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Triameen® Y12D – High Performance, Broad Spectrum Biocide for Disinfection and Preservation

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Introduction

Control of harmful microorganisms is important in many application fields to ensure human and animal welfare and product integrity. The trend to more sustainable products/processes like use of natural based raw materials, reduction of solvents and increasing recycling of industrial water enhance the risk of microbial deterioration. Hence biocides play an important role in safeguarding our current standard of living and realizing a more sustainable future.

In Europe the making available on the market and use of biocidal products is regulated by the Biocidal Products Regulation (BPR, Regulation (EU) No 528/2012). It replaces the European Biocidal Product Directive (BPD, Directive 98/8/EC).

The purpose of the BPR is to improve the free movement of biocidal products within the Union while ensuring a high level of protection of both human and animal health and the environment.

Due to high cost involved in registration many biocidal active substances were not supported and disappeared in the meantime from the market. Others were not approved in the still ongoing BPR review process. Hazard labelling according to Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP regulation), e.g. as sensitizer, mutagen or carcinogen lead to further decrease of appropriate actives. Last, but not least, also public opinion plays an important role in acceptance of active substances.

All these factors resulted in strong decrease of available active substances for specific application fields and increasing risk falling below an adequate number of different molecules for safe and effective biocide applications.

In this article we will explore the particular performance characteristics that make Triameen® Y12D such an interesting and differentiated biocide for use in disinfection and preservation today.

Triameen® Y12D

Triameen® Y12D (N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine, CAS no 2372-82-9) is a highly effective biocidal active substance applicable in a broad variety of disinfectant and preservative applications. The molecular structure of Triameen® Y12D is shown in **Fig. 1**. As can be seen it has the shape of a "Y" with primary amine groups (blue circles) at the end of the 2 propyl groups and a tertiary amine group (black circle) at the cross-point.

Fig. 1 Molecular structure of Triameen® Y12D.

Triameen® Y12D is in the review program of the BPR under the short name "Diamine".

Nouryon Surface Chemistry supports the following BPR product types (**Tab. 1**).

Disinfection applications

Triameen® Y12D is broadly used in the field of disinfection. Below we will give specific attention to it's use in indirect food/feed contact applications.

As mentioned above, Triameen® Y12D is in the BPR review process for PT4 application, Food and feed area disinfection. In the dossier we have shown that usual concentrations are safe regarding indirect food contact. We are still waiting for evaluation of our dossier by the authorities for final approval, but we do not expect any issues. In the meantime, Triameen® Y12D can be continued to be used for this application.

Main Group	Product Type
1: Disinfectants	2: Disinfectants and algaecides not intended for direct application to humans or animals 3: Veterinary hygiene 4: Food and feed area
2: Preservatives	6: Preservatives for products during storage 11: Preservatives for liquid-cooling and processing systems 12: Slimicides 13: Working or cutting fluid preservatives

Tab. 1

For some active substances being also used as pesticides so-called MRL=maximum residue limits have been fixed. An MRL is the highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly (Good Agricultural Practice). For those active substances for which an MRL has been fixed this is also applicable for disinfection in the food/feed area. An example for this MRL setting coming out of the pesticide application and transferred to surface disinfection are the quaternary ammonium compounds DDAC (Didecyldimethylammonium chloride, CAS number 7173-51-5) and BKC (C12-16-alkyldimethylbenzylammonium chloride, CAS number 68424-85-1). Their MRL has been set to 0.1 mg/kg foodstuff (REGULATION (EU) No 1119/2014).

For Triameen® Y12D there is currently no MRL set. If this will be needed, it will be decided in several years after careful monitoring and risk assessment starting after the BPR approval of the active substance for PT4. Background for this is the EU Note for agreement (CA-March17-Doc.7.6.c-final) describing an interim approach to decide in which situations and/or under which conditions it is necessary to establish MRL values.
Another piece of legislation used as orientation help if specific molecules may be used in food contact application in the cleaning/disinfection area is the so-called French Positive List. This is a list based on French Decree No. 73-138 of 12 February 1973 (amended several times) on cleaning products for materials and articles intended to come into contact with foodstuffs, products and beverages for human and animal consumption.
Triameen® Y12D is in compliance with the French Positive List.

Preservative applications

Also, in the field of preservation the question of allowance in food contact applications is of significant interest. In this context the recommendations of the German Federal Institute for Risk Assessment (BfR) on Food Contact Materials are often used for orientation. They are based on European law and its transposition into national law.
Triameen® Y12D is listed in the following BfR recommendations:

- XIV: Polymer dispersions for coating commodities
- XXXVI: Paper and board.

Many active substances for preservation show strong limitation in usability due to hazard labelling as being a sensitizer, mutagen or carcinogen. Triameen® Y12D does not have any of these properties.
It is also free of aldehydes and halogens.

Another limitation of a number of active substances is their suitability as preservatives for EU Ecolabel formulations. Many have a concentration limit mentioned which is below the level of efficacy. For Triameen® Y12D there is no limit mentioned.
It is largely naturally sourced, with the natural component of vegetable origin resulting in 67% renewable carbon index (RCI). Triameen® Y12 will be soon available in an RSPO certified version.

Efficacy

An overview on the spectrum of efficacy of Triameen® Y12D is given in **Fig. 2**. It shows the concentration in ppm active substance needed to pass the quantitative suspension tests EN 1276 and EN 1650. For simulation of practical conditions, the tests are performed in the presence of water hardness (17°dH (German hardness) = 300 mg/kg as $CaCO_3$) and bovine albumin as protein load. The tests in **Fig. 2** were conducted at a level of 0.03% bovine albumin (clean conditions). Different gram negative and gram positive bacteria were tested as well as fungi. Decreasing concentrations in the figure point to increasing efficacy.

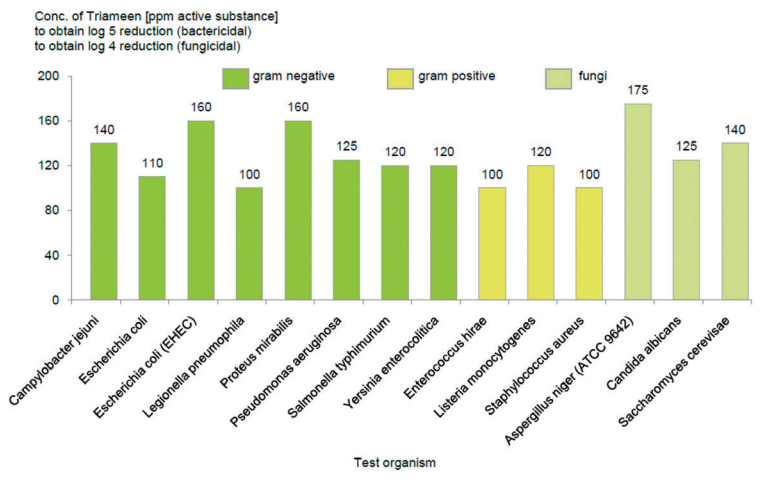

Fig. 2 Efficacy of Triameen® Y12D versus different gram negative and gram positive bacteria according to EN 1276 and versus fungi according to EN 1650.

It is remarkable that Triameen® Y12D has a very even level of concentration needed to pass the test versus the different organisms meaning that it has a broad spectrum of efficacy.
Triameen® Y12D is a fatty amine-based biocide, similar to quaternary ammonium compounds but without the permanent positive charge on the nitrogen. The nitrogen atoms of Triameen® Y12D may become partially positively charged in application, based on the pH value in relation to the pKa value of the amine groups.
When comparing the efficacy of Triameen® Y12D with that of the quats it is remarkable that the quats show a much stronger deviation in the spectrum. They are strongly effective against gram positive bacteria but significantly less effective against fungi and

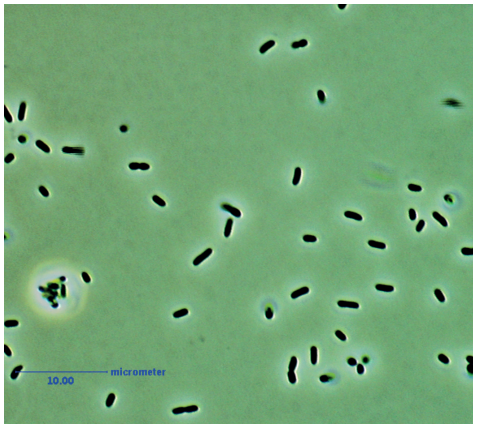

Fig. 3 Microscopic picture of the gram negative bacteria *Pseudomonas aeruginosa*.

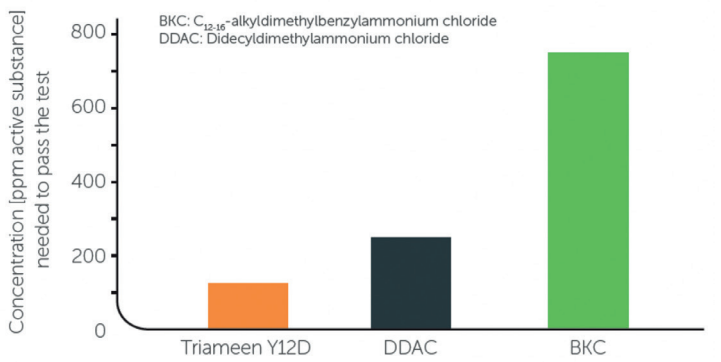

Fig. 4 Efficacy of Triameen® Y12D and the quaternary ammonium compounds DDAC and BKC versus the gram negative bacteria *Pseudomonas aeruginosa* according to EN 1276, clean conditions.

gram negative bacteria in the standard tests EN 1276 and EN 1650. Specifically, *Pseudomonas aeruginosa* (**Fig. 3**) needs relatively high concentrations of quats to be controlled. This can be seen in **Fig. 4**.

Efficacy against mycobacteria

Another group of bacteria being highly important in the field of disinfection and preservation are mycobacteria. Their extremely lipophilic cell wall is impermeable to the gram staining dyes which is why mycobacteria cannot be sorted into this category. *Mycobacterium tuberculosis*, the causative organism for tuberculosis is the most infamous representative of this group but there are further important pathogens and contaminants in it. Control of mycobacteria is of utmost importance in specific application fields such as disinfection in the medical and veterinary area and preservation of metal working fluids.
Quats are known as being ineffective against mycobacteria. In contrast to this Triameen® Y12D is effective. It passes the corresponding test EN 14348 (0.03% protein load) at 600 ppm for mycobactericidal and tuberculocidal efficacy.

Efficacy against viruses

Triameen® Y12D is also effective against enveloped (lipophilic) viruses.
For example, against influenza virus H1N1 the test EN 14476 (0.03% protein load) is passed at 300 ppm active substance and a contact time of 10 minutes.

pH influence on the efficacy of Triameen® Y12D versus *Pseudomonas aeruginosa* in the presence of water hardness

In general is can be stated that the efficacy of Triameen® Y12D increases with increasing pH value. *Pseudomonas aeruginosa* however shows a specific behaviour in the presence of water hardness in the standard test. It's efficacy also increases with increasing pH value but gets lost above the pH of 9. This effect is only observed with *Pseudomonas aeruginosa* and not with the other bacterial test strains in EN 1276. At pH 9 there is the pKa value to the tertiary amine group of Triameen® Y12D.
Pseudomonas aeruginosa as gram negative bacteria has an outer membrane in the cell wall. It is known that water hardness, so Ca^{2+} and Mg^{2+} ions stabilize this outer membrane leading to general decrease of efficacy of biocides. It seems that in this context a partly positive charge at the molecule of Triameen® Y12D is important for adherence and penetration through the outer membrane if high concentrations of the bivalent water hardness ions are present.

In **Fig. 5** we plotted on the y-axis the concentrations of Triameen® Y12D (ppm active substance) needed to pass the test at different pH values on the x-axis. The pH value was ad-

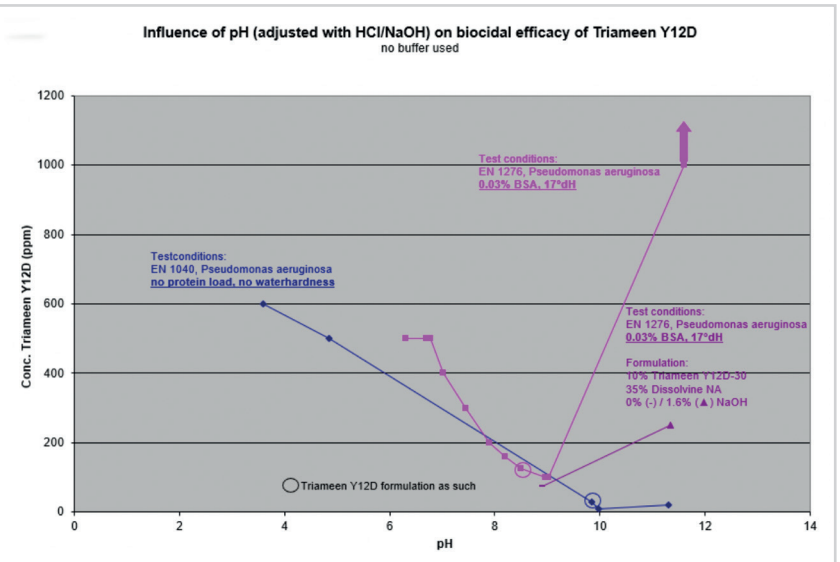

Fig. 5 Influence of pH value on the biocidal efficacy Triameen® Y12D versus *Pseudomonas aeruginosa* at different levels of water hardness respectively with and without chelant

justed with HCl or NaOH to avoid the influence of any other more complex ions which would be needed e.g. with application of specific buffer systems. The test organism is *Pseudomonas aeruginosa*. The pH values plotted were measured after finalization of the tests in the test tubes (including test organisms, biocidal active substance, water hardness and protein load as described).
The pink line shows the influence of the pH value on the efficacy of Triameen® Y12D at standards test conditions of EN1276, so standard hardness of 17°dH. The breaking point at pH 9 can be clearly seen. The blue line shows the efficacy at different pH values at basic test conditions, so without protein load and water hardness. As can be seen there is no breaking point at pH 9 but further increase of efficacy up to an optimum level being reached at about pH 10.
The purple line shows test results on a formulation with an amount of EDTA (added as Dissolvine NA) appropriate to "neutralize" the water hardness in the disinfectant test. Here it is seen that with addition of the sequestering agent the strong decrease of efficacy above pH 9 is nearly neutralized.

If in practice efficacy at final use concentration is needed at a pH higher than 9 there are different possibilities to ensure sufficient efficacy versus *Pseudomonas aeruginosa*. The method of choice could be reducing the water hardness with adequate concentrations of sequestering agents like EDTA (Ethylenediaminetetraacetic acid) or GLDA (Glutamic acid, N,N-diacetic acid). Both chelating agents are available in the market under the trade name Dissolvine®.
It must be born in mind that in practice application of disinfectants in most cases lower water hardness will be present than in the standard test representing the "worst case". So, recommending dilution of disinfectant concentrates with deionized water or bringing corresponding ready to use products onto the market might be another possibility getting good effects versus *Pseudomonas aeruginosa* at pH values higher than 9.

In preservative applications, the correlation between water hardness (Ca^{2+} and Mg^{2+}) ions and pH value is also important to bear in mind because *Pseudomonas aeruginosa* as water borne organism is an important contaminant. Products without any Ca^{2+} or Mg^{2+} ions might need only low concentrations of Triameen® Y12D for adequate preservation even if the pH is above 9.

Market products

There are 2 products available on the market. Triameen® Y12D as nearly 100% active substance and Triameen® Y12D-30 as 30% aqueous dilution of Triameen® Y12D. The latter is more easily miscible with water. Although Triameen® Y12D is highly soluble in water it has first to overcome a so-called gel-phase upon dilution. Dilution of Triameen® Y12D needs strong stirring and heating to avoid gel formation, which can otherwise lead to unequal distribution of the active substance in formulations. For those customers having problems to realize such conditions in their own production we recommend the use of the pre-diluted 30% product Triameen® Y12D-30.

Guideline formulation with Triameen® Y12D

The following guideline formulation was developed and evaluated:
- 10% Triameen® Y12D-30
- 8% Dissolvine® GL-47-S
- 82% water

We have chosen Triameen® Y12D-30 as being easily formulated. Dissolvine GL-47-S contains GLDA (CAS 51981-21-6), a chelating agent with superior environmental and (eco)toxicological properties.

Tab. 2 shows selected phys-chem. properties of the concentrated formulation as well as different dilutions in hard water.

	Concentrate	Dilution		
		1 : 50*	1 : 75*	1 : 200*
Appearance at 20°C	Clear liquid	Clear liquid	Clear liquid	Clear liquid
Cloud point	55 °C	> 75 °C	> 75 °C	> 75 °C
Density	1.02 g/cm³	1.00 g/cm³	1.00 g/cm³	1.00 g/cm³
pH	12.0	10.1	9.9	9.4
Foam height**				
Immediately		157 mm	140 mm	128 mm
After 1 minute		152 mm	138 mm	125 mm
After 5 minutes		145 mm	136 mm	121 mm
Surface Tension (Du Noüy)	31.0 mN/m	30.5 mN/m	30.6 mN/m	30.2 mN/m
CMC	17 mg/L			

* dilution is done in water 17°dH
** 20°C, according to Vindan

Tab. 2 Physikalisch-chemische Eigenschaften der Richtformulierung mit Triameen® Y12D.

This guideline formulation

Results on efficacy tests for surface disinfection, clean conditions:
Different standard efficacy tests for surface disinfection were performed with a protein load of 0.03% bovine albumin and water hardness of 17°dH:

- EN 1276, bactericidal efficacy: passed at 0.4% (= 120 ppm active substance) for all 4 test organisms
- EN 1650, yeasticidal efficacy: passed at 0.3% (= 90 ppm active substance) for Candida albicans
- EN 13697 (Surface test without mechanical action for bactericidal and yeasticidal efficacy): passed for all 5 test organisms at
  - 1.5 % (= 450 ppm active substance) if 0.03% bovine albumin was used as protein load for Pseuomonas aeruginosa (new method)
  - 2% (600 ppm active substance) if 0.85% skim milk was used as protein load for *Pseudomonas aeruginosa* (old method)

Summary

Triameen® Y12D is a highly efficient biocide for use in disinfectant and preservative applications. It has a broad spectrum of efficacy and is free of aldehydes, halogens and quats. It is not a sensitizer and is not classified as a mutagen or carcinogen. It is suitable for indirect food contact applications and does not have an MRL.

contact

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