# Biocidal Products Committee (BPC)

Opinion on the application for approval of the active substance:

***Willaertia magna c2c maky***

**Product type: 11**

ECHA/BPC/206/2018

Adopted

26 April 2018

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# Opinion of the Biocidal Products Committee

**on the approval of the active substance *Willaertia magna c2c maky***
**for product type 11**

In accordance with Article 8(4) of Regulation (EU) No 528/2012 of the European Parliament and of the Council 22 May 2012 concerning the making available on the market and use of biocidal products (BPR), the Biocidal Products Committee (BPC) has adopted this opinion on the application for approval in product type 11 of the following active substance:

| | |
|---|---|
| **Common name:** | ***Willaertia magna c2c maky*** |
| **Chemical name(s):** | **Not applicable** |
| **EC No.:** | **Not applicable** |
| **CAS No.:** | **Not applicable** |

**New active substance**

This document presents the opinion adopted by the BPC, having regard to the conclusions of the evaluating Competent Authority. The assessment report, as a supporting document to the opinion, contains the detailed grounds for the opinion.

## Process for the adoption of BPC opinions

Following the submission of an application by Amoéba on 17 March 2014, the evaluating Competent Authority France submitted an assessment report and the conclusions of its evaluation to ECHA on 15 March 2017. In order to review the assessment report and the conclusions of the evaluating Competent Authority, the Agency organised consultations via the BPC (BPC-25) and its Working Groups (WGMO-2-2017 and WGMO-3-2018). Revisions agreed upon were presented and the assessment report and the conclusions were amended accordingly.

## Adoption of the BPC opinion

**Rapporteur: France**

The BPC opinion on the approval of the active substance *Willaertia magna c2c maky* in product type 11 was adopted on 26 April 2018.

The BPC opinion was adopted by consensus. The opinion is published on the ECHA webpage at: http://echa.europa.eu/regulations/biocidal-products-regulation/approval-of-active-substances/bpc-opinions-on-active-substance-approval.

# Detailed BPC opinion and background

## 1. Overall conclusion

The overall conclusion of the BPC is that the *Willaertia magna c2c maky* in product type 11 may not be approved. The detailed grounds for the overall conclusion are described in the assessment report.

## 2. BPC Opinion

### 2.1. BPC Conclusions of the evaluation

#### a) Presentation of the active substance including the classification and labelling of the active substance

This evaluation covers the use of *Willaertia magna c2c maky* in product type 11 (preservatives for liquid-cooling and processing systems). The mode of action *of Willaertia magna c2c* (amoeba) is not clearly demonstrated. It is assumed that it results from phagocytosis of microbial cells.

The identity of the active substance is demonstrated. The Restriction Fragment Length Polymorphism method (RFLP) presented may be considered as a valid method to identify the active substance. However, raw data (electrophoresis figures) of the RFLP gels are needed to fully validate this method. Moreover the phylogenetic tree should be updated with the three *W. magna* strains included in the RFLP study.

Specifications for the reference source can be established. However as the method used for the determination of the active substance cannot distinguish if the cells are dead or alive, a new five batch analysis with an appropriate analytical method should be provided in order to confirm the specifications.

The physico-chemical properties of the active substance and biocidal product have been evaluated and are deemed acceptable for the appropriate use, storage and transportation of the active substance and biocidal product.

There is no classification and labelling for *Willaertia magna c2c maky* according to Regulation (EC) No 1272/2008 (CLP Regulation) as microorganisms are not in the scope of the CLP regulation. However, all microbial active substances are regarded based on the precautionary principle as potential skin and respiratory sensitisers and the labels will require the following phrase “Microorganisms may have the potential to provoke sensitising reactions”. The active substance is not biohazardous according to Directive 200/54/EC on the protection of workers from risks related to exposure to biological agents at work.[1]

#### b) Intended use, target species and effectiveness

*Willaertia magna c2c maky* is intended to be used by professionals to prevent the growth of *Legionella pneumophila* in industrial processing water systems.

[1] OJ L 262, 17.10.2000 p.21

Efficacy data were only submitted for cooling towers. Assessment of the efficacy data leads to the following conclusions:

- According to the results from laboratory studies and peer-reviewed publications, the efficacy of *W. magna c2c maky* is not always reproducible.

  Indeed contradictions within the results from the different laboratory studies and peer-reviewed publications are pointed out. Notably some co-culture studies with *Willaertia magna c2c maky* and *L. pneumophila* (Lp) showed that if some Lp strains are effectively phagocytised by *W. magna c2c maky* and a decrease of Lp was observed, some other Lp strains remain alive within the amoeba, while others multiplied. Therefore in laboratory, permissivity of *W. magna c2c maky* is strain and MOI[2] dependent, which is not compatible with a treatment in a cooling tower where Lp strains and level of contamination are not known.

  As a conclusion of the laboratory tests, the intracellular stability of some bacterial strains within *W.magna c2c maky* means that the ingested bacteria can remain viable (demonstrated by CFU method[3]), and sometimes in high concentration, showing the role of reservoir of *Willaertia magna c2c maky*.

  To conclude, the presence of *W. magna c2c maky:*
  (1) does not guarantee the prevention of the *Legionella pneumophila* growth and,
  (2) can favor, in some conditions (strains, ratio biocide/target) its survival or even its growth.

  The applicant reported experiments on 10 sites (R&D in France) in order to evaluate the efficiency of the representative product containing the active substance. The treatment strategy implemented in these tests is intended to prevent *L. pneumophila* growth in industrial cooling water systems. However, the criterion used to decide when and how the water of the cooling towers was treated with *W. magna c2c maky* was not available in these field studies. For the majority of sites, the water was not contaminated with *L. pneumophila* (levels of Lp under the action threshold) at the beginning of the assays as a chemical treatment occurred (chlorine) prior to the injection of the active substance in the system. So the treatment with *W. magna c2c maky* was performed on cooling systems that had apparently no major problem with legionella. For this reason these tests cannot be used as a demonstration of the *W. magna c2c maky* efficacy. In addition, for the majority of sites no control system without *W. magna c2c maky* treatment was performed in parallel (i.e. *W. magna c2c maky* in one tower and chemical biocide in another one) although it is realized that having proper controls in field studies is problematic.

  Moreover for some tested industrial sites, poor or absence of efficacy is observed even if the product is injected several times per day. It has to be noted that for one site where there was a comparison between *W. magna c2c maky* and a chemical treatment, the chemical treatment was able to stabilize the concentration of *L. pneumophila* below the detection limit. However, when the *W. magna c2c maky* treatment was experienced, peaks of cultivable *L. pneumophila* (CFU) occurred even if high volumes of the product were applied, without any effect on the bloom of *L. pneumophila*.

[2] The multiplicity of infection or MOI is the ratio of agents (e.g. *Legionella*) to infection targets (e.g. amoebae).
[3] A colony-forming unit (CFU) is a unit used to estimate the number of viable microbes cells in a sample

Furthermore, the efficacy studies presented do not allow determining the effective dose(s) since no dose-effect was achieved. The rationale for the product application rate is without a coherent explanation, since the criteria triggering the treatment are not identified.

Therefore, innate efficacy of *W. magna c2c maky* is not sufficiently demonstrated and the application rate cannot be determined.

**c) Overall conclusion of the evaluation including need for risk management measures**

**Human health**

No evidence of pathogenicity, infectivity, mortality or clinical signs of toxicity was observed when tested up to the maximum recommended dose following a single administration.

However, due to the mode of action of the active substance, pathogens can be taken up by phagocytosis and internalized in *Willaertia magna c2c maky* in vesicles without being digested (i.e surviving intracellularly). *Willaertia magna c2c maky* becomes a reservoir (Trojan horse effect) of possible pathogens.

No information is available on the toxicity or infectivity of the active substance when containing internalized pathogenic microorganisms, or in the free vesicles[4] liberated when *Willaertia magna c2c maky* is lysed, or on its cyst form.

The table below summarises the different exposure scenarios that were assessed.

| **Summary table: human health scenarios** | | | |
|---|---|---|---|
| **Scenario** | **Primary or secondary exposure Description of scenario** | **Exposed group** | **Conclusion** |
| Loading of biocidal product into the injection cabinet | By loading the product cubitainer into the injection cabinet (injection pump), the water treatment operator may have dermal contact with spills of the biocidal product. | Professional | Acceptable with PPE (gloves, goggles and appropriate clothing) |
| Maintenance of the industrial water system | During maintenance of the industrial water system, cleaning and maintenance of the equipment, monitoring the cooling water system and the disposal of waste, dermal contact and/or inhalation (when an aerosol is generated) via treated water may occur. | Professional | Acceptable with PPE (gloves, goggles, and RPE when aerosols are generated) |
| Atmospheric emission from cooling system | General public around cooling towers are exposed to the plume generated, through dermal or inhalation exposure. | General public | Not acceptable |

PPE: Personal Protective Equipment

[4] During the process of phagocytosis, the amoeba engulfs microorganisms in vesicles. When internalized pathogenic microorganisms exhibit resistance to the host digestion they are released in a package of variable sizes of vesicles once the host which is in this case is *Willaertia magna c2c maky* is lysed.

Pathogens can be taken up by phagocytosis and internalized in *Willaertia magna c2c maky* in vesicles without being digested (i.e surviving intracellularly). *Willaertia magna c2c maky* thus becomes a reservoir (Trojan horse effect), and in case of lysis of the amoeba, the internalized microorganisms can be released into the environment freely or in vesicles. Vesicles can contain numerous pathogens. In addition, for certain strains of *Legionella,* an acquisition or an increase of virulence and pathogenicity after internalization into amoeba has been reported.

For professional users, no risk is identified due to the automated loading of the product into the system and to the application of appropriate risk mitigation measures (PPE, prevention of aerosols generation, use of RPE in case of aerosol generation).

However, according to the available data, general public exposure via plume to *Willaertia magna c2c maky* and to any pathogens carried by it or its vesicles cannot be ruled out. The droplets size released from the cooling towers might be up to 40 µm. Therefore, *Willaertia magna c2c maky* (mainly the cyst form <18 µm) and the vesicles (<10µm) could be released into the droplets. The extent of the exposure cannot however be quantified. Considering that the exposure of the general public to cysts and vesicles cannot be excluded and that the Trojan horse effect still remains as a concern, it is concluded here that the risk is considered as not acceptable for the general public.

**Environment**

As *Willaertia magna c2c maky* is a microorganism, issues related to degradation half-lives or possible routes of degradation are not relevant. Ecotoxicity tests showed no toxicity of *Willaertia magna c2c maky* for tested densities between 6E+07 and 1E+08 cells/L for the aquatic tests and between 2.7E+09 and 5.8E+11 cells/kg for the terrestrial tests. There is no information regarding the form (cyst / trophozoites) of added *Willaertia magna c2c maky* in the tests. According to the Guidance on the Biocidal Products Regulation Volume V on Active Microorganisms and Biocidal Products[5], no PNEC should be derived for microorganisms substances. Therefore, endpoints from ecotoxicity tests have been directly compared to estimated environmental density (EED).

No information is available on the ecotoxicity of the active substance when containing internalized pathogenic microorganisms, or on the free vesicles liberated when *Willaertia magna c2c maky* is lysed.

[5] Guidance on the Biocidal Products Regulation Volume V, Guidance on Active Microorganisms and Biocidal Products, ECHA, version 2.1, March 2017

The table below summarises the exposure scenarios assessed.

| **Summary table: environment scenarios** | | |
|---|---|---|
| Large open recirculating cooling systems | Direct emission to surface water. Direct emission to air due to evaporation and spray and wind drift, subsequent deposition on soil. | Acceptable for short term exposure. No conclusion on chronic exposure, which needs to be addressed through monitoring of effluents and plume on sites where *Willaertia magna* is used. |
| Small open recirculating cooling systems without Sewage Treatment Plant (STP) | Direct emission to air, surface water and soil through air deposition. | Acceptable for short term exposure. No conclusion on chronic exposure, which needs to be addressed through monitoring of effluents and plume on sites where *Willaertia magna* is used. |
| Small open recirculating systems with emission of waste water to STP | Direct emission to air, soil through air deposition. Emission to surface water, soil via STP | Acceptable for short term exposure. No conclusion on chronic exposure, which needs to be addressed through monitoring of effluents and plume on sites where *Willaertia magna* is used. |

According to a semi quantitative assessment for environment, it is not expected that short term releases of *Willaertia magna c2c maky* in the environment could have adverse effects on non-target-organisms. However, available information tends to indicate that numerous *Willaertia magna c2c maky* applications should occur for the treatments of the systems leading to continuous releases in the environment. Nevertheless, no reliable data have been provided to address chronic exposure of non-target organisms. Therefore it is difficult to state if in such conditions acceptable risks for the environment are expected.

The relevance of known chronic ecotoxicity tests to assess the chronic risk of *Willaertia magna* is open to question as the impact of amoebae on other organisms could be in first place on microorganisms which are not well studied in usual tests. Besides, considering that *Willartia magna c2c maky* is a new active substance for biocidal purposes, monitoring studies or impact assessment on the environment following the use of biocidal products containing *Willartia magna c2c maky* are not available. Therefore, it is recommended to request (as a condition for product authorisation where this information would need to be submitted one year after the authorisation of the biocidal product) monitoring in effluents and plume on sites where *Willaertia magna* is used.

Mitigation or correction measures should be proposed for the cases where significant *Willaertia* are quantified. In the case of failure to avoid the release of *Willartia magna c2c maky* to the environment, the impact on biodiversity should be assessed either by direct monitoring in situ or through representative micro- or mesocosm tests. At last, the potential Trojan horse issue, leading to selection and amplification of other microbial species has not been taken into account. It is however confirmed that *W. magna* c2c maky can act as a reservoir of certain pathogenic strains either in trophozoite or cyst form. The release of different microbial species and the resulting effects on organisms of the environment are not known.

**Overall conclusion**

Regarding the identity of the active substance, the Restriction Fragment Length Polymorphism method (RFLP) as submitted may be considered as a valid method to identify the active substance. However, the raw data (electrophoresis figures) of the RFLP gels are required in order to fully validate this method. Moreover the phylogenetic tree should be updated with the three *W. magna* strains included in the RFLP study.

Regarding the efficacy and the risk to human health or to the environment, several concerns are identified in consequence of the hazard and exposure assessment for the active substance when considering the intended use and are listed in each relevant section as mentioned above. The major concerns are cited below:

- In laboratory studies, the efficacy of *W. magna c2c maky* is not always reproducible. The presence of *W. magna c2c maky*

  (1) does not guarantee the limitation of the *Legionella pneumophila* growth and,

  (2) can favour, in some conditions (strains, ratio biocide/target) its survival or even its growth.

  Therefore innate efficacy of *W. magna c2c maky*, an active substance that is intended to control Legionella which poses a high risk to human health, is not sufficiently demonstrated.

- *W. magna c2c maky* can act as a reservoir of certain pathogenic strains either in trophozoite or cyst form which leads to a potential Trojan horse effect of the active substance.

  Considering that the exposure of the general public to cysts and vesicles cannot be excluded and that the Trojan horse effect still remains as a concern, it is concluded here that the risk is considered as not acceptable for the general public.

In view of these conclusions of the evaluation, it cannot be concluded that a safe use of *Willaertia magna c2c maky* is demonstrated.

## 2.2. Exclusion, substitution and POP criteria

### 2.2.1. Exclusion and substitution criteria

The table below summarises the relevant information with respect to the assessment of exclusion and substitution criteria:

<table>
<tr><th colspan="2">Property</th><th colspan="2">Conclusions</th></tr>
<tr><td rowspan="3">CMR properties</td><td>Carcinogenicity (C)</td><td>Not applicable *</td><td rowspan="3">*Willaertia magna c2c maky* does not fulfil criterion (a), (b) and (c) of Article 5(1) and criterion (a) of Article 10(1)(a)</td></tr>
<tr><td>Mutagenicity (M)</td><td>Not applicable *</td></tr>
<tr><td>Toxic for reproduction (R)</td><td>Not applicable*</td></tr>
<tr><td rowspan="3">PBT and vPvB properties</td><td>Persistent (P) or very Persistent (vP)</td><td>Not applicable**</td><td rowspan="3">*Willaertia magna c2c maky* does not fulfil criterion (e) of Article 5(1) and criterion (d) of Article 10(1)</td></tr>
<tr><td>Bioaccumulative (B) or very Bioaccumulative (vB)</td><td>Not applicable **</td></tr>
<tr><td>Toxic (T)</td><td>Not applicable **</td></tr>
<tr><td>Endocrine disrupting properties</td><td colspan="3">Not applicable**<br><br>*Willaertia magna c2c maky* does not fulfil criterion (d) of Article 5(1) and criterion (a) of Article 10(1)</td></tr>
<tr><td>Respiratory sensitisation properties</td><td colspan="3">No classification required. No data indicating respiratory sensitization, but based on the precautionary principle all microorganisms may be considered as potential sensitisers.<br><br>*Willaertia magna c2c maky* does not fulfil criterion (b) of Article 10(1)</td></tr>
<tr><td>Concerns linked to critical effects</td><td colspan="3">*Willaertia magna c2c maky* does not fulfil criterion (e) of Article 10(1)</td></tr>
<tr><td>Proportion of non-active isomers or impurities</td><td colspan="3">Not relevant.<br><br>*Willaertia magna c2c maky* does not fulfil criterion (f) of Article 10(1)</td></tr>
</table>

*The active substance *Willaertia magna c2c maky* as a microorganism is not in the scope of Regulation (EC) No 1272/2008 (CLP Regulation)

** The active substance *Willaertia magna c2c maky* as a microorganism is excluded from an ED assessment and from the PBT assessment based on Annex XIII of the REACH Regulation 1907/2006

Consequently, the following is concluded:

For microorganisms the assessment of exclusion criteria is not relevant. The assessment of substitution criteria is relevant for microorganisms and were assessed in line with the "Note on the principles for taking decisions on the approval of active substances under the BPR"[6] agreed at the 54th meeting, of the representatives of Member States Competent Authorities for the implementation of Regulation 528/2012 concerning the making available on the market and use of biocidal products. This implies that the assessment of the substitution criteria is based on Article 10(1)(a, b, and d). Of these Article 10(1)(b) may be relevant although microorganisms are not covered by CLP. All microorganisms are considered as potential sensitizers. In the absence of data indicating respiratory sensitization *Willaertia magna c2c maky* does not meet Article 10(1 b). The other criteria (Article 10(1)(a and b) are not applicable for microorganisms.

Therefore, *Willaertia magna c2c maky* does not meet the conditions laid down in Article 10 of Regulation (EU) No 528/2012, and is therefore not considered as a candidate for substitution.

#### 2.2.2. POP criteria

For microorganisms, the assessment of POP criteria is not relevant.

### 2.3. BPC opinion on the application for approval of the active substance Willaertia magna c2c maky in product type 11

In view of the evaluation, it is concluded that biocidal products containing *Willaertia magna c2c maky* as an active substance for the use as preservatives for liquid-cooling and processing systems may not be expected to meet the criteria laid down in points (b)(i) and (b)(iii) of Article 19(1) of Regulation (EU) 528/2012. Consequently, it is proposed that *Willaertia magna c2c maky* shall not be approved and included in the Union list of approved active substances.

As all microorganisms are considered as potential sensitisers, based on the precautionary principle, the active substance may not fulfil the criteria according to Article 28(2) to enable inclusion in Annex I of Regulation (EU) 528/2012.

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[6] See document: Note on the principles for taking decisions on the approval of active substances under the BPR (available from https://circabc.europa.eu/d/a/workspace/SpacesStore/c41b4ad4-356c-4852-9512-62e72cc919df/CA-March14-Doc.4.1%20-%20Final%20-%20Principles%20for%20substance%20approval.doc)