GUIDANCE ON THE USE OF GLOBALLY-RELEVANT MODERN BIOCIDES

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Abstract

There are two major types of biocides: those designed to prevent spoilage of water-based liquids during manufacture and storage (in-can biocides) and those designed to prevent biological establishment on cured or solid compositions, regardless of whether they were originally water or solvent-based (dry film biocides). Selection of the most appropriate biocide requires a systematic approach encompassing consideration of, not only the physicochemical characteristics of the product, but also regulatory or marketing label requirements, relevant spoilage organism types, product lifespan requirements, manufacturing environment and economic viability. The industry trend toward reduction or replacement of organic solvents and impurities has given rise to compositions with abnormally high susceptibility to biological spoilage, complicating matters further. This, coupled to ever-increasing regulatory restrictions on biocides has challenged biocide manufacturers to formulate novel innovative multicomponent biocides which have broad versatility within and across various product ranges. The latest in-can and dry film biocide innovations are discussed.

Introduction

Replacement of solvent-based compositions with water-based equivalents due to VOC concerns or certification programs has gained steady momentum. Reduction of unreacted monomer in synthetic polymers, coupled to complete or partial replacement of organic solvents in compounded formulations, has given rise to products with dramatically increased susceptibility to in-can microbiological spoilage[1]. As such, consideration of an appropriate biocide in a formulation has become every bit as important as any other functional formulation component. There are only a handful of biocide chemistries to choose from, each with their own merits and limitations. Selection of the most appropriate biocide for any given product requires consideration of multiple factors, many of which may not be obvious to the formulator. To account for this, carefully formulated multicomponent biocides are typically recommended above ones based on a single active ingredient. This not only enables the biocide composition to work against a broader spectrum of target organisms by virtue of synergistic or complimentary effects, but also enables the formulator more latitude in using a single biocide composition over a wider range of products with different physicochemical characteristics.

Dry film biocides, where required, are added separately, and function to prevent biological establishment on the cured or dry product. The actives used in these biocides typically have low water solubilities, and are therefore often formulated as dispersions or micro-emulsions where low VOC is a requirement. Dry film biocides are typically fungicidal or algicidal in nature, as opposed to in-can biocides, which are mostly bactericidal or fungicidal in nature.

In-Can biocides:

Although there are numerous in-can biocide molecules on the market today, five molecule types constitute more than 80% of the volume used in water-based industrial products[2] (Table 1). Each of these perform best under defined physicochemical conditions, and each have their own efficacy profiles. When used singly, preservation potential and versatility are often limited, hence the preference for blends of these for optimum preservation. Most of these molecules have electrophilic character, which makes them highly effective in shutting down critical metabolic pathways of offending microorganisms at low concentration. At the same time, however, reactive functional groups of formulation components may also negatively impact these molecules, and therefore consideration needs to be given to the most appropriate addition point in the manufacturing process. In our experience, the two most effective and versatile in-can preservation systems are based on either a MIT/CIT platform (mostly for products with a pH of less than 8), or a MIT/BIT platform (for products with pHs ranging between 2 and 11). Other suitable molecules can be included with these platforms to broaden their effectiveness or versatility.

Table 1. Summary of the most commonly used In-can biocidal actives.

Molecule	Efficacy / Stability
5-chloro-2-methyl-4-isothiazolin-3-one (CIT)*	Broad-spectrum, high efficiency, lower stability
2-methyl-4-isothiazolin-3-one (MIT)	Excellent bactericide, poor fungicide, good stability
1,2-benzisothiazolin-3-one (BIT)	Good bactericide, moderate fungicide, fair stability
2-bromo-2-nitropropane-1,3-diol (BNPD)	Effective bactericide, lower stability
Formaldehyde-donors - various	Weak fungicides, some bacteria can tolerate high levels

* sold commercially in combination with MIT in a ratio of 3:1 (CIT:MIT)

Factors to consider in a systematic approach to successful in-can biocide selection include the following: product pH, Redox potential, reactive nucleophile content, compatibility, susceptibility and regulatory compliance. Consideration of relevant spoilage organisms is also very important. Wild strains isolated from spoiled product or contaminated plant equipment should be considered when evaluating biocide efficacy. Specialist knowledge and experience are essential in this respect. For example, a published MIC value for *Pseudomonas putida* laboratory strain is 250 ppm for BIT, whereas (in practice) more than 750 ppm may be required to prevent establishment of an equivalent wild strain (Table 2). The use of biocide blends will, in most instances, mitigate consideration of this variable. In addition, the chances of bacteria developing tolerance against any one biocide active is reduced significantly when using blends.

Since biocides are designed to kill living organisms, they are subjected to ever-increasing global regulatory scrutiny. In Europe, in particular, the Second Adaptation to Technical Progress of the Classification, Labeling and Packaging (CLP) regulation (1272 / 2008 / EC) will restrict the level of many commonly-used actives classified as sensitizers, above which would require a warning phrase (Table 3). The proposed levels will be $1/10^{th}$ of published 'Specific Concentration Limits' for applicable molecules, and will come into effect in June 2015. Although this regulation is not relevant in the United States, it will apply to manufacturers exporting to countries forming part of the European Union, and other countries following the European model. For most single biocide actives, the level not requiring a warning phrase may be completely inadequate for preservation (e.g. 1.5 ppm CIT/MIT or 50 ppm BIT).

Table 2. Minimum Inhibitory Concentration (MIC) values (ppm) of biocide actives against wild-type bacteria

Organism	MIC value (ppm) at 72 hours in Mueller-Hinton Broth				
	MIT	**BIT**	**MIT/BIT**	**CIT/MIT**	**Formaldehyde***
Corynebacterium sp.	75	>300	100	75	>600
Pseudomonas aeruginosa	40 *[30]*	>300 *[250]*	75	5 *[9]*	400
Pseudomonas sp.	50	400	75	15	750
Burkholderia cepacia	NT	450	50	NT	500
Pseudomonas putida	NT	>750 *[250]*	100	NT	500
Pseudomonas fluorescens	NT	300	100	NT	750

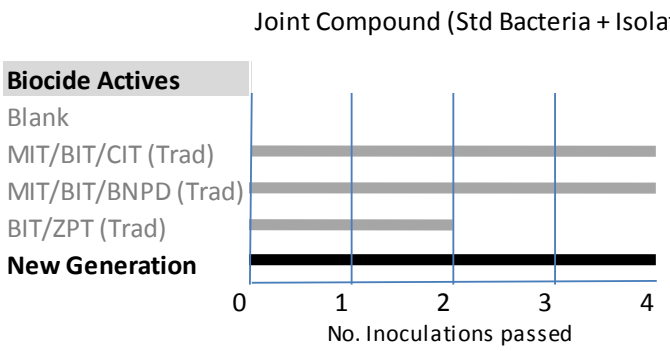
NT – not tested. Values in *[italics]* are those of laboratory strains published in reliable literature[3]
* Refers to biocides which rely on release formaldehyde for their efficacy

A range of New Generation in-can biocides has recently been developed, which can out-perform traditional biocides by factors of two or more without cautionary labeling relating to the forthcoming European legislation (Figures 1a-1e).

Table 3. Concentrations of commonly used actives given in Table 1, requiring sensitization labeling (ppm) in accordance with the June 2015 European regulation.

Active Ingredient	Sensitizer Category	Concentration requiring labeling (ppm)
CIT/MIT	1A	>/= 1.5
BIT	1A	>/= 50
MIT	1A	>/= 100
BNPD	Not Categorized	No Limit*
Formaldehyde Donors	1	1000[#]

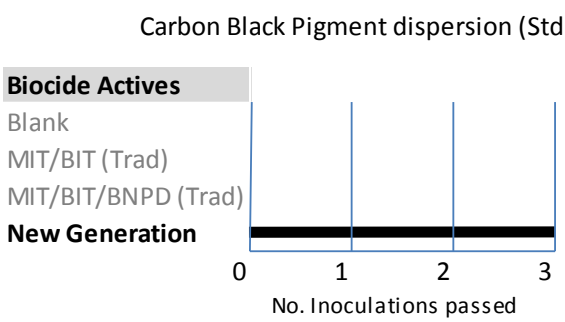
*High level can cause discoloration or affect cross-linking
[#]Depends on type



Mold Growth (4 wk)	Viable spores (4 wk)	Yeast (4 wk)	ppm Biocide	Sensit Label
Fail	Fail	Fail	0	N/A
Pass	**Pass**	**Pass**	80	Yes
Fail	Fail	**Pass**	130	**No**
Pass	**Pass**	**Pass**	115	Yes
Pass	**Pass**	**Pass**	50	**No**

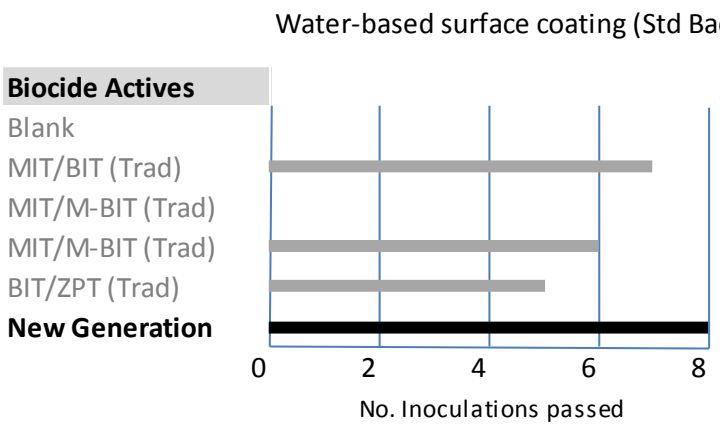
ZPT = Zinc-bis (2-thiolpyridine-N-oxide)

Figure 1a. In-can biocidal performance of a New Generation Biocide versus current Traditional Biocides in an alkaline tape joint compound. *Bacterial testing reflects four inoculation cycles; mold and yeast reflects a single inoculum with evaluation of viability following four weeks incubation under ideal conditions. Only the New Generation biocide was able to pass all microbiological testing without forthcoming EU cautionary labeling implications.*



Mold Growth (4 wk)	Viable spores (4 wk)	Yeast (4 wk)	ppm Biocide	Sensit Label
Fail	Fail	Fail	0	N/A
Fail	Fail	Fail	200	Yes
Fail	Fail	Fail	260	Yes
Pass	**Pass**	**Pass**	199	**No**

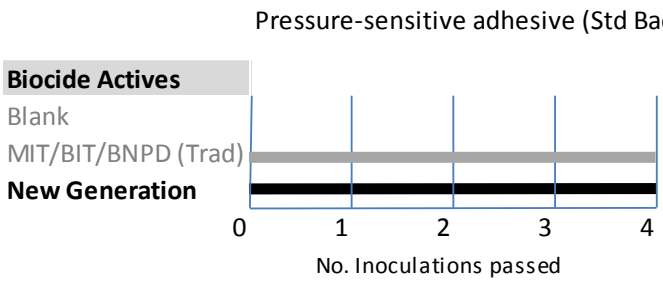
Figure 1b. In-can biocidal performance of a New Generation Biocide versus current Traditional Biocides in an alkaline carbon black pigment dispersion, known to be difficult to preserve. *Bacterial testing reflects three inoculation cycles; mold and yeast reflects a single inoculum with evaluation of viability following four weeks incubation under ideal conditions. Only the New Generation biocide had any measure of antimicrobial effect without forthcoming EU cautionary labeling implications.*



Yeast (*Candida*) (8 cycles)	ppm Biocide	Sensit Label
Fail	0	N/A
Fail	150	Yes
Fail	67	**No**
Fail	88	**No**
Pass	115	Yes
Pass	75	**No**

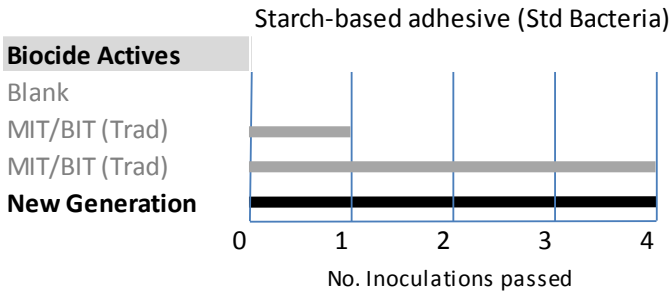
M-BIT = Methyl-BIT / DTBMA (Dithio-bis-methyl amide)

Figure 1c. In-can biocidal performance of a New Generation Biocide versus current Traditional Biocides in an alkaline acrylic surface coating. *Bacterial testing reflects eight inoculation cycles with an aggressive acclimated inoculum. Yeast testing reflects eight inoculation cycles with a wild-type yeast. Only the New Generation biocide was able to pass all microbiological testing without forthcoming EU cautionary labeling implications.*



Mold Growth (4 wk)	Viable spores (4 wk)	Yeast (4 wk)	ppm Biocide	Sensit Label
Fail	Fail	Fail	0	N/A
Pass	**Pass**	Fail	247	**No**
Pass	**Pass**	**Pass**	199	**No**

Figure 1d. In-can biocidal performance of a New Generation Biocide versus current Traditional Biocides in a Pressure-sensitive adhesive. *Bacterial testing reflects four inoculation cycles; mold and yeast reflects a single inoculum with evaluation of viability following four weeks incubation under ideal conditions. Only the New Generation biocide satisfied all efficacy requirements without forthcoming EU cautionary labeling implications.*



Mold Growth (4 wk)	Viable spores (4 wk)	Yeast (4 wk)	ppm Biocide	Sensit Label
Fail	Fail	Fail	0	N/A
Fail	Fail	Fail	75	**No**
Pass	**Pass**	**Pass**	375	Yes
Pass	**Pass**	**Pass**	199	**No**

Figure 1e. In-can Biocidal performance of a New Generation Biocide versus Traditional Biocides in an alkaline starch-based adhesive. *Bacterial testing reflects four inoculation cycles; mold and yeast reflects a single inoculum with evaluation of viability following four weeks incubation under ideal conditions. Only the New Generation biocide satisfied all efficacy requirements without forthcoming EU cautionary labeling implications.*

Dry film biocides:

As was the case for in-can biocides, only a handful of dry film biocide actives make up more than 80% of the volume used in industrial applications[2] (Table 4). Many others have, over the past 20 years, been selected against due to unfavorable human or environmental attributes. Each of the molecules given in Table 4 has some unfavorable technical, human/environmental or performance attribute. However, as was the case for in-can biocides, blends of two or more actives in favorable ratios can remedy one or more unfavorable attribute.

The greatest development regarding dry film biocides in the past 15-20 years has been the ability to encapsulate the biocide actives in an organic matrix to improve not only their in-can stability[7], but also to extend their longevity against harsh environmental influences in-service (heat, UV light, rain etc) compared to non-encapsulated equivalents. Advanced organic embedding encapsulation technology (referred to as AMME[5] technology) is the industry benchmark, and has grown exponentially over the past 10 years. Apart from extensive internal accelerated and field testing data showing the performance

and safety benefits of organically encapsulated actives over standard (traditional) actives,[2,4,6,7] various independent organizations have confirmed the concept, particularly with respect to reduced environmental emission and eco-toxicological effects.[8,9,10]

Table 4. Summary of the most commonly used dry film biocide actives.

Active Ingredient	Principle function
2-n-octyl-4-isothiazolin-3-one (OIT)	Fungicide
4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT)	Fungicide
3-iodopropynyl butylcarbamate (IPBC)	Fungicide
2,4,5,6-tetrachloro-1,3-dicyanobenzene(Chlorothalonil - CHL)	Fungicide
Methyl-N-(2-benzimidazolyl) carbamate (Carbendazim)	Fungicide
Zinc-bis(2-thiolpyridine-N-oxide) (ZPT)	Fungicide
3-(3,4-dichlorophenyl)-1,1-dimethylurea (Diuron)	Algicide
2-tert-butylamino-4-ethylamino-6-methylthio1,3,5-triazine (Terbutryn)	Algicide

There are many different dry film biocide blends, specifically formulated for regional requirements, regulatory compliance or formulation variations. In the USA, although organically encapsulated single active products are available, most interest centers around blends containing these actives. Biocides 1 & 2 (Table 5) are two such blends being sold to the US industry. Fungal and algal growth occurred on the blank and coating containing a standard biocide blend at 3840 ppm total biocide. No fungal or algal growth occurred on the coating with either organically encapsulated (AMME) biocides at substantially lower total biocide concentration (including the one without Diuron).

The same performance advantage of an organically encapsulated (AMME) biocide blend versus a standard biocide blend is given in Table 6. In this study, two different exterior coatings (semi-gloss and matte) were evaluated. Photographs of the test coupons of this study are given in Figure 2.

Table 5. Fungal and algal growth on a white exterior pure acrylic coating.

Inocula: Fungal Inoculum: 1.9×10^6 cfu/ml, Algal Inoculum: $>10^5$ cells/ml
Hours Leached: 48

SAMPLE	Fungal Growth	Algal Growth	Biocide (ppm)
Sample 1 (No Biocide)	**5**	**4**	Nil
Sample 1 + OIT + Carbendazim + Diuron (Std)	**4**	**2**	3840
Sample 1 + AMME Biocide 1 (contains Diuron)	0	0	2360
Sample 1 + AMME Biocide 2 (no Diuron)	0	0	2050

Film Fungal Growth Rating Chart for Test Method 800.2:

0 = No growth on sample, halo zone
1 = Trace fungal growth on edge of sample
2 = Growth from the edge 30% coverage of growth
3 = Growth of single colonies (30-50%)
4 = Sample surface widespread growth (50-75%)
5 = Sample surface strong or completely grown (75-100%)

Table 6. Fungal and algal growth on a white exterior semi-gloss and white exterior matte pure acrylic coating.

Fungal Inoculum: 2.8 x 10^6 cfu/ml, Algal inoculum: > 10^5 cells/ml
Hours Leached: 48

SAMPLE	Fungal Growth	Algal Growth	Ref / Figure
Semi Gloss (No Biocide)	**4**	**5**	2 a
Semi Gloss + IPBC, Carbendazim, Diuron (Standard)	**2**	**3**	2 b
Semi Gloss + AMME Biocide 1	0	0	2 c
Matte (No Biocide)	**5**	**5**	2 d
Matte + IPBC, Carbendazim, Diuron (Standard)	**3**	**4**	2 e
Matte + AMME Biocide 1	0	0	2 f

Film Fungal Growth Rating
0 = No growth on sample
1 = Trace fungal growth on edge of sample
2 = Growth from the edge 30% coverage of growth
3 = Growth of single colonies (30-50%)
4 = Sample surface widespread growth (50-75%)
5 = Sample surface strong or completely grown (75-100%)

Algal Growth Rating
0 = No growth
1 = Trace growth
2 = Slight growth
3 = Moderate growth
4 = Heavy growth
5 = Dense growth

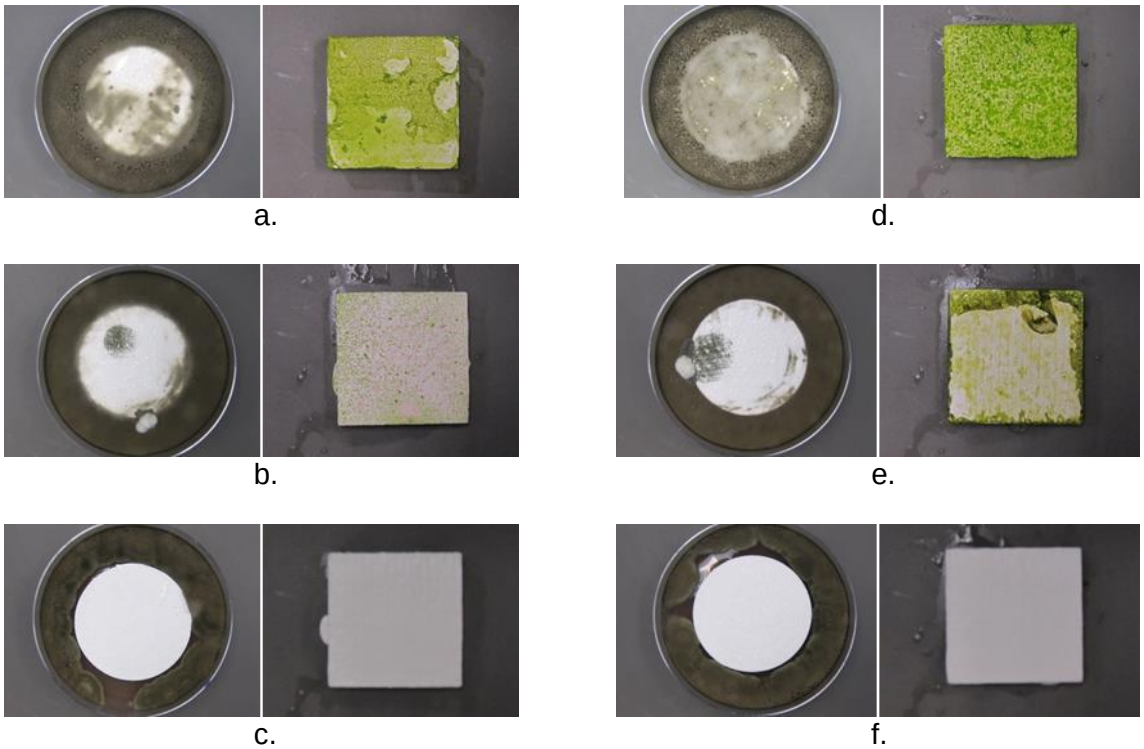


Figure 2. Fungal & Algal coupons relating to Table 6. Left are fungal coupons, right are algal coupons.

Summary:

Despite regulatory pressures reducing the number of biocide molecules from which to choose (or their concentrations in end-products), blends of carefully selected actives in optimal ratios, coupled with new technical advancements are able to provide improved preservation solutions without the need for cautionary labeling. Significantly better film retention of dry film biocide actives in organic capsules results in dramatic reductions in human and environmental impact whilst prolonging antimicrobial performance in-service. Choosing the correct biocide for any particular application requires consideration of multiple variables, and is best done with specialist assistance from a reputable biocide manufacturer.

References:

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