Section A5 Effectiveness against target organisms and intended uses

Subsection (Annex Point)			Official use only
5.1	**Function (IIA5.1)**	Bactericide	
5.2	**Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)**		
5.2.1	**Organism(s) to be controlled (IIA5.2)**	Bacteria	
5.2.2	**Products, organisms or objects to be protected (IIA5.2)**	Table A5.2-1 provides an overview of uses of DBNPA and products and objects to be protected. DBNPA is used for short-term preservation of mineral slurries prior to use.	
5.3	**Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)**	--	
5.3.1	**Effects on target organisms (IIA5.3)**	The active substance DBNPA is effective against bacteria. See Table A5.3.1-1.	
5.3.2	**Likely concentrations at which the A.S. will be used (IIA5.3)**	Please refer to Table A5.2-1. 2,2-Dibromo-3-nitrilopropionamide (DBNPA) is used in the paper industry for short-term preservation of mineral slurries prior to use. DBNPA may also be used to maintain a low microbial load during storage of the slurry in tanks at the paper mill. The applicant has claimed a recommended dosage of 50 ppm (0.005%). DBNPA is used as in-use 20% solution for short-term preservation of mineral slurries during manufacturing, transport and/or storage. A separate validated analysis of the degradation of DBNPA in mineral slurry has been performed and shows that the decomposition of DBNPA is 80% from the mineral slurry over 4 hours (Askew (2020).	
5.4	**Mode of action (including time delay) (IIA5.4)**	--	
5.4.1	**Mode of action**	DBNPA acts via bromine, which inactivates enzymes by converting functional -SH groups to the oxidised S-S form. DBNPA is a fast acting biocide. The biocidal action is exerted	

Section A5 Effectiveness against target organisms and intended uses

		directly after application (Paulus, 2005[1]). If some biocides have very specific target sites, DBNPA has a multisite effect. Some studies on the interaction of radioactively labelled [^{14}C] DBNPA with bacteria have shown that the ^{14}C label never penetrates the cell, as would be necessary to become involved with energy metabolism. Instead, it binds strongly and rapidly to the cell wall of the bacteria (Rossmore, 1991[2]). The easy reaction of DBNPA with sulphur-containing nucleophiles common to micro-organisms such as glutathione or cysteine, is the basis of its mode of antimicrobial action. DBNPA reacts through its bromine chemistry, i.e. via bromine, which inactivates thiol-based (R-SH) amino-acids and enzymes by converting their functional -SH groups to the oxidised S-S form and forming disulphide bridges: N ≡ C-CBr_2-$CONH_2$(DBNPA) + 2R-SH + 2HS-R' => 2HBr + 2R-S-S-R' + N ≡ C-HCH-$CONH_2$ Unlike other thiol-reactive biocides, its action is such that thiol-based amino acids, like cysteine, are oxidized beyond the formation of disulphide species. This reaction irreversibly disrupts the function of cell-surface components, interrupting transport across cell membranes, and inhibiting key biological functions (Gartner, 1998[3]). DBNPA is therefore not a typical oxidizing or halogen-releasing biocide. It does not release bromine (Br_2) or hypobromous acid (HOBr).
5.4.2	**Time delay**	DBNPA is a fast-acting biocide and is exerting its biocidal action directly after its application.
5.5	**Field of use envisaged (IIA5.5)**	Please refer to Table A5.2-1.
5.6	**User (IIA5.6)**	Please refer to Table A5.2-1.
	Industrial	Yes
	Professional	Yes
	General public	No
5.7	**Information on the occurrence or possible occurrence of the development of resistance and**	--

[1] Paulus W. (2005). Relationship between chemical structure and activity or mode of action of microbicides. In: Paulus W., editor. Directory of Microbicides for the Protection of Materials – a Handbook. Publ. Springer-Verlag; 2005.
[2] Rossmore, H.W (1991). Nitrogen Compounds. Disinfection, Sterilization and Preservation, Seymour S. Block, 4th edition chapter 7, 1991, pages 290- 333.
[3] Gartner C. (1998). 2,2-Dibromo-3-Nitrilopropianamide (DBNPA): Proposed Mechanism of Activity. The Dow Chemical Company report number 981334.

Section A5 Effectiveness against target organisms and intended uses

	appropriate management strategies (IIA5.7)	
5.7.1	**Development of resistance**	No cases of the development of resistance are known to the applicants (Buske, 2008[4]), (Gartner, 1998[3]). The development of resistance is unlikely, as DBNPA is a fast-acting biocide and hydrolyses rapidly (Exner et al., 1973[5]). Therefore, microbes are not exposed over a longer time period to DBNPA to allow the development of resistance. Microbes will not come in contact with DBNPA in a natural environment. DBNPA has multiple reaction sites on the surfaces of microorganisms. As a result, organisms have great difficulty in developing an effective resistance mechanism because multiple reactions and reaction sites are involved. Developing resistance to an antimicrobial is a direct function of a combination of the mode-of-action and the reactions that occur on/in a microorganism. If an antimicrobial has a specific reaction with a specific cell component, the organism often develops a resistance mechanism. For example, antibiotics will have a target cellular component or metabolic reaction that will be blocked. A prime example of this is penicillin, which blocks a specific reaction involved in cell wall formation in bacteria; organisms can develop resistance to such specific reactions. Organisms have great difficulty in developing an effective resistance mechanism against DBNPA, because multiple reactions and reaction sites are involved. However, if the organisms are in a biofilm, they can be protected from the action of DBNPA because it will react with exopolymeric materials that act as the framework of the biofilm (Grobe, et al., 2002[6]). The cells are not resistant to DBNPA – they are simply protected from its inhibitory effect because the biocide reacts with the exopolymeric materials rather than the cells (Gartner, 1998)[3].
5.7.2	**Management strategies**	If resistance would be observed, another biocide should be used.
5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	See confidential part of the dossier.

[4] Buske A. (2008). Biocidal Activity of DBNPA. Dow ICM-Research Intelligence Group Research bibliography.
[5] Exner J.H., Burk G. A. and Kyriacou, D. (1973). Rates, and products of decomposition of 2, 2-dibromo-3-nitrilopropionamide, J. Agric. Food. Chem. 21: 838-842.
[6] Grobe K.J., Zahller J., Stewart P. S. (2002). Role of dose concentration in biocide efficacy against *Pseudomonas aeruginosa* biofilms. Center for Biofilm Engineering and Department of Chemical Engineering, Montana State University, Bozeman, MT, 59717-3980, USA SO, Journal of Industrial Microbiology & Biotechnology, 29(1), 10-15.

Section A5 Effectiveness against target organisms and intended uses

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30.10.2022
Materials and methods	The applicant's version is acceptable.
Conclusion	Applicant's version is adopted. Based on the submitted efficacy studies the use of 50 ppm DBNPA is sufficiently effective for short-term preservation of mineral slurry for up to 7 days. For uses longer than 7 days new efficacy data must be submitted. It is considered that Tier 2 testing (ageing studies) are not relevant for the representative use as submitted Tier 1 testing covers the short-term preservation (≤ 7 days).
Reliability	Not relevant
Acceptability	acceptable
Remarks	
	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.* *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	

Table A5.2-1: Overview on biocidal uses of DBNPA in PT6

PT	Use	Typical micro-organisms to be controlled	Likely concentrations at which the A.S. will be used	User category
6	Short-term preservation of mineral slurries prior to use.	Bacteria	50 ppm DBNPA	Industrial

Table A5.3.1-1: Summary of experimental data on the effectiveness of DBNPA against target organisms at different fields of use envisaged

<table>
<tr><th>Test substance</th><th>Test organism(s)</th><th>Test system / concentrations applied / exposure time</th><th>Test results: effects, mode of action, resistance</th><th>Reference*)</th></tr>
<tr>
<td>[REDACTED] 20% DBNPA (2,2-Dibromo-2-cyanoacetamide) solution, [REDACTED]</td>
<td>Klebsiella aerogenes NCIMB 10102
Alcaligenes faecalis NCIMB 13147
Providencia rettgeri NCIMB 10842
Pseudomonas putida NCIMB 9494
Pseudomonas aeruginosa NCIMB 15442
Micrococcus luteus NCIMB 9278
Staphylococcus aureus ATCC 6538
Myroides odoratus ATCC 4651

Organisms commonly found in mineral slurries</td>
<td>The minimum inhibitory concentration (MIC) and the minimum biocidal concentration (MBC) were tested according to modified ISO 20776-1:2019: Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – Part 1: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.

Concentrations tested:
<table>
<tr><th>Product concentration in test</th><th>DBNPA in [REDACTED]</th></tr>
<tr><td>0.8000%</td><td>1600 ppm</td></tr>
<tr><td>0.4000%</td><td>800 ppm</td></tr>
<tr><td>0.2000%</td><td>400 ppm</td></tr>
<tr><td>0.1000%</td><td>200 ppm</td></tr>
<tr><td>0.0500%</td><td>100 ppm</td></tr>
<tr><td>0.0250%</td><td>50 ppm</td></tr>
<tr><td>0.0125%</td><td>25 ppm</td></tr>
<tr><td>0.0063%</td><td>12.50 ppm</td></tr>
<tr><td>0.0031%</td><td>6.25 ppm</td></tr>
<tr><td>0.0016%</td><td>3.125 ppm</td></tr>
<tr><td>0.0008%</td><td>1.5625 ppm</td></tr>
<tr><td>0.0004%</td><td>0.7813 ppm</td></tr>
</table>
Exposure time: 24hrs, 5 days
Test temperature: 30°C ± 2°C</td>
<td>The bacteria tested showed a minimum inhibitory concentration (MIC) for the mixture of microorganisms of 0.0063% of [REDACTED] in both cases equivalent to 12.5 ppm DBNPA.
[REDACTED] presented a MIC of ≥ 0.8%.

The bacteria tested showed a minimum biocidal concentration (MBC) for the mixture of microorganisms of 0.0125% of [REDACTED] in both cases equivalent to 25 ppm DBNPA.
[REDACTED] presented a MBC of ≥ 0.8%.

Both DBNPA formulations tested ([REDACTED]) demonstrated the same microbial effect; the products are therefore accepted as equivalent.

Furthermore, the results show that the co-formulants [REDACTED] does not demonstrate antimicrobial activity.</td>
<td>Iredale, G and Allison, K. (2020): Report number; IMSL2020/02/009.2C1, B5.10/01.</td>
</tr>
</table>

Test substance	**Test organism(s)**	**Test system / concentrations applied / exposure time**	**Test results: effects, mode of action, resistance**	**Reference*)**
[REDACTED] (20% DBNPA solution), Ground calcium carbonate (GCC) mineral slurry (Hydrocarb 75 ME-78%) (solids 78%, pH 10.10)	*Klebsiella aerogenes* NCIMB 10102 *Alcaligenes faecalis* NCIMB 13147 *Providencia rettgeri* NCIMB 10842 *Pseudomonas putida* NCIMB 9494 *Pseudomonas aeruginosa* NCIMB 15442 *Micrococcus luteus* NCIMB 9278 *Staphylococcus aureus* ATTC 6538 *Myroides odoratus* ATCC 4651	The efficacy of DBNPA as a short-term preservative in ground calcium carbonate mineral slurry was tested according to the IBRG Tier 1 Method for Determining the Basic Efficacy of Biocidal Active Substances used to Preserve Aqueous-Based Products; Version IBRG PDG 16-007.03 (April 2019). The test was performed using multiple challenges of the consortium of bacteria. Mineral slurries prepared without a preservative were treated with different concentrations of DBNPA or sterile distilled water. The pass criterion was no growth in treated samples and growth in the untreated controls. Concentrations tested: 0 ppm (untreated control), 5, 10, 25, 50 ppm Exposure time: < 5 minutes, 1 and 7 days (inoculated twice, 7 days apart) Test temperature: 30°C ± 2°C	Growth was observed in the un-treated control following the first incubation, while no growth was observed for up to 7 days in the GCC mineral slurry treated with 5, 10, 25 and 50 ppm DBNPA after the first inoculation following a 7-day incubation period. Growth was observed in the GCC mineral slurry treated with 5, 10, 25 and 50 ppm DBNPA following the 2nd inoculation. No growth was observed in the untreated control after the second inoculation.	Iredale, G and Webb, R (2020): Report number; IMSL2020/02/009.2A.1, B5.10/02

Test substance	Test organism(s)	Test system / concentrations applied / exposure time	Test results: effects, mode of action, resistance	Reference*)
[REDACTED] (20% DBNPA solution), Kaolin Mineral Slurry (Hydragloss 90 Clay-ML 71%)	*Klebsiella aerogenes* NCIMB 10102 *Alcaligenes faecalis* NCIMB 13147 *Providencia rettgeri* NCIMB 10842 *Pseudomonas putida* NCIMB 9494 *Pseudomonas aeruginosa* NCIMB 15442 *Micrococcus luteus* NCIMB 9278 *Staphylococcus aureus* ATTC 6538 *Myroides odoratus* ATCC 4651	The efficacy of DBNPA as a short-term preservative in kaolin slurry was tested according to the IBRG Tier 1 Method for Determining the Basic Efficacy of Biocidal Active Substances used to Preserve Aqueous-Based Products; Version IBRG PDG 16-007.03 (April 2019). The test was performed using multiple challenges of the consortium of bacteria. Mineral slurries prepared without a preservative were treated with different concentrations of DBNPA or sterile distilled water. The pass criterion was no growth in treated samples and growth in the untreated controls. Concentrations tested: 0 ppm (untreated control), 5, 10, 25, 50 ppm Exposure time: < 5 minutes, 1 and 7 days (inoculated twice) Test temperature: 30°C ± 2°C	Growth was observed in the un-treated control following the first incubation, while no growth was observed for up to 7 days in the kaolin mineral slurry treated with 5, 10, 25 and 50 ppm DBNPA after the first inoculation following a 7-day incubation period. Following the second inoculation, growth was observed in the kaolin mineral slurry treated with 5, 10 and 25 ppm DBNPA. No significant growth was observed in the slurry preserved with 50 ppm DBNPA, however, growth was observed in one of three replicate sub-samples treated with 50 ppm DBNPA. This resulted in the difference between the < 5 minutes observations and the 7 days observations being statistically non-significant. Growth in one sub-sample is regarded as an outlier by the applicant and assumed to be the result of the 50 ppm application rate being at the limit of efficacious treatment of kaolin mineral slurry for up to 14 days. However, using IQR on the data from the 7 days observations, the eCA did not identify the sub-sample with growth as an outlier, the absence of significance is therefore considered as the pass criteria not being met. Growth was observed in the untreated control after the second inoculation.	Iredale, G and Webb, R (2020): Report number; IMSL2020/02/009.2B.1, B5.10/03

*) References:

- Iredale, G and Allison, K. (2020): MINIMUM INHIBITORY CONCENTRATION AND MINIMUM BIOCIDAL CONCENTRATION OF TWO DBNPA BIOCIDES (20% SOLUTIONS), [REDACTED], Industrial Microbiological Services Ltd, Pale Lane, Hartley Wintney, Hampshire, UK, Report number IMSL2020/02/009.2C1, Section Point B5.10/01

- Iredale, G and Webb, R. (2020): DETERMINATION OF EFFICACY OF DBNPA BIOCIDE FOR USE AS A SHORT-TERM PRESERVATIVE IN A CALCIUM CARBONATE MINERAL SLURRY, Industrial Microbiological Services Ltd, Pale Lane, Hartley Wintney, Hampshire, UK, Report number. IMSL2020/02/009.2A.1, Section Point B5.10/02
- Iredale, G and Webb, R (2020), DETERMINATION OF EFFICACY OF DBNPA BIOCIDE FOR USE AS A SHORT-TERM PRESERVATIVE IN A KAOLIN SLURRY, Industrial Microbiological Services Ltd, Pale Lane, Hartley Wintney, Hampshire, UK, Report number IMSL2020/02/009.2B.1, Section Point B5.10/03