Safety Data Sheet according to regulation (EC) 1907/2006 (REACH) and regulation (EC) 2015/830

Trade name: Bacoban® DL

Date of Issue: 06.08.2019 Replace Data Sheet of: 13.04.2018 "*" alterations as compared to previous version

SECTION 1. Identification of the substance/mixture and of the company/undertaking

1.1. Product identifiers

Trade name:	Bacoban DL
Article-No. (manufacturer/supplier):	X-BAC500DL, X-BACDLTUE
Preparation No:	n.a.
Registration no:	n.a.
UFI-Code(*):	n.a.

1.2. Relevant identified uses of the substance or mixture and uses advised against

relevant identified uses:

Identified used: Surface Disinfectant

1.3. Details of the supplier of the safety data sheet

Supplier (manufacturer/importer/downstream user/distributor)

Ropimex R. Opel GmbH (Geschäftsbereich Hygiene-Systeme Adexano)

Bildstocker Straße 12 Telephone: +49 - (0)6821 / 91277 60

D – 66538 Neunkirchen Telefax: +49 - (0)6821 / 91277 79

E-Mail info@ropimex.com

Dept. responsible for information:

E-Mail (competent person) info@ropimex.com

1.4. Emergency telephone number

Emergency Telephone of Company / Undertaking	+49 - (0)6821 / 91277 - 0 (08:00 am- 4:00 pm)
Counselling centre for poisoning	+49 (0)551 19240 (Giftnotrufzentrale GIZ Nord)

SECTION 2. Hazards identification

2.1. Classification of the substance or mixture

Classification according to Regulation (EC) No. 1272/2008 [CLP]

This mixture is not classified as hazardous according to regulation (EC) No. 1272/2008 [CLP].

-

2.2. Label elements

The product is not classified and labelled according to EC directives or corresponding national laws.

Labelling according to Regulation (EC) No. 1272/2008 [CLP]

Hazard pictograms: **Signal word:**

none none

Hazard statements

-

Precautionary statements

P102 Keep out of reach of children.

contains:

n.a.

Supplemental Hazard information (EU)

n.a.

Supplemental Hazard information
n.a.

2.3. Other hazards

SECTION 3. Composition / information on ingredients

3.2. Mixtures
Description: aqueous solution with biocides
Hazardous ingredients:
Classification according to Regulation (EC) No. 1272/2008 [CLP]

EG-No CAS-No INDEX-No	REACH-No. Chemical name classification	wt% / Remark
270-325-2 68424-85-1	01-2119983287-23-xxxx Quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl-, chlorides Acute Tox. 4 H302 / Acute Tox. 4 H312 / Skin Corr. 1B H314 / Aquatic Acute 1 H400 (M=10)	0,26
223-296-5 3811-73-2	01-2119493385-28-0000 Pyridine-2-thiol-1-oxide, sodium salt Acute Tox. 4 H302 / Acute Tox. 4 H312 / Acute Tox. 4 H332 / Skin Irrit. 2 H315 / Aquatic Acute 1 H400	0,025

Additional information
Full text of classification: see section 16

SECTION 4. First aid measures

4.1. Description of first aid measures
General information
In all cases of doubt, or when symptoms persist, seek medical advice. In case of unconsciousness give nothing by mouth, place in recovery position and seek medical advice
In case of inhalation
Remove casualty to fresh air and keep warm and at rest. In case of irregular breathing or respiratory arrest provide artificial respiration.
Following skin contact
Remove contaminated, saturated clothing immediately. After contact with skin, wash immediately with plenty of water and soap. Do not use solvents or thinners.
After eye contact
Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Seek medical advice immediately.
After ingestion

If swallowed, rinse mouth with water (only if the person is conscious). Seek medical advice immediately. Keep victim calm. Do NOT induce vomiting.

4.2. Most important symptoms and effects, both acute and delayed

In all cases of doubt, or when symptoms persist, seek medical advice.

4.3. Indication of any immediate medical attention and special treatment needed

SECTION 5. Firefighting measures

5.1. Extinguishing media

Suitable extinguishing media

Resistant foam, carbon dioxide, Powder, spray mist, (water)

Extinguishing media which must not be used for safety reasons:

Strong water jet

5.2. Special hazards arising from the substance or mixture

Provide a conveniently located respiratory protective device.

5.3. Advice for firefighters:

Provide a conveniently located respiratory protective device..

Additional information

Cool closed containers that are near the source of the fire. Do not allow water used to extinguish fire to enter drains, ground or waterways. Treat runoff as hazardous.

SECTION 6. Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

Keep away from sources of ignition. Ventilate affected area. Do not breathe vapours. See protective measures under point 7 and 8.

6.2. Environmental precautions

Do not allow to enter into surface water or drains. If the product contaminates lakes, rivers or sewages, inform competent authorities in accordance with local regulations.

6.3. Methods and material for containment and cleaning up

Isolate leaked material using non-flammable absorption agent (e.g. sand, earth, vermiculite, diatomaceous earth) and collect it for disposal in appropriate containers in accordance with the local regulations (see chapter 13). Clean using cleansing agents. Do not use solvents.

6.4. Reference to other sections

Observe protective provisions (see chapter 7 and 8).

SECTION 7. Handling and storage

7.1. Precautions for safe handling

Advices on safe handling

Avoid formation of flammable and explosive vapour concentrations in the air and exceeding the exposure limit values. Only use the material in places where open light, fire and other flammable sources can be kept away.

Electrical equipment must be protected meeting the accepted standard. Keep away from heat sources, sparks and open flames. Use only spark proof tools. Avoid contact with skin, eyes and clothes. Do not inhale dusts, particulates and spray mist when using this preparation. Avoid respiration of swarf. When using do not eat, drink or smoke. Personal protection equipment: refer to chapter 8. Do not empty containers with pressure - no pressure vessel! Always keep in containers that correspond to the material of the original container. Follow the legal protection and safety regulations.

Precautions against fire and explosion:

Vapours are heavier than air. Vapours form explosive mixtures with air.

7.2. Conditions for safe storage, including any incompatibilities

Requirements for storage rooms and vessels

Storage in accordance with the Ordinance on Industrial Safety and Health (BetrSiVO). Keep container tightly closed. Do not empty containers with pressure - no pressure vessel! Smoking is forbidden. Access only for authorised persons. Store carefully closed containers upright to prevent any leaks. Soils have to conform to the "Guidelines for avoidance of ignition hazards due to electrostatic charges (BGR 132)".

Hints on joint storage

Keep away from strongly acidic and alkaline materials as well as oxidizers.

Further information on storage conditions

Take care of instructions on label. Store in a well-ventilated and dry room at temperatures between 15 °C and 30 °C. Protect from heat and direct sunlight.

Due to the content of organic solvents in the preparation:

Protect from heat and direct sunlight. Keep container tightly closed. Remove all sources of ignition. Smoking is forbidden. Access only for authorised persons. Store carefully closed containers upright to prevent any leaks..

7.3. Specific end use(s)

Observe technical data sheet. Observe instructions for use. Read label before use.

SECTION 8. Exposure controls / personal protection

8.1. Control parameters

Occupational exposure limit values:

n.a.

Additional information:

n.a.

8.2. Exposure controls

Provide good ventilation. This can be achieved with local or room suction. If this should not be sufficient to keep aerosol and solvent vapour concentration below the exposure limit values, a suitable respiratory protection must be used.

Occupational exposure controls

Respiratory protection

If concentration of solvents is beyond the occupational exposure limit values, approved and suitable respiratory protection must be used. Observe the wear time limits according GefStoffV in combination with the rules for using respiratory protection apparatus (BGR 190). Use only respiratory protection equipment with CE-symbol including four digit test number.

Hand protection
For prolonged or repeated handling the following glove material must be used: nitrile rubber or butyl rubber with at least 0.7 mm thickness
Thickness of the glove material>= 0,7 mm
Breakthrough time (maximum wearing time)
> 480 min.
Observe the instructions and details for use, storage, maintenance and replacement provided by the protective glove manufacturer. Penetration time of glove material depending on intensity and duration of exposure to skin. Recommended glove articles DIN EN 374
Barrier creams can help protecting exposed skin areas. In no case should they be used after contact.
Eye protection
Wear closely fitting protective glasses in case of splashes.
Protective clothing
Wear antistatic clothing of natural fibres (cotton) or heat resistant synthetic fibres.
Protective measures
After contact clean skin thoroughly with water and soap or use appropriate cleanser.
Environmental exposure controls
Do not allow to enter into surface water or drains. See chapter 7. No additional measures necessary.

SECTION 9. Physical and chemical properties

9.1. Information on basic physical and chemical properties

Physical state: liquid, **Colour:** colourless, **Odour:** perfume, **Odour threshold:** n.d.

9.1.2	pH-value, undiluted:	7
	pH-value, 1% aqueous solution: n.d.	
9.1.3	Boiling point / Boiling - range (°C):	100, Melting point / Melting range (°C): 0
9.1.4	Flash point (°C):	n.ap., closed cup
9.1.5	Flammability (EEC A10/A13):	no
9.1.6	Ignition temperature (°C):	n.ap.
9.1.7	Autoflammability (EEC A16):	n.ap.
9.1.8	Oxidising properties:	None.
9.1.9	Explosion hazard:	No.
9.1.10	Explosion limits (Vol.%) lower:	n.ap., upper: n.ap.
9.1.11	Vapour pressure:	n.ap.
	Vapour density (Air = 1):	n.av.
9.1.12	Density (g/ml):	1
9.1.13	Solubility (in Water):	mixible
9.1.14	Partition coefficient, n-Octanol / Water:	n.av.
9.1.15	Viscosity:	1,5 – 2,5 mPa*s (20°C)
9.1.16	Solvent content (m %):	<1
9.1.17	Thermal decomposition (°C):	n.av.
9.1.18	Evaporation rate:	n.av.

9.2 **Other information**
n.av.

9.2. Other information

SECTION 10. Stability and reactivity

10.1 Reactivity
None.
10.2 Chemical stability
Stable under normal conditions.
10.3 Possibility of hazardous reactions
No dangerous reaction known under conditions of normal use.
10.4 Conditions to avoid
Protect from frost and heat.
10.5 Incompatible materials
Incompatible with strong oxidizing agents.
10.6 Hazardous decomposition products
No decomposition by typical storage and use. Do not heat to prevent thermal decomposition.

SECTION 11. Toxicological Information

Classification for mixtures and used evaluation method according to regulation (EC) 1207/2008 [CLP]
No data on preparation itself available.
11.1. Information on toxicological effects
Acute toxicity

Inhalation	n. a.
Ingestion	ATEmix: >10000 mg/kg
Skin contact	n. a.
Irritation / corrosion (to skin/eye)	May cause skin irritation in susceptible persons
Sensitation	May cause eye irritation in susceptible persons.
Carcinogenicity	n. a.
Mutagenicity	n. a.
Teratogenicity	n. a.
Specific target organ toxicity	n.a.
Specific target organ toxicity, repeated exposure	n.a.
Narcotic effect	none

Practical experience/human evidence
Other observations:
Inhaling of solvent components above the MWC-value can lead to health damage, e.g. irritation of the mucous membrane and respiratory organs, as well as damage to the liver, kidneys and the central nerve system. Indications for this are: headache, dizziness, fatigue, amyosthenia, drowsiness, in serious cases: unconsciousness. Solvents may cause some of the aforementioned effects through skin resorption. Repeated or

prolonged contact with the preparation may cause removal of natural fat from the skin resulting in non-allergic contact dermatitis and/or absorption through skin. Splashing may cause eye irritation and reversible damage.

Overall Assessment on CMR properties

The ingredients in this mixture do not meet the criteria for classification as CMR category 1A or 1B according to CLP.

Remark:

There is no information available on the preparation itself.

SECTION 12. Ecological information

Overall evaluation

Classification for mixtures and used evaluation method according to regulation (EC) 1207/2008 [CLP] There is no information available on the preparation itself.

12.1. Toxicity

Quaternary ammonium compound:

EC_{50}/48h/Daphnia = 0,0059 – 0,052 mg/l

LC_{50}/96h/Oncorhynchus mykiss = 0,923 mg/l

EC_{50}/72h/Alge = 0,8 mg/l

12.2. Persistence and degradability

No data available for the product itself.

12.3. Bioaccumulative potential

n.v.

Bioconcentration factor (BCF)

Toxicological data are not available.

12.4. Mobility in soil

Toxicological data are not available.

12.5. Results of PBT and vPvB assessment.

The substances in the mixture do not meet the PBT/vPvB criteria according to REACH, annex XIII.

12.6. Other adverse effects

12.6.1	COD-Value [mg/g]	n. a.
12.6.2	BSB5-Value [mg/g]	n. a.
12.6.3	AOX-Remarks	The product include organic halogen compounds
12.6.4	Ecological significant components	-
12.6.5	Other adverse effects	Not applicable

SECTION 13. Disposal considerations

13.1. Waste treatment methods

Appropriate disposal / Product

Recommendation

Do not allow to enter into surface water or drains. This material and its container must be disposed of in a safe way. Waste disposal according to directive 2008/98/EC, covering waste and dangerous waste.

D10

List of proposed waste codes/waste designations in accordance with EWC
07 06 01
Packaging
Recommendation
Non-contaminated packages may be recycled. Vessels not properly emptied are special waste.
13.2 Contaminated Packaging
13.2.1 Recommendation: Wash with suitable cleaner. Otherwise as described under Residues. Uncontrolled disposal or recycling of this packaging is not permitted and can be dangerous.
13.2.2 Safe Handling: As described under Residues.
Handle in accordance with good industrial hygiene and safety practice

SECTION 14. Transport information

No dangerous good in sense of this transport regulation

	ADR	IMDG	IATA
14.1	UN number		
	no DGR	no DGR	no DGR
14.2	UN proper shipping name		
	-	-	-
14.3	Transport hazard class(es)		
	-	-	-
14.4	Packing group		
14.5	Environmental hazards		
14.6	Special precautions for user		
	Tunnel restriction code: Transport category: - Classification Code: - Hazard-No.: - LQ: -	-	Packing Instructions (passenger): - Packing Instructions (cargo): -
14.7	Transport in Bulk according to Annex II of MARPOL73/78 and the IBC Code		
		None	

SECTION 15. Regulatory information

15.1. Safety, health and environmental regulations/legislation specific for the substance or mixture
EU legislation
National legislation
Restrictions of occupation
n.a.

15.2. Chemical safety assessment
Chemical safety assessments for substances in this preparation were not carried out.

SECTION 16. Other information

Full text of classification in section 3:

EUH70 Toxic by eye contact
H302 Harmful if swallowed
H312 Harmful in contact with skin
H314 Causes severe skin burns and eye damage
H315 Causes skin irritation
H332 Harmful if inhaled
H373 May cause damage to organs <or state all organs affected, if known> through prolonged or repeated exposure <state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard>.
H400 Very toxic to aquatic life

Abbreviations and acronyms:
n.av. not available
n.ap. not applicable
n.d. not determined

Additional information
Classification for mixtures and used evaluation method according to regulation (EC) 1207/2008 [CLP].
The statements in this Material Safety Data Sheet were made to the best of our knowledge and are as accurate as possible. They are given for information only. They do not constitute a contractual guarantee of a product's properties. They must neither be altered nor transferred to other products.

DIPL.-BIOL. DR. RER. NAT. D. PAULMANN

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ROPIMEX R. OPEL GmbH
Bildstocker Straße 12
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Bremen, 15/04/2020

Statement: Modified vaccinia virus Ankara (MVA) as surrogate virus to confirm virucidal activity against all enveloped viruses

In Europe, the modified vaccinia virus Ankara (MVA) represents the official surrogate test virus for all enveloped viruses. For surface disinfectants it is defined in the EN 14476 (quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in human medicine (phase 2/step 1) and the EN 16777 (chemical disinfectants and antiseptics – Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area – Test method and requirements (phase 2/step 2).

The claim is resulting from the prEN 14885:2020 in combination with the above-mentioned standards where details are given how to use the European standards for making claims. From the table in 4.3.2.6 the conclusion can be drawn that after passing examinations with MVA an activity against all enveloped viruses including members of the virus family *coronaviridae* (like MERS-CoV, SARS-CoV-1 and SARS-CoV-2) is achieved.

Dr. D. Paulmann

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Bremen, 04/05/2020

Summary: Virus-inactivating properties of Bacoban WB of ROPIMEX R. OPEL GmbH on treated ceramic carriers based on ASTM E 2180

This summary is based on the following test report of Dr. Brill + Partner GmbH for the surface disinfectant Bacoban WB produced by ROPIMEX R. OPEL GmbH:

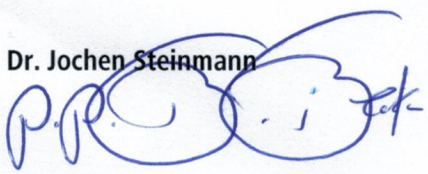
modified vaccinia virus Ankara test report (L20/0143MV.1) dating 14/04/2020

Based on the test method as described in ASTM E 2180 ceramic carriers treated with Bacoban WB 10 days before the inactivation test were overlaid with the test virus suspension and after different exposure times residual virus infectivity was determined.

Evaluating this method with modified vaccinia virus Ankara (MVA), the ceramic tiles coated with 1.0 % Bacoban WB were able to demonstrate a significant ($P < 0,01$) $\log_{10}$ reduction of MVA after an exposure time of 5, 15 and 30 minutes.

After passing evaluation with modified vaccinia virus Ankara an activity against all enveloped viruses including members of the virus family *coronaviridae* (like MERS-CoV, SARS-CoV-1 and SARS-CoV-2) with the surface disinfectant Bacoban WB is achieved.

Dr. Jochen Steinmann



DR. BRILL + DR. STEINMANN
INSTITUTE FOR HYGIENE AND MICROBIOLOGY

ilac-MRA DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01 D-PL-13412-01-02
Anerkannt durch/Recognized by Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten www.zlg.de
ZLG-AP-216.11.02

TEST REPORT

14/04/2020

Test report L20/0143MV.1

Evaluation of the effectiveness of

Bacoban WB

Test virus: modified vaccinia virus Ankara (MVA)

Method: based on **ASTM E2180** (Standard Test Methods for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Materials)

Sponsor:
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1. Introduction

The objective of this study was to evaluate the virus-inactivating efficacy of Bacoban WB against modified vaccinia virus Ankara (MVA) using a quantitative carrier test based on the ASTM E2180 (1).

Ceramic tiles treated with Bacoban WB (and untreated controls) are contaminated with test virus suspension in an agar slurry. The ceramic tiles were incubated at room temperature for 5, 15 and 30 minutes. The inactivation of the test virus was studied in one run with three parallels for each exposure time. The ceramic tiles were checked after eluation for residual virus at the end of the experiment. The virus-inactivating properties of Bacoban WB under the chosen conditions can be calculated by comparing the virus titres of treated ceramic tiles with the controls (non-treated carriers).

2. Identification of test laboratory

Dr. Brill + Partner GmbH Institute for Hygiene and Microbiology, Norderoog 2, DE - 28259 Bremen

3. Identification of sample

Manufacturer	ROPIMEX R. OPEL GmbH
Name of product	Bacoban WB
Confirmation no.	212558
Product diluent recommended by the manufacturer	-
Batch number	2002130
Application	surface disinfection
Production date	-
Expiry date	02/2022
Active compound (s) (100 g)	QAV
Appearance, odour	clear, yellow, viscous liquid product specific
pH-values	undiluted: 5.70 (20 °C) 1.0 %: 6.47 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	19/02/2020

4. Materials

4.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM, Biozym Scientific GmbH, catalogue no. 880121)
- fetal calf serum (Biochrom AG, article no. S 0115)
- Aqua dest. (SG ultrapure water system, type Ultra Clear; serial no. 86996-1)
- PBS (Invitrogen, article no. 18912-014)
- Penicillin/ streptomycin (Sigma-Aldrich, article no. P-0781)
- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153)
- Agar-Agar (Carl Roth GmbH, article no. 5210.2)
- NaCl (Carl Roth GmbH, article no. 3957.1)
- Propan-2-ol (Carl Roth GmbH, article no. 6752.1)

4.2 Virus and cells

The modified vaccinia virus Ankara (MVA) originated from Dr. Manteufel, Institut für Tierhygiene und Öffentliches Veterinärwesen, DE - 04103 Leipzig. Before inactivation assays, virus had been passaged three times in *BHK 21-cells* (Baby Hamster Kidney).

BHK 21-cells (passage 104) originated from the Friedrich-Löffler-Institut, Bundesforschungsinstitut für Tiergesundheit (formerly Bundesforschungsanstalt für Viruskrankheiten der Tiere, isle of Riems).

The cells were inspected regularly for morphological alterations and for contamination by mycoplasmas. No morphological alterations of cells and no contamination by mycoplasmas could be detected.

4.3 Apparatus, glassware and small items of equipment

- CO_2 incubator, Nunc GmbH & Co. KG, model QWJ 350)
- Agitator (Vortex Genie Mixer, type G 560E)
- pH measurement 315i (WTW, article no. 2A10-100)
- Centrifuge (Sigma-Aldrich-Chemie GmbH, type 113)
- Microscope (Olympus, type CK 30)
- Centrifuge 5804 R (Eppendorf AG)
- Water bath (JULABO, Julabo U 3)
- Adjustable and fixed-volume pipettes (Eppendorf AG)

- Pipette ErgoOne (STARLAB)
- Polysterol 96-well microtitre plate (Nunc GmbH & Co. KG, Wiesbaden)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden)
- Container, flat bottom, 25 cm, with cap (Sarstedt AG & Co., Nümbrecht)
- Glass petri dishes (Nunc GmbH & Co. KG, Wiesbaden)
- Ceramic tiles Ø 2 cm (provided by the sponsor)

5. Experimental conditions

Test temperature	room temperature (21.0 ± 2.0 °C)
Test product concentration (Bacoban WB)	1.0 %
Diluent of the test product solution	water of standardised hardness (WSH)
Size of test samples	2.0 cm in diameter
Coating of the ceramic tiles	10 days before inactivation test
Contact time of the Bacoban WB-coated ceramic tiles to the virus inoculum	5, 15 and 30 minutes
Volume of virus inoculum	50 µl (40 µl virus suspension + 10 µl interfering substance)
Interfering substances in the virus inoculum	0.3 g/l bovine serum albumin (clean conditions, EN 14476)
Procedure to stop action of disinfectant	immediate dilution
Test virus	modified vaccinia virus Ankara (MVA) (ATCC VR-1508)
Period of analysis	23/03/2020 – 14/04/2020
End of testing	14/04/2020

6. Methods

6.1 Preparation of test virus suspension

For preparation of test virus suspension *BHK 21-cells* were cultivated with MEM and 10 % or 2 % fetal calf serum. *Cells* were infected with a multiplicity of infection of 0.1. After cells showed a cytopathic effect, they were subjected to a freeze/thaw procedure followed by a low speed centrifugation in order to sediment cell debris. After aliquotation, test virus suspension was stored at – 80 °C.

6.2 Preparation of the test product solution

Bacoban WB was used as 1.0 % solution. This solution was prepared with water of standardiser hardness immediately before coating of the carriers.

6.3 Preparation and coating of ceramic tiles

The ceramic tiles were provided by the sponsor. These were carriers with a diameter of 2 cm, which had been briefly immersed in 100 % isopropanol before coating. After removal, they were wiped off with a cloth and placed in square 8-well plates for air drying (4 carriers per plate in the inner 4 wells). The coating was then carried out with 50 µL Bacoban WB (1.0 % solution), the surface disinfectant being distributed to the edge by swirling, until they were visible dry. The untreated carriers for the control assays were also placed in square 8-well plates. The storage time in the covered plates at room temperature was 10 days until used in the experiments.

6.4 Preparation of agar slurry

0.3 g Agar-Agar were solved in 100 ml of a 0.85 % saline solution by stirring on a heat plate. Afterwards, the agar slurry was autoclaved (121 °C, 15 min) and cooled down to 37 – 45 °C.

6.5 Preparation of the virus inoculum

Four volumes of test virus suspension were mixed with one volume of interfering substance solution and five volumes of the agar slurry (see 6.4).

6.6 Inactivation assays and controls

Tests were carried out at room temperature (21.0 ± 2.0 °C). For each exposure time three ceramic tiles treated with Bacoban WB (non-treated ceramic tiles as controls) were prepared. The inactivation experiments were run in one assay.

Two of the prepared carriers (treated with Bacoban WB or untreated controls) are placed in the inner wells of a 6-well plate. The outer wells are filled with Aqua bidest (moist chamber). The carriers are each inoculated with 100 µl virus inoculum and incubated in the closed plate for the respective exposure time. At the end of the respective exposure time, 2 ml ice-cold cell culture medium (without FCS) are removed from the prepared eluate container with 9.9 ml cell culture medium and added to the carrier. The carrier is then rinsed 14 times with 1 ml of the medium and then the complete eluate is returned to the eluate container (total volume 9.9 ml). Directly after elution, series of ten-fold dilutions of the eluate in ice-cold maintenance medium were prepared and inoculated on cell culture.

The non-treated ceramic tiles were processed as described above. Three test pieces were incubated for each respective exposure time.

To determine the initial virus titre a virus input control (VIC) at the beginning and the end of testing were included. Therefore, 100 µl of the virus inoculum were added to 9.9 ml cell culture.

Determination of cytotoxicity was performed as follows: Ceramic tiles treated with Bacoban WB as described above were inoculated with a mixture of 40 µl cell culture medium, 10 µl interfering substance and 50 µl agar slurry instead of the virus inoculum. This assay was incubated for the longest exposure time (30 minutes) in a moist chamber at room temperature and afterwards eluted as described above. The cytotoxicity control is needed for definition of the lower detection limit.

In addition, a control of efficiency for suppression of disinfectant's activity was included. Therefore, 4.95 ml of the undiluted eluate from the cytotoxicity controle (see above) were mixed with 50 µl virus inoculum and incubated on ice for 30 minutes. Afterwards this assay was diluted, and the infectivity was determined. The result is compared with the mean of the virus input control and the difference should be ≤ 0.5 (based on EN 14476 (4)).

Furthermore, a cell control (only addition of medium) was incorporated.

6.7 Determination of infectivity

Infectivity was determined by means of end point dilution method by transferring 0.1 ml of each dilution into eight wells of a microtitre plate with 0.1 ml of freshly trypsinized *BHK 21-cells* (10-15 x 10^3 cells per well), beginning with the highest dilution. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read after six days by using an inverted microscope. Calculation of the infective dose $TCID_{50}$/ml was calculated with the method of Spearman (2) and Kärber (3).

6.8 Calculation of virucidal activity

The virucidal activity of Bacoban WB as coating on ceramic tiles was evaluated by calculating the difference in the logarithmic virus titres between treated ond non-treated ceramic tiles after inoculation and incubation, giving the reduction factor.

7. Verification of the methodology

The following criteria were fulfilled:

a) The mean virus titre of the virus input controls (VIC) at the beginning (6.50 ± 0.35) and the end of testing (6.25 ± 0.44) has a similarity of > 90 %.
b) The recovery of the virus on the non-treated carriers immediately after addition of the virus inoculum (VCt5, 6,25 ± 0.25) is ≥ 80 % compared to the mean VIC (6.38 ± 0.28).
c) The initial virus titre allows a significant reduction in virus titre.
d) The cytotoxicity of the test sample did not influence cell morphology or growth in no way that a significant reduction of virus titre could not be shown.
e) The difference of the virus titres of the control for suppression of disinfectant's activity and the VIC (based on EN 14476) is ≤ 0.5 log (6.50 ± 0.46 (test sample) versus 6.38 ± 0.28 (mean VIC)).

Since all criteria were fulfilled, examination with MVA based on ASTM E2180 was valid.

8. Results

The ceramic tiles coated with Bacoban WB were examined for 5, 15 and 30 minutes at room temperature. The results are shown in table 1.

The mean virus titres on the ceramic tiles coated with a 1.0 % solution of Bacoban WB ten days before the inactivation tests were ≤ 4.00 ± 0.87 $\log_{10}$ $TCID_{50}$/ml after 5 minutes, ≤ 3.96 ± 0.52 $\log_{10}$ $TCID_{50}$/ml after 15 minutes and ≤ 3.54 ± 0.14 $\log_{10}$ $TCID_{50}$/ml after 30 minutes. The reduction factors were therefore ≥ 2.25 ± 0.90 after 5 minutes, ≥ 2.21 ± 0.74 after 15 minutes and ≥ 2.58 ± 0.29 after 30 minutes.

9. Conclusion

The ceramic tiles coated with Bacoban WB were able to demonstrate a significant ($P < 0,01$) log_{10} reduction of MVA after an exposure time of 5, 15 and 30 minutes.

Bremen, 14/04/2020

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10. Quality control

The Quality Assurance of the results was maintained by performing the determination of the virus-inactivating properties of the disinfectant in accordance with Good Laboratory Practice regulations:

1) Chemicals Act of Germany, Appendix 1, dating of 01.08 1994 (BGBI. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBI. I, 1997, page 1060).
2) OECD Principles of Good Laboratory Practice (revised 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring – Number 1. Environment Directorate, Organization for Economic Co-operation and Development, Paris 1998.

The plausibility of the results was additionally confirmed by controls incorporated in the inactivation assays.

11. Records to be maintained

All testing data, protocol, protocol modifications, the final report, and correspondence between Dr. Brill + Partner GmbH and the sponsor will be stored in the archives at Dr. Brill + Partner GmbH.

12. Literature

1. Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Materials ; ASTM E2180-18.

2. Spearman, C.: The method of `right or wrong cases` (constant stimuli) without Gauss's formulae. Brit J Psychol; 2 1908, 227-242.

3. Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch Exp Path Pharmak; 162, 1931, 480-487.

4. EN 14476:2013+A2:2019: Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity of chemicals disinfectants and antiseptics in human medicine test - Test method and requirements (phase 2, step 1)

Appendix

Legend to the tables

Table 1: Results of ceramic tiles treated wit a 1.0 % solution of Bacoban WB and untreated ceramic tiles as controls against MVA

Table 1: Results of ceramic tiles treated wit a 1.0 % solution of Bacoban WB and untreated ceramic tiles as controls against MVA (#6487)

	Conc.	Exposure time	Interfering substance in virus inoculum	Cytotoxicity log_{10} CD_{50}/ml	log_{10} $TCID_{50}$/ml before drying		log_{10} $TCID_{50}$/ml after drying					reduction	
							Carrier 1	Carrier 2	Carrier 3	MV	2xSD	RF	95 % CI
VIC (virus inoculum)	n.a.	n.a.	clean	n.a.	6.50	6.25	n.a.	n.a.	n.a.	**6.38**	**n.d.**	**n.a.**	**n.a.**
VC	n.a.	5 min	clean	n.a.	n.a.	n.a.	6.13	6.25	6.38	**6.25**	**0.25**	**0.13**	**n.a.**
VC	n.a.	15 min	clean	n.a.	n.a.	n.a.	6.25	5.88	6.38	**6.17**	**0.52**	**0.21**	**n.a.**
VC	n.a.	30 min	clean	n.a.	n.a.	n.a.	6.13	6.00	6.25	**6.13**	**0.25**	**0.25**	**n.a.**
Bacoban WB	1,0%	5 min	clean	3.50	n.a.	n.a.	≤ 3.75	≤ 4.50	≤ 3.75	**≤ 4.00**	**0.87**	**≥ 2.25**	**0.90**
Bacoban WB	1,0%	15 min	clean	3.50	n.a.	n.a.	≤ 4.25	≤ 3.88	≤ 3.75	**≤ 3.96**	**0.52**	**≥ 2.21**	**0.74**
Bacoban WB	1,0%	30 min	clean	3.50	n.a.	n.a.	≤ 3.50	≤ 3.63	≤ 3.50	**≤ 3.54**	**0.14**	**≥ 2.58**	**0.29**

n.a. = not applicable
n.d. = not done

VIC = virus input control (virus control)
MV = mean value
SD = standard deviation

VC = virus control
CI = confidence

RF = reduction factor