

September 20th, 2018**Reclassification proposal related to the substance Diethylamino Hydroxybenzoyl Hexyl Benzoate, CAS 302776-68-7; Additionally provided data**

BASF has provided the following additional information (i.e. study reports and supplemental data) regarding the above-mentioned intention for reclassification of Diethylamino Hydroxybenzoyl Hexyl Benzoate which has been published via the ECHA website to be included within the current public consultation. This document is the related cover letter, giving an overview of the additional data submitted, their reason and background as well as the summarized outcome and conclusion of the data.

1. Analysis Reports 18N01168 and 18A01078: Effect of stirring on M4 media composition

M4 medium is stirred under different conditions to evaluate the effect of stirring on the nutrient composition (i.e. iron concentration). Variation in stirring time, temperature, stirring velocity as well as the presence of the test substance up to the water saturation concentration had been tested for effects on the metal content. In comparison to the non-stirred medium, the content of iron (and slightly manganese) decreased with increasing times of stirring, independent from temperature, velocity or presence of test substance while other metal nutrients measured, remained constant.

2. Supplemented Report for 18N01168 and 18A01078 Detailed analytical procedure

The supplemented report to Analysis Reports 18N01168 and 18A01078 gives the detailed analytical results of the single measurements, the method deviation as well as the different linearities of the calibration curves together with the results of the recovery rate determinations.

3. M4 medium with and without iron: BASF SE (2018): Report Effects of the absence of soluble iron, Fe(II) in standard M4-Medium on the reproduction of the water flea *Daphnia magna* STRAUS 1820 (Screening Test), unpublished data, report no. 51E0000/18E006.

The report examines the reproduction behaviour of daphnids with and without the presence of iron in the nutrient M4 medium. The result shows, that daphnids with iron deficiency showed delayed and more irregular brood deposition compared to daphnids kept in OECD 211 compliant M4 medium (i.e. with complete nutrient supply).

4. Diethylamino Hydroxybenzoyl Hexyl Benzoate using *Daphnia magna* clone 5 (IBACON): BASF SE (2018) [REDACTED] Report *Daphnia magna* reproduction test, unpublished data, report no. 51E0636/02E019.

Clone 5 had been used at the IBACON study of 2007 and was now tested again in order to evaluate whether the strain used by IBACON is more sensitive than the same clone 5 used in the BASF study from 2009 and cultured in the test facilities of BASF since the late 1970ies.

Parental clone 5 daphnids were supplied from IBACON facility, shipped to BASF, cultured at the BASF test facility and corresponding second brood daphnids from that synchronized culture were used for the subsequent testing. The results of the *Daphnia*

reproduction study did not show any significant differences compared to the control up to the solubility limit under test conditions (i.e. NOEC \geq 11.4 $\mu\text{g/L}$; mean measured). Furthermore, the day of brood deposition was very much synchronized among both control and treatment group. Thus, the *Daphnia* strain from IBACON is considered as not more sensitive in comparison to the one from BASF.

5. Diethylamino Hydroxybenzoyl Hexyl Benzoate using *Daphnia magna* clone M10 (ECT Ökotoxikologie): BASF SE (2018), [REDACTED] Report *Daphnia magna* reproduction test, unpublished data, report no. **51E0636/02E020**

This additional *Daphnia* reproduction study should give information, whether different clones might be more sensitive to chemicals than the one typically used at BASF or at IBACON. Like before (see 2.), parental clone M10 daphnids were supplied from ECT Ökotoxikologie facility, shipped to BASF, cultured at the BASF test facility and corresponding synchronized second brood daphnids were used for the testing. Again, clone M10 did not show substance related effects (NOEC \geq 12.7 $\mu\text{g/L}$; mean measured) and the parental daphnids showed a high synchronicity in the day of brood deposition of both control and treatment group. The study confirms, that a different strain of daphnids is not more sensitive than the one used at BASF or IBACON.

6. Determination of solubility of the test substance in M4 Water: BASF SE (2009), Report Bestimmung der Wasserlöslichkeit in M4 Medium und in Milli-Q-Water, unpublished data, report no. **08E03159**

The determination of water solubility of the test substance according to the column elution method yielded a mean value of

13 \pm 6 $\mu\text{g/L}$ for M4 medium and
16 \pm 3 $\mu\text{g/L}$ for pure water (Milli-Q)

The solubility of the test substance is lower in M4 medium than in pure water.

The target concentrations of the *Daphnia* studies above were related to the maximum concentrations in pure water, but not to the solubility in M4 medium. As the maximum concentration of the test substance is lower in M4 medium, it can be concluded that in both recent studies with the test substance (i.e. report no. **51E0636/02E019** and **51E0636/02E020**), the mean measured test substance concentration was within \pm 20 % of the maximum concentration, which can be achieved under test conditions.

Overall, the data package shows:

- Stirring of M4 medium reduces the iron content of the nutrient M4 medium and thus impacts the final medium composition used for the chronic *Daphnia* toxicity testing.
- Iron deficiency in the M4 medium has a negative impact on the reproduction behavior of daphnids: The delay of brood deposition and loss of brood deposition synchronicity affects the overall number of broods towards the end of the experiment and thus the overall number of daphnids produced per female.
- Despite the fact, that the different clones (i.e. 5 and M10) have different parental sizes and show a different reproduction behavior (i.e. number of offspring and time to first brood), no asynchronous brood deposition behavior was given among the control animals leading to a

comparable number of broods (i.e. 4 broods per female) in the controls and the treatment group at test end.

- Repetition of the *Daphnia* reproduction tests even with different clones of daphnids prove that the test substance does not have a negative impact on the reproduction behavior on the test organisms up to the limit of solubility under test conditions irrespective from the clone used.
- Control and treatment group within the IBACON study 2007 have different nutrient compositions (i.e. iron deficiency due to visible iron precipitation during the preparation of the test media of the various treatment groups), so that the control is inadequate to be compared with the treatment group.

The recent results demonstrate and confirm that the test substance Diethylamino Hydroxybenzoyl Hexyl Benzoate has no negative chronic effects on the daphnids and that the IBACON study from 2007 has serious shortcomings and therefore should not be used for Classification and Labelling purposes due to significant shortcomings in the test medium preparation procedure.

The additional *Daphnia* reproduction tests confirm and substantiate the finding, that Diethylamino Hydroxybenzoyl Hexyl Benzoate does not impact the reproduction behavior of daphnids by testing also different daphnid strains. The increase number of studies along with the supplemental information on the measured iron concentration in stirred M4 medium under various conditions shall enable the experts to derive an informed decision.