 20. Recent literature searches have not revealed any information indicating that resistance to Ampholyt 20 may have occurred. As a general rule, careful working practice, comprising complete and thorough cleaning of the surfaces and objects to be disinfected, may be considered as a suitable means of preventing development of resistance.	
Management strategies	Not applicable, in view of the fact that resistance has to date not been observed. However, as a general strategy, sufficiently efficient concentrations should be applied and the residence times should be considered. In other words: Adherence to the manufacturer's use instructions may be considered as a suitable management strategy.	

Section A5 Annex Point IIA5	Effectiveness against target organisms and intended uses	
		Official use only
Likely tonnage to be placed on the market per year (IIA5.8)	Data on produced/ imported tonnages are considered to be commercially sensitive and are therefore to be treated as CONFIDENTIAL. These data are provided separately in Appendix 1 to Document III-A (confidential information).	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	15th July 2013	
Materials and Methods	Adopt mode of action	
Results and discussion	N/A	
Conclusion	N/A	
Reliability	N/A	
Acceptability	Acceptable	
Remarks	None	
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Section A5.10.

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
1.0, 0.5 and 0.125 % (v/v) of Ampholyt 20	*Pseudomonas aeruginosa, Staphylococcus aureus*	Dilution-neutralization method DIN EN 1040	Temperature: 20 ± 1°C Contact time: 5 min ± 10 sec	A bacterial viability reduction of more than 10^5 was achieved in both organisms with all of the three concentrations.	B5.10.2/01
0.125, 0.25, and 0.5 % (v/v) of Ampholyt 20	*Staphylococcus aureus Enterococcus hirae Escherichia coli Pseudomonas aeruginosa*	Quantitative suspension test DIN EN 1276	Temperature: 20 ± 1°C Contact time: 5 min ± 10 sec Interfering substance: 0.3 g/L bovine albumin	A bacterial viability reduction of more than 10^5 was achieved in all organisms with all of the three concentrations under clean conditions (0.3 g/L bovine albumin).	B5.10.2/02
0.25, 0.5, and 1.0 % (v/v) of Ampholyt 20	*Staphylococcus aureus Enterococcus hirae Escherichia coli Pseudomonas aeruginosa*	Quantitative suspension test DIN EN 1276	Temperature: 20 ± 1°C Contact time: 5 min ± 10 sec Interfering substance: 0.3 g/L bovine albumin	A bacterial viability reduction of more than 10^5 was achieved in all organisms with all of the three concentrations under clean conditions (0.3 g/L bovine albumin).	B5.10.2/03

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
Aspergillus niger: 10, 5 and 2.5 % of Ampholyt 20 *Candida albicans*: 1.0, 0.5 and 0.25 % of Ampholyt 20	*Candida albicans* *Aspergillus niger*	Quantitative suspension test (membrane filtration method) DIN EN 1650	Temperature: 20 ± 1°C Contact time: 15 min exposure time (*C. albicans*, *A. niger*) 60 min exposure time (*A. niger*) Interfering substance: 0.3 g/L bovine albumin	*C. albicans* viability was reduced within 15 min treatment for more than log 4 even with concentrations of 0.25% Ampholyt 20. *A. niger* was not affected sufficiently by Ampholyt 20 in concentrations up to 10% for 60 min (less than log 4).	B5.10.2/04
0.75% Ampholyt 20	Vaccinia virus strain Elstree	Effectiveness against viruses (Federal Office of Health and the German Association for the Control of Virus Diseases)	Temperature: 20 ± 1°C Contact time: 5, 10 and 15 min Interfering substance: 0.2 % BSA or 10.0 % BSA	A titre reduction of > 4 $\log_{10}$ was achieved in all 3 test samples (without, with 0.2% BSA, with 10% FCS) independent of exposure times (5, 10 and 15 min).	B5.10.2/05
0.75% Ampholyt 20	Bovine viral diarrhea virus (surrogate of HCV)	Infectivity was determined by means of end point dilution titration in a micro-procedure. The difference of test titre with the control is given as reduction factor (RF) or Δ log ID_{50}. The infectious dose (ID_{50}) was calculated according to the method of Spearman (1908) and Kärber (1931).	Temperature: 20 ± 1°C Contact time: 5, 10 and 15 min Interfering substance: without, 0.2 % BSA, 10 % FCS	A titre reduction of > 4 $\log_{10}$ was achieved in all 3 test samples (without, with 0.2% BSA, with 10% FCS) after 15 min exposure time	B5.10.2/06

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
0.75% Ampholyt 20	Herpes simplex virus, type 1	Infectivity was determined by means of end point dilution titration in a micro-procedure. The difference of test titre with the control is given as reduction factor (RF) or Δ log ID50. The infectious dose (ID50) was calculated according to the method of Spearman (1908) and Kärber (1931). (Quantitative suspension test method, Vero cell cultures were observed for cytopathic effects)	Temperature: 20 ± 1°C Contact time: 5, 10 and 15 min Interfering substance: without, 0.2 % BSA, 10 % FCS	Efficacy of TEGO 2000 (0.75%) on herpes simplex virus is measured by reduction of virucidal effect on Vero cells. The reduction of recommended RF ≥4.00 is achieved after 5 min with or without interfering substances.	B5.10.2/07
0.75% Ampholyt 20	Bovine coronavirus	Infectivity was determined by means of end point dilution titration in a micro-procedure. The difference of test titre with the control is given as reduction factor (RF) or Δ log ID_{50}. The infectious dose (ID_{50}) was calculated according to the method of Spearman (1908) and Kärber (1931).	Temperature: 20 ± 1°C Contact time: 5, 10 and 15 min Interfering substance: without, 0.2 % BSA, 10 % FCS	A titre reduction of ≥ 4 log_{10} was achieved in all test samples (without, with 0.2% BSA, with 10% FCS) already after 5 minutes of exposure.	B5.10.2/08
1.0, 2.0 % Ampholyt 20	Hepatitis B Virus (HBV)	Determination of the modification /destruction of envelope protein HBsAg of HBV by Ampholyt 20	exposure time: 30, 60, and 120 minutes interfering substance: without, 0.2 % BSA, 10 % FCS	<u>Effect of 1% TEGO 2000:</u> After 30 min incubation with 1% TEGO 2000, the HBsAg present in the test system was not detectable any more in the assay without interfering substance (indicating deactivation of HBV). After 60 min HBsAg was also destroyed completely in the assay containing a medium protein concentration, and after 120 min in the assay containing a high protein concentration. <u>Effect of 2% TEGO 2000:</u> After 30 minutes incubation with 2% TEGO 2000 HBsAg was not detectable any more, even in the assay with high protein concentration.	B5.10.2/09

<table>
<tr><th>Test substance</th><th>Test organism(s)</th><th>Test method</th><th>Test conditions</th><th>Test results: effects, mode of action, resistance</th><th>Reference</th></tr>
<tr><td>Serial dilution of Ampholyt 20</td><td>Staphylococcus aureus
Streptococcus faecium
Proteus mirabilis
Pseudomonas aeruginosa
Candida albicans</td><td>B5.10.2/10 is a summary report on B5.10.2/11 to B5.10.2/14
Qualitative and quantitative suspension test method, Guidelines for testing chemical disinfectants for veterinary medicine (1984) by the Committee “Disinfection in Veterinary Medicine” of the German Veterinary Medical Association e.V. (DVG)</td><td>B5.10.2/10 is a summary report on B5.10.2/11 to B5.10.2/14
Temperature: 10 or 20 °C
Exposure time: 5, 15, 30 and 60 min
Interfering substance: without, or 10 % bovine serum, or 1% skimmed milk</td><td>
<table>
<tr><td rowspan="3">AB</td><td rowspan="3">°C</td><td colspan="8">indicated are the use concentrations in % v/v</td></tr>
<tr><td colspan="4">slightly soiled area</td><td colspan="4">highly soiled area</td></tr>
<tr><td colspan="2">bactericidal</td><td colspan="2">fungicidal</td><td colspan="2">bactericidal</td><td colspan="2">fungicidal</td></tr>
<tr><td></td><td></td><td>30 min</td><td>60 min</td><td>30 min</td><td>60 min</td><td>30 min</td><td>60 min</td><td>30 min</td><td>60 min</td></tr>
<tr><td rowspan="2">A</td><td>20</td><td>0.25</td><td>0.25</td><td>0.1</td><td>0.1</td><td>0.5</td><td>0.5</td><td>0.2</td><td>0.1</td></tr>
<tr><td>10</td><td>2.0</td><td>0.5</td><td>0.5</td><td>0.25</td><td>3.0</td><td>1.0</td><td>2.0</td><td>1.0</td></tr>
<tr><td rowspan="2">B</td><td>20</td><td>0.25</td><td>0.25</td><td>0.1</td><td>0.1</td><td>0.25</td><td>0.25</td><td>0.1</td><td>0.1</td></tr>
<tr><td>10</td><td>2.0</td><td>0.5</td><td>0.5</td><td>0.25</td><td>2.0</td><td>0.5</td><td>0.25</td><td>0.25</td></tr>
</table>
</td><td>B5.10.2/10</td></tr>
<tr><td>Serial dilution of Ampholyt 20 between 1.0 and 0.004 % (v/v)</td><td>Staphylococcus aureus
Streptococcus faecium
Proteus mirabilis
Pseudomonas aeruginosa
Candida albicans</td><td>Qualitative and quantitative suspension test method, Guidelines for testing chemical disinfectants for veterinary medicine (1984) by the Committee “Disinfection in Veterinary Medicine” of the German Veterinary Medical Association e.V. (DVG)</td><td>Temperature: 20 °C
Exposure time: 5, 15, 30 and 60 min
Interfering substance: without, or 10 % bovine serum</td><td>Bacteriostatic/fungistatic effect:
A bactericidal effect was achieved at a concentration of 0.25 % (v/v) without and 0.5 % (v/v) TEGOL 2000 with protein load after an exposure time of 15 to 30 minutes.
A fungicidal effect occurred at a concentration of 0.06 % (v/v) without and 0.125 % (v/v) with protein load after 30 minutes exposure.</td><td>B5.10.2/11</td></tr>
<tr><td>Serial dilution of Ampholyt 20 between 5.0 and 0.0625 % (v/v)</td><td>Staphylococcus aureus
Pseudomonas aeruginosa
Candida albicans</td><td>Qualitative and quantitative suspension test method, Guidelines for testing chemical disinfectants for veterinary medicine (1984) by the Committee “Disinfection in Veterinary Medicine” of the German Veterinary Medical Association e.V. (DVG)</td><td>Temperature: 20 °C
Exposure time: 5, 15, 30 and 60 min
Interfering substance: without, or 10 % bovine serum</td><td>Bacteriostatic/fungistatic effect:
A bactericidal effect occurred at a concentration of 2.0 % (v/v) without and 3.0 % (v/v) with protein load after 15 minutes exposure. 30 minutes exposure to 0.5% (without protein load) or to 1.0% (with protein load) for 30 minutes was sufficiently bactericidal (reduction factor > log 4).
A fungicidal effect was achieved at a concentration of 0.5% or 0.25% (v/v) without and 2.0% or 1.0% (v/v) TEGOL 2000 with protein load after an exposure time of 15 or 30 minutes respectively.</td><td>B5.10.2/12</td></tr>
</table>

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
Serial dilution of TEGOL 2000 between 1 and 0.0312 % (v/v)	*Staphylococcus aureus* *Streptococcus faecium* *Proteus mirabilis* *Pseudomonas aeruginosa* *Candida albicans*	Qualitative suspension test according to the DGHM guideline for the testing and evaluation of chemical disinfection procedures	Temperature: 20 °C Exposure time: 5, 15, 30 and 60 min Protein load: 1 % skimmed milk	A bactericidal effect for *S. areus*, *P. mirabilis* and *P. aeruginosa* was achieved at a concentration of 0.25 % (v/v) TEGOL 2000 with protein load (1% skimmed milk) after an exposure time of 15 minutes, while under the same conditions 0.125% TEGOL 2000 was effective against *S. faecium*. 30 minutes 0.25% TEGOL 2000 was effective against *P. mirabilis* and *P. aeruginosa* and 0.125% against *S. aureus* and *S. faecium*. A fungicidal effect (*C. albicans*) occurred at a concentration of 0.0625 % (v/v) with protein load after 15 and 30 minutes exposure.	B5.10.2/13
Serial dilution of TEGOL 2000 between 2 and 0.25 % (v/v)	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Candida albicans*		Temperature: 10°C Exposure time: 5, 15, 30 and 60 min Protein load: 1 % skimmed milk	A bactericidal effect for *P. aeruginosa* was achieved at a concentration of 2 % (v/v) TEGOL 2000 with protein load (1% skimmed milk) after an exposure time of 15 minutes, while under the same conditions 0.25% TEGOL 2000 was effective against *C. albicans*. 30 minutes 0.5% TEGOL 2000 was effective against *S. aureus* and 0.25% against *C. albicans*	B5.10.2/14
2.0, 1.0, 0.75, 0.5, 0.25, 0.10 and 0.05 % of Ampholyt 20	*Staphylococcus aureus* *Escherichia coli* *Proteus mirabilis* *Pseudomonas aeruginosa*	Qualitative suspension test according to the DGHM guideline for the testing and evaluation of chemical disinfection procedures	Temperature: 20 ± 1°C Interfering substance: without, or 20 % bovine serum, 0.2%, 1.0% bovine albumin	Under clean conditions all bacteria strains were inactivated at 0.5% Ampholyt 20 after 5 min. Also with 20% bovine serum *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* were inactivated at 0.5% after 5 min, though *Staphylococcus aureus* was eliminated at 0.5% after 10 min. With 0.2% bovine albumin all bacteria strains were inactivated at 0.5% Ampholyt 20 after 5 minutes. With 1.0% bovine albumin *Staphylococcus aureus* and *Escherichia coli* were inactivated at 0.5% after 5 min, though, *Proteus mirabilis* and *Pseudomonas aeruginosa* were inactivated at 0.5% after an exposure time of ≥ 20 minutes or with a higher disinfectant concentration.	B5.10.2/15

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
0.75 %, 1.5 %, 2.0 % Ampholyt 20	human rotavirus	Quantitative suspension test	Temperature: 20 °C Exposure time: 15 min, 30 min, 60 min Interfering substance: no	Ampholyt 20 at concentrations of 0.75% and 1.5% showed an efficacy on rota virus (reduction of the titre, $\Delta\log ID_{50}$) after 60 min of exposure. The reduction of recommended $\Delta\log ID_{50}$ $\geq$4.00 is achieved with 2% Ampholyt 20 after 30 min.	B5.10.2/17
0.75 % Ampholyt 20	avian influenza virus A	Inactivation tests (micro-procedure) carried out in accordance to Bundesgesundheitsamt (BGA) and Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten (DVV) – guideline with interfering substances as mentioned in EN 14476 (2005).	Exposure time: 5 min, 10 min, 15 min, 30 min	After an exposure time of 5 minutes a reduction of the virus titre was measured being 4.25. After 10 minutes no virus could be detected any longer (RF $\geq$ 5.25).	B5.10.2/18

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
1% Tego 51	Poxvirus Herpesvirus Enterovirus Adenovirus Rhabdovirus Orthomyxovirus	At room temperature 1 ml of TEGO 51 was mixed with 1 ml of the virus stock suspension to be tested. After each exposure time the mixture was rapidly diluted to 10^{-8} in MEM with 5 % of inactivated bovine fetal serum. Five wells of a microtitre plate with a confluent monolayer were inoculated with 50 µl of the mixture, for 60 minutes placed at 37 °C (virus adsorption time) before the inoculums was removed from each well and 0.1 ml medium (MEM or ELH with 5 % inactivated bovine fetal serum) was added. For the incubation the plates were placed at 37 °C in CO_2 incubator.	1, 5 and 30 min exposure time	Efficacy of TEGO 51 (1%) on Poxvirus, Herpesvirus, Orthomyxovirus, Adenovirus and Rhabdovirus is measured by reduction of the virucidal effect in the test cell line. TEGo 51 is able to inactivate within one minute over 99 % of the viruses. Enterovirus are totally resistant and after 30 minutes of contact their titre remains equal to the control. Summarising the results, it can be recommended to use the surface disinfectant TEGO 51 for inactivation of the lipophilic viral groups (Poxvirus, Herpesvirus, Orthomyxovirus, Adenovirus and Rhabdovirus) as follows: 1 %, at least 1 min. The Enterovirus Poliovirus type 1 strain proves absolutely resistant.	B5.10.2/19

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
1.0, 0.5 and 0.25 % (v/v) of Ampholyt 20	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Enterococcus hirae* ATCC 10541 *Escherichia coli* ATCC 10536 *Staphylococcus aureus methycillin resistant (MRSA)* ATCC 33592 *Candida albicans*	EN 1040 (2005) EN 1275 (2005) EN 1276 (1997) EN 1650 (1997) EN 13697 (2001)	5, 15 and 30 min exposure time	Evidence of inherent biological and yeasticidal activity shown. No evidence of activity against *A. niger*, therefore fungicidal activity cannot be claimed. Test methods not performed in full accordance with related EN standards and evidence of errors in report.	B5.10.2/20