# ATC comments on the proposed Harmonised Classification and Labelling for:

**Reaction products of diphenylamine with nonene, branched (EC 701-385-4)**

## Submission to the ECHA Public Consultation

**Document 157**
**April 2024**

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## Introduction

The Technical Committee of Petroleum Additive Manufacturers in Europe (ATC) was established in 1974 for member companies to discuss topics of a technical and statutory nature which are of concern to our industry. ATC works to develop sound scientific responses to regulatory changes, for the benefit of end consumers and environmental protection.

ATC's position is that the proposed Harmonised Classification and Labelling (CLH) of Toxicity to Reproduction Category 1B and Aquatic Chronic Category 1 with an MF of 10 for EC 701-385-4 (which will lead to an unwarranted and overly conservative Restriction on consumer uses per REACH Annex XVII as well as a "Dangerous Goods" classification on formulated oils), is not justified based on the available toxicology data for the reasons summarised below and in the annexes to this document.

Along with EC 270-128-1 (subject to a parallel CLH proposal), this substance has proven to be a highly effective antioxidant for lubricants which are essential for transport, power generation and a range of other industries. Use of this substance in lubricants allows equipment and vehicle manufacturers to comply with increasingly stringent fuel efficiency and emission targets, to enhance hardware durability, and to reduce the use of chemicals and mineral oils, thereby benefitting the European economy, society and environment and contributing to the sustainability goals of the EU Green Deal.

## Summary of the ATC's position on the proposed classifications

ATC disagree with the assessment of the dossier submitter (DS) and does not consider the classification of Reaction products of diphenylamine with nonene, branched as Repr. 1B (H360 FD) and Aquatic Chronic 1 (H410) warranted based on the reasoning provided in this document. We have identified multiple shortcomings in data presentation and evaluation by the DS. These affect the overall assessment of toxicological relevance and severity of the findings observed. Since a correct and complete compilation of all available evidence and a scientifically sound data assessment is essential for a robust decision on classification, ATC asks the Dossier Submitter (DS), rapporteur and the Risk Assessment Committee (RAC) to consider the comments provided in Annex 1 – Additional comments to the CLH dossier as well as further data evaluations presented in Annexes 2-13 before concluding on the proposed hazard classification for the substance.

## 1. Limitations and reasons for rejection of read across

On a general note, ATC would like to point out that most of the evidence put forward by the DS on effects of Reaction products of diphenylamine with nonene, branched on both sexual function / fertility as well as developmental toxicity are based on data available for another substance, Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. The DS correctly states that "In the registration dossier, a read-across from Benzenamine, N-phenyl-, reaction products with 2,4,4- trimethylpentene, was initially proposed by the registrants to fulfil REACH Annex X, Section 8.7.3., before the registrants submitted a testing proposal (17/12/2021) for an EOGRTS performed with the substance itself." This initial proposition was offered several years ago, following the approaches of some groups such as the COLLA pilot project (European Chemicals Agency., 2018; Health Canada, 2017; OECD, 2016) and using general theoretical assumptions of structural similarity with a view to completing the requirements under REACH and reducing animal use. However, since that time, new studies have been conducted (reproduction/developmental toxicity screening studies, OECD Testing

Guideline (TG) 421, extended, 2020) for both substances which substantially alter the viability of the read-across justification. Based on the data obtained in these extended OECD TG 421 studies, the lead registrant submitted a testing proposal for an OECD TG 443 study with Reaction products of diphenylamine with nonene, branched on October 6th, 2021, as the registrants no longer supported the read-across.

The OECD TG 421 studies currently available are the only two bridging studies used by the DS in their read-across justification. Further, the DS provided QSAR analysis as supporting information, despite the substances being complex UVCBs, which impacts the reliability of *in silico* predictions. The data obtained from the OECD TG 421 studies show distinct differences between the two substances, particularly in the areas most likely to be responsible for the mode of action proposed for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. Therefore, the read-across approach proposed for the reproductive and developmental endpoints is considered not sufficiently reliable, even if basic structural considerations and bioavailability may allow application of read across for other endpoints such as those in the aquatic environment. However, based on ECHA's read-across assessment framework (RAAF) guidance, there is no applicable scenario for direct read-across between these two substances as described in Annex 10 – Comments on the Read-across approach.

Based on these arguments, the registrants of Reaction products of diphenylamine with nonene, branched consider the read-across put forward by the DS as being of very low confidence for this endpoint. Section 3.7.2.3.1. of CLP Regulation specifies that for the decision on classification as reproductive toxicant, read-across substances "may also be included, particularly when information on the substance is scarce." However, as a robust GLP study according to OECD TG 421 with extended study design as well as OECD TG 414 studies in two species have been conducted with the test substance itself, the results of these studies should not be overruled by results of a read-across substance.

2. Toxicity to reproduction: fertility

Lower numbers of implantation sites with subsequently smaller litter sizes, decreased ovary weight and altered cyclicity are identified by the DS as the basis for the proposed classification regarding fertility. These are discussed in more detail below.

*Implantation sites and litter size*

While the DS evaluates the available toxicological data as consistent and clear evidence for a reduced number of implantation sites caused by the two substances, they make scientific errors by ignoring differences in the effects between the two substances, disregarding absence of statistical significance on some occasions, modifying historical control ranges to artificially generate toxicological relevance, and ignoring accompanying systemic toxicity.

In an OECD TG 421 study (BASF SE, 2020a) with Reaction products of diphenylamine with nonene, branched, the mean number of implantation sites remained within the historical control range and are therefore considered of no biological relevance. The corresponding mean litter size remained within the historical control range at the mid dose level and was just slightly below the historical control range at the top dose level.

The data on Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene show that implantation sites and subsequent litter size changes reached statistical significance only at the highest dose level and were accompanied by clear systemic toxicity. This pattern of toxicity

and potential mode of action for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene has been discussed in detail in the comments for that substance, which is assessed in a parallel harmonized classification process. This mode of action is not evident for Reaction products of diphenylamine with nonene, branched, which further underlines that the read-across proposed by the DS is not applicable for the endpoint of toxicity to reproduction. Data on effects of Reaction products of diphenylamine with nonene, branched on implantation sites are only available from an OECD TG 421 study (BASF SE, 2020a), in which the values observed were within the biological variation usually observed for this rat strain and age. Therefore, the data available for the substance under evaluation does not support a hazard classification. A more detailed discussion of the available data on implantation sites and subsequent impact on litter size is provided in Annex 2 – Detailed information on effects on implantation sites, litter size and systemic toxicity**.**

*Ovary weights*

A statistically significantly decreased absolute ovary weight is identified by the DS in the available OECD 421 study with the substance (BASF SE, 2020a). While in the main study the high dose group did show statistically significant reductions in absolute and relative organ weights, a 14-day recovery group included in the study showed that the absolute ovary weights mostly recovered whereas relative ovary weights were fully recovered following a 14-day treatment-free period. This is supported by the notion that only those females with lower body weights were below the range of the concurrent control animals with regards to ovary weights. Importantly, no histopathological correlations were found for the ovary weight change. In an OECD 408 study (BASF SE, 2013) available for the substance, no changes were observed on male or female reproductive organs, despite dosing up to limit dose. More details on this are provided in Annex 3 – Detailed information on ovary weight changes**.** Applying internationally accepted standards for data interpretation, these changes in ovary weights should not be considered as toxicologically relevant and most importantly, not considered relevant to support a classification as Repr. 1B.

*Estrous cycle changes*

Altered cyclicity is another argument raised by the DS to substantiate the need to classify the substance as reproductive toxicant, Repr. 1B. No significantly increased estrous cycle length was found at any dose level of the OECD 421 study for this substance. More details are provided in Annex 4 – Detailed information on data for proposed estrus cycle changes**.** Based on the lack of statistical significance as well as the low biological relevance of the effect, these arguments should not be taken into consideration for classification as Repr. 1B.

*Comparison with CLP criteria*

According to the CLP Annex I section 3.7.2, classification of a substance in Category 1B requires data showing “clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.” In contrast, a substance shall be classified in Category 2 for reproductive toxicity when there is “some evidence” of an adverse effect on sexual function and fertility, or on development, “and where the evidence is not sufficiently convincing to place the substance in Category 1”. Also, for classification in Category 2, the relevant “effects shall have been observed in the absence of other toxic effects, or if

occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."

Section 3.7.2.2.1 further specifies that "classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction". In addition, section 3.7.2.3.3 states that effects which "are considered to be of low or minimal toxicological significance, classification may not necessarily be the outcome."

CLP also acknowledges that the quality of the evidence observed in a screening study (OECD TG 421 or 422) is less reliable than that obtained through a full study such as the EOGRTS according to OECD TG 443 (CLP Annex I, section 3.7.2.5.2). Thus, care must be taken when deciding on a classification as reproductive toxicant based on only a screening study, especially when the data are within the historical control range of the testing laboratory. Moreover, the effect occurred only in the presence of systemic toxicity, which introduces further uncertainty on a direct mode of action.

*Conclusions*

Taken together, the weak and borderline effects observed in the study available for the substance do not provide robust evidence for a clear, intrinsic property of the substance to adversely affect female fertility. Moreover, as also described in detail by the DS, systemic toxicity was observed in the study including decreased body weights and food consumption at all dose levels where the borderline reproductive effects were present. The available study for this substance is only a screening study and thus has some limitations such as reduced statistical power to ultimately conclude on a potential substance-specific adverse effect on female fertility. Therefore, the effects do not fulfill the CLP criteria for classification as reproductive toxicant Category 1B.

In addition, to support their proposal by using data from another substance, the DS has ignored the significant differences in both magnitude and quality of effects between the proposed bridging studies, which are especially important as they constitute the proposed mode of action for the source substance proposed by the DS. On the basis of these findings, it is highly questionable whether any effect can be predicted (even in support) based on such weak read-across evidence. Of note, the Registrants have notified their removal of the read-across for fertility and submitted a testing proposal for an OECD TG 443 study on October 6$^{th}$, 2021 as conclusive study with more statistical power. The DS, rapporteur and RAC are kindly asked to consider this proposal prior to taking a decision on classification (further information is provided in section 4 below).

3. Toxicity to reproduction: developmental toxicity

On a general note, the ATC would like to point out that most of the evidence put forward by the DS on effects of Reaction products of diphenylamine with nonene, branched on development are based on data available for another substance, Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. As indicated above (section 1) and elaborated in detail in Annex 10 – Comments on the Read-across approach, this read-across is considered not reliable. Therefore, the OECD TG 414 studies for this substance should be given the most weight in terms of hazard assessment for this endpoint. As already mentioned, it should be noted that the lead registrant submitted a testing proposal for an OECD TG 443 study with this substance Reaction products of diphenylamine with nonene, branched on October 6$^{th}$, 2021. Therefore, the effects raised by the DS are not being ignored by the

Registrants, rather they require a substance-specific full study to more clearly establish the hazard potential for this endpoint.

Changes in neurohistopathology and neuromorphometric parameters together with findings in auditory startle response (ASR) identified in a study with another substance (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene) are stated by the DS as basis for the proposed classification regarding developmental toxicity on this substance. Further, effects on offspring viability as well as pup growth and development are identified by the DS. In their evaluation of the data, the DS made several scientific errors in data assessment, statistical evaluation, and scientific data interpretation - these are explained below, as well as in detailed annexes.

*Structural abnormality*

The DS concludes on the main critical effect for developmental toxicity being clear evidence for structural abnormalities in the central nervous system of animals exposed during the developmental period based on neurohistopathological and neuromorphometric findings in the high dose group of the OECD 443 performed with another substance (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene) (BASF SE, 2021a). Importantly, no data on developmental neurotoxicity is available for Reaction products of diphenylamine with nonene, branched, which is the substance under evaluation for harmonized classification and labelling in this dossier. The effects found in the study available for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene are described in detail in Annex 5 – Detailed information on neurohistopathology and neuromorphometrics. While males of the developmental neurotoxicity (DNT) cohort investigated at postnatal day (PND) 77 did show increased incidences of axonal degeneration in the thoracic cord, he concurrent controls also showed an unusually high background of this parameter. Further, this finding was not observed in females and no clear dose-dependent increase of severity was observed. Additional investigations in pups of the DNT cohort at PND 22 could not confirm this observation, either. Therefore, it was concluded that this finding is due to neurotoxicity following repeated exposure rather than developmental neurotoxicity. The neuromorphometric changes observed were not considered statistically significant in the study report, however the DS conducted statistical re-analysis of the data showing statistical significance in a two-way ANOVA (sex, treatment). Of note, no other brain morphometric parameter investigated was impacted. However, investigation of the historical control data available on neuromorphometric parameters showed the findings were both within the historical control data of the laboratory as well as within the variation coefficient for this parameter and can therefore not be regarded as biologically relevant.

*Functional deficiency*

Again, relying on the read-across rather than the studies for the substance under evaluation, the DS claims that there is some evidence that the substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene induces functional neurological deficiency based on changes in the ASR investigated as part of the OECD 443 DNT module (BASF SE, 2021a). Analyzing this data package, the DS has made several grave mistakes which distort the data reported in the CLH dossier and do not reflect the data available in the study report. The study report did not identify any influence of the test substance on this endpoint, and the slight differences observed by the DS were due to an outlier in the control group. Importantly, no data on developmental neurotoxicity is available for the substance Reaction products of diphenylamine with nonene, branched, which is the substance under evaluation for harmonized classification and labelling. A detailed discussion of the effects found for

Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene can be found in the comments for that substance, but is also in this document in Annex 6 – Detailed information on data for proposed neurological functional deficits.

*Death of the developing organism*

The DS concludes that there is slight evidence that the substance induces death of the developing organism based on increased abortions in a rabbit prenatal developmental toxicity study (OECD TG 414) with Reaction products of diphenylamine with nonene, branched (BASF SE, 2019). However, the DS also points out that there is a well-known association with reduced food consumption in rabbits and increased incidences of abortions. As shown in Annex 7 – Detailed information on proposed information for death of the developing organism, all females affected by abortions showed particularly low food consumption and therefore would need to be considered showing excessive toxicity throughout the study phase.

*Altered growth*

The DS claims that the substance alters growth of the developing organism at dose levels also affecting maternal/parental weight due to the observation of delayed ossification in the OECD TG 414 study in rabbits with Reaction products of diphenylamine with nonene, branched (BASF SE, 2019). Based on the data available, the delays in ossification were observed in parts of the skeleton that occur during later development and it could be shown that the mean fetal weight of those fetuses affected was clearly below the mean fetal weight of all fetuses in the high dose group. It is well-described in the literature that fetal growth retardation is secondary to maternal toxicity and is often associated with delays in ossification. Additional information can be found in Annex 8 – Detailed discussion of effects on delays in ossification.

Further evidence for effects of the substance on growth of the developing organism is claimed based on reduction of postnatal weights in high dose pups of the OECD TG 421 study with Reaction products of diphenylamine with nonene, branched (BASF SE, 2020a). Importantly, the reductions in fetal postnatal weights were limited to the high dose group and only occurred together with pronounced maternal toxicity. It is well-known that maternal toxicity can impact several developmental parameters, including fetal weights as a secondary unspecific mechanism. For further information on the data available for Reaction products of diphenylamine with nonene, branched please refer to Annex 9 – Detailed information on data for proposed effects on postnatal development**.**

*Comparison with CLP criteria*

According to CLP Annex I section 3.7.2, classification of a substance in Category 1B requires data showing “clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.” In contrast, a substance shall be classified in Category 2 for reproductive toxicity when there is “some evidence” of an adverse effect on sexual function and fertility, or on development, “and where the evidence is not sufficiently convincing to place the substance in Category 1”. Also, for classification in Category 2, the relevant “effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.”

Section 3.7.1.4 of Annex I, CLP state “The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.”

- Structural abnormality: No data on developmental neurotoxicity is available for the substance Reaction products of diphenylamine with nonene, branched, which is the substance under evaluation for harmonized classification and labelling in this dossier. The DS concludes on the main critical effect for developmental toxicity being clear evidence for structural abnormalities in the central nervous system of animals exposed during the developmental period based on neurohistopathological and neuromorphometric findings in the high dose group of the OECD 443 performed with another substance (Benzenamine,
  N-phenyl-, reaction products with 2,4,4-trimethylpentene). Based on the low reliability of the read-across, the slight magnitude of the effect and the lack of any functional impairment, the biological relevance of these findings is uncertain. In addition, these effects were not observed at the end of the lactation period (PND 22) and a clear correlation of developmental exposure and subsequently observed neurotoxicity cannot be made. CLP section 3.7.1.4 states “for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure.”, therefore these findings should not be considered a developmental effect but rather a finding following repeated administration and should not be applicable for classification as developmental toxic.
- Functional deficiency: No evidence for developmental functional deficiency is available for the substance itself. The DS claims that there is some evidence that the substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene induces functional neurological deficiency based on changes in the ASR investigated as part of the OECD 443 DNT module. Analyzing this data package, the DS has made several grave mistakes which distort the data reported in the CLH dossier and do not reflect the data available in the study report. Based on thorough scientific analysis of the data, no functional deficiency could be identified. Thus, these data do not support any hazard classification.
- Death of the developing organism: In a prenatal developmental toxicity study in rabbits, the high dose group showed increased incidences of abortions which were not statistically significant. Additionally, excessive toxicity was observed (as severe inappetence) in all affected animals which is well-known to be associated with increased abortion rates. CLP Annex I, section 3.7.2.5.8 states “In principle, adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would not normally lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate.” In the absence of data to support higher susceptibility of humans, the findings described should be disregarded for classification purposes.
- Altered growth: In a prenatal developmental toxicity study, delays in ossification were observed together with severe maternal toxicity. It is well-described in the literature that fetal growth retardation is secondary to maternal toxicity often associated with delays in ossification. Further, reduced postnatal weights were observed in high dose pups of the OECD 421 study. Importantly, the reductions in fetal postnatal weights were limited to the high dose group and only occurred together with pronounced maternal toxicity. It is well-known that maternal toxicity can impact several developmental parameters, including fetal weights as a secondary unspecific mechanism. Based on the well-established links of these observations to maternal toxicity, these findings should be disregarded for classification in accordance with CLP, Annex I, Sections 3.7.2.4.2 and 3.7.2.4.3.

*Conclusions*

The available studies on the substance itself show effects on pup viability and growth of the organism which only occurred conjunct with excessive maternal toxicity and should therefore be disregarded based on the criteria laid down in Regulation (EC) 1272/2008 (CLP).

For another substance proposed by the DS as read-across source substance (which the Registrants do not agree on), some evidence for a neurotoxic effect following repeated exposure was seen; however, no correlation to the developmental period could be established. In the absence of functional impairment, or any indication of neurotoxicity to the substance under evaluation, no classification for developmental toxicity is warranted. These changes in neurohistopathology and neuromorphometric parameters together with findings in auditory startle response (ASR) identified for another substance are the basis for the proposed classification by the DS regarding developmental toxicity on this substance. However, it is shown in this document that:

1) the effects in the read-across substance used are not sufficiently reliable enough to warrant classification
2) the read-across between the two substances is not sufficiently reliable for the endpoint addressed and should not be used as the sole basis for the classification proposed – particularly given the differences noted between the substances (further information on limitations of read-across are presented in section 1).

Overall, the basis for the proposed classification is not sufficiently reliable and cannot be accorded a level of certainty required under CLP for Category 1B.

## 4. Toxicity to reproduction: outlook and generation of additional data

Of note, the ATC do not intend to dismiss any concerns raised, and indeed support better evaluation of these potential hazards.

Based on the data obtained in these extended OECD TG 421 studies, the lead registrant submitted a testing proposal for an OECD TG 443 study with Reaction products of diphenylamine with nonene, branched on October 6$^{th}$, 2021, as the registrants no longer supported the read-across. Following a public commenting phase, no further steps have been taken in the testing proposal evaluation process, thus prohibiting the registrants to conduct this study under EU REACH.

However, since studies such as the OECD TG 443 study are required for jurisdictions outside of the EU (e.g., obtaining registration in the Republic of Korea pursuant to the Act on the Registration and Evaluation of Chemicals (“Korean REACH”)), ATC members intend to conduct an OECD TG 443 study on Reaction products of diphenylamine with nonene, branched. Furthermore, ATC has initiated an enhanced OECD TG 421 study including additional mechanistic examinations. These studies will deliver additional data to clarify the reproductive toxicity concerns for Reaction products of diphenylamine with nonene, branched. ATC therefore asks the DS, Rapporteur and RAC to consider these additional data during RAC Opinion development. It is anticipated that study reports for the OECD TG 421 study will be available in Q1/2025, and mid-2026 for the OECD TG 443 study.

5. Bioaccumulation

For bioaccumulation, the DS derived a BCF of 2219 L/kg using an OECD 305 study conducted in 2000 on the MNDPA constituent of the substance. The experimental study was performed to meet the requirements of the Japanese legislation (Chemical Substances Control Law of Japan (MITI)) and it was not designed to meet the requirements of the updated OECD 305 Guideline or the criteria required to evaluate the 'B' criterion of the PBT assessment under REACH. There are, therefore, a number of shortcomings in the study, as recognized by the DS in the CLH report (e.g. use of surfactant and dissolvent, measurements were made for a group of 2 fish instead of individually). However despite this the DS has re-assessed this data against these new criteria and has produced an unrealistic worst-case evaluation.

Bioaccumulation potential has been assessed by the Registrants using experimental data, with support of QSAR modelling. An OECD 305 bioaccumulation study in fish was performed with the test substance Mono-Nonyl diphenylamine (EC248-295-7) at the MITI institute in 2000. Different modelling approaches identified the constituent MNDPA as the most critical component affecting bioaccumulation for the registered UVCB. Considering the available experimental data and the QSAR data of the different models, the BCF based on experimental data is used to conservatively assess the bioaccumulation potential and used for the chemical safety assessment. This value as calculated by the study directors was 1730 L/kg. The position of the Registrants is that the substance is not bioaccumulative, as per Annex XIII of regulation 1907/2006/EC and the Guidance on information requirements and chemical safety assessment Chapter R.11 (PBT assessment, June 2017).

There are many issues when re-assessing an older study under new criteria and inherent uncertainty in these estimations. In addition, the registrants consider that the shortcomings in the performed fish study (similar to OECD TG 305, 2000) contribute to an unrealistic inaccurate evaluation which cannot be used to robustly assess bioaccumulation. It is also true that the data are close to the threshold of 2000, and a valid assessment of "B" is crucial to the PBT assessment. Thus, the Registrants consider that the multiple uncertainties would best be dealt with by re-performing the bioaccumulation study in fish - as per the current testing proposal based on the registration dossier update. Annex 11 – Detailed information on bioaccumulation in fish provides further discussion on this endpoint.

*Conclusion:*

The performed bioaccumulation study revealed several shortcomings when compared against the today relevant criteria. In summary, these shortcomings impair a robust and valid evaluation of the bioaccumulation potential in fish. Therefore, the registrants strongly recommend to re-perform the bioaccumulation study in fish.

6. Chronic aquatic toxicity

The DS derived a NOEC of 1.28 µg/L from an OECD 211 study on the substance as the basis for the proposed classification on aquatic chronic hazards. This long-term daphnia study (OECD 211) is based on nominal loading rates and analytics performed on the substance. While the testing laboratory derived valid effect values based on the nominal exposure rates (WAF, whole UVCB), the DS determined the effect values based on the measured concentrations for only one component group (C9DPA) of the UVCB substance. Considering the predicted toxicity of other constituents in the UVCB besides this one measured constituent, Registrants are of the opinion that it is unjustified to base aquatic toxicity of this substance

solely on quantification of C9DPA as this will lead to an inaccurate assessment of toxicity. This is because daphnia are exposed to the whole UVCB (with unquantified concentrations of other constituent groups).

Currently available data and arguments provided in this document indicate that there is no aquatic toxicity within the range of water solubility. Also, effect values in the available daphnia study should be related to nominal loading rates, rather than to measured concentrations representing only one constituent of the UVCB substance. Based on the applied approach there is no toxicity at the limit of water solubility and consequently no classification and labelling is warranted. Please see Annex 12 - Detailed information on long term toxicity on daphnia (*Daphnia magna*) for further discussion on this endpoint.

In addition, the registrants proposed to re-conduct the chronic daphnia study using a more sophisticated approach (passive dosing), developed recently by Registrants in agreement with the Member States Committee in the relevant ECHA CORAP of 2023 for this UVCB substance (see information on other regulatory processes in Annex 13 - Further work proposed and other regulatory processes). It is considered that these data will confirm the true hazard potential for this substance.

*Conclusion:*

Effect values in the available daphnia study should be related to nominal loading rates, rather than to measured concentrations representing only one constituent of the UVCB substance. Based on the applied approach there is no toxicity within the water solubility and consequently no classification and labelling is warranted.

# Annex 1 – Additional comments to the CLH dossier

| page | Citation from CLH report, proposal for Harmonized Classification and Labelling for Reaction products of diphenylamine with nonene, branched | Comments on the respective section of the CLH proposal |
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| 28 | Estrous cycle: At the high dose level, a slight non-statistically significant increase of estrous cycles length (4.3 days vs. control 4.0 days) associated with a slight increased in the mean percentage of days in diestrous stage (35% vs 28% in control). | The slight increase in the high dose group was not statistically significant and due to only one animal showing one cycle with long diestrus duration. All other animals were in the range of the control group for estrus cycle length and number of days in diestrus. The one animal with one long diestrus cycle showed normal cycles with 1-2 days in diestrus for the remainder of the observation period. For further information, please refer to Annex 4 – Detailed information on data for proposed estrus cycle changes. |
| 28 | The mean number of implantation sites was significantly lower in the high-dose group (-31%) and in the mid-dose group (-24%) with a mean number of implantation sites of 9.9 and 10.9 respectively (concurrent controls 14.4). | The mean numbers of implantation sites were significantly reduced compared to the concurrent control. However, the concurrent control was unusually high and all values were within the historical control data of the laboratory and therefore within the range of commonly observed biological variation for this rat strain. For futher information, please see Annex 2 – Detailed information on effects on implantation sites, litter size and systemic toxicity. |
| 28 | However, the range of HCD for the number of implantation sites from 2008 to 2018 period provided in the study report of the OECD TG 443 (Unpublished study report, 2021) performed with the analogue by the same laboratory, was 11.1 - 15.3 sites and 11.2-15.3 when considering a more appropriate timeframe (2015-2018). When considering the later HCD, the value of the concurrent control is well within the HCD range while the values of the mid-dose and high-dose groups are outside (Table 14). HCD were largely based on studies conducted by gavage while the present study was performed in diet (only 2/40 in HCD of OECD 421 or 422). […]<br>Consequently to the decreased number of implantation sites, the mean number of delivered F1 pups per dam was significantly decreased (-19%) in the high-dose group with 8.7 pups per litter and in the mid-dose group (-31%) with 10.2 pups per litter. The historical control range provided in the FSR is 9.0-13.2 pups delivered per litter, while the HCD range from the EOGRTS is 10.3-14.9 and 10.9-14.9 when considering a more appropriate timeframe (2015-2018). | Historical control data from one study protocol can only be adapted to another study protocol under the considerations of e.g. animal age, treatment protocol, group size, etc. and expert judgement on the study conduct is required in order to assess which data are adequate for consideration as historical control data. Based on expert judgement by the performing laboratory, historical control data of OECD TG 443 studies are not adequate for discussion of effects observed in OECD 421/422 studies. Updated historical control data from OECD 421/422 studies were compiled which were conducted between 2015 and 2022 by the laboratory. These include 79 studies, with the following exposure routes: diet (11), gavage (58), drinking water (6), inhalation (4). More information can be found in the section “Historical control data” of Annex 2. |
| 29 | For females (see Table 21), a dose-related and significant decrease in absolute ovarian weights was observed from the mid-dose group. The relative weight was also impacted from the mid-dose level but statistical significance was only reached at the high dose level. This decrease in absolute ovarian weight seemed to be attenuated in the recovery group (-14%) but was still statistically significant. No histopathologic findings were noted in the ovaries. | While in the main OECD 421 study the high dose group did show statistically significant reductions in absolute and relative organ weights, a 14-day recovery group included in the study showed that the absolute ovary weights mostly recovered whereas relative ovary weights were fully recovered following a 14-day treatment-free period. This is supported by the notion that only those females with lower body weights were below the range of the concurrent control animals with regards to ovary weights. Importantly, no histopathological correlates were found for the ovary weight change. In an OECD 408 study available for the substance, no changes were observed on male or female reproductive organs, despite dosing up to limit dose. More details on this are provided in Annex 3 – Detailed information on ovary weight changes. |

| page | **Citation from CLH report, proposal for Harmonized Classification and Labelling for Reaction products of diphenylamine with nonene, branched** | **Comments on the respective section of the CLH proposal** |
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| 29 | Table 22: Absolute and Relative ovarian weights (Reaction products of diphenylamine with nonene,branched, OECD TG 421) | The relative ovary weights for recovery groups showing no effect are missing from the table and the Standard deviations were omitted, also. Additonal information on ovary weights can be found in this document in Annex 3 – Detailed information on ovary weight changes. |
| 29 | In the mid-dose, decreased mean body weight was limited to females at the end of the gestation period and during the lactation period (-7% compared to controls) associated to non-significant minor adverse alterations of the food consumption. No effect on body weight was observed during the premating period. | Decreased mean body weights at the end of gestation were -8.8%* vs. controls and the described “non-significant minor adverse alteration of food consumption” was a significantly reduced food consumption during gestion of -8.8%** vs. controls. Additionally, body weight change as relevant parameter for systemic toxicity was not considered. Detailed information on food consumption, body weights and body weight change can be found in Annex 2 – Detailed information on effects on implantation sites, litter size and systemic toxicity. |
| 30-38 | Discussion on studies available for Benzenamine, N-phenyl-,<br>reaction products with 2,4,4-trimethylpentene | Data on Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene are discussed in detail in the comments to the CLH proposal for this substance. Further information on why the read-across is not supported can be found in Annex 10 – Comments on the Read-across approach. |

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| 39 | Animal studies provide clear evidence of an adverse effect on fertility. Indeed, in the four reliable guideline and GLP-compliant studies addressing explicitly reproduction (one OECD TG 421 with the substance itself and one OECD TG 421, one OECD TG 422 and one OECD TG 443 with the analogue), Reaction products of diphenylamine with nonene, branched and its analogue consistently induced lower numbers of implantation sites with subsequently smaller litter sizes as compared to control animals. This main critical effect was dose-related in all the studies, statistically significant even in screening OECD 421 TG studies with a small number of animals (10/sex/dose) and was repeated in both generations of the EOGRTS.<br>At the top doses tested, the litter size was reduced by 31% and 36% in dams exposed to 5000 ppm of Reaction products of diphenylamine with nonene, branched and 3000 ppm of the analogue respectively, which is considered a severe impact on fertility/reproductive function (a decrease of one third in the progeny). At those dose levels the systemic toxicity was not marked (i.e.: no lethality, no dramatic reduction in absolute body weight, no coma), body weights at the end of the premating period were decreased by 10% and liver and thyroid effects were observed. However, a direct correlation between systemic toxicity and reduced implantation sites could not be established based on the data available and lower numbers of implantation sites with subsequently smaller litter sizes were already observed at lower dose levels where body weight was not affected.<br>Other supportive effects relevant for fertility/sexual function classification noted with both Reaction products of diphenylamine with nonene and its analogue were statistically significant decrease of absolute ovary weights (without histopathological correlates) and increased estrous cycles length associated with a slight increase in the mean percentage of days in diestrous stage (reaching statistically significance only in high-dose dams of the OECD TG 421 and in the high-dose dams of the EOGRTS performed with the analogue).<br>The consistency of the effects observed with the two structural analogues through the available studies further strengthens the causality between exposure to two SDPAs and the observed reproductive outcomes.<br>The reliability of the available studies compliant to GLP and current OECD TGs, make the quality of evidence convincing. | Only one OECD 421 study is available for the substance under evaluation. The read-across performed by the DS is not reliable as described in Annex 10 – Comments on the Read-across approach.<br>Taken together, the weak and borderline effects observed in the study available for the substance do not provide robust evidence for a clear, substance-intrinsic property of the substance to adversely affect female fertility. Moreover, as also described in detail by the DS, systemic toxicity was observed in the study including decreased body weights and food consumption at all dose levels where the borderline reproductive effects were present. The available study for this substance is only a screening study and thus has some limitations such as reduced statistical power to ultimately conclude on a potential substance-specific adverse effect on female fertility. Therefore, the effects do not fulfill the CLP criteria for classification as reproductive toxicant Category 1B.<br>Further information on data available and its discussion can be found in<br>- Annex 2 – Detailed information on effects on implantation sites, litter size and systemic toxicity<br>- Annex 3 – Detailed information on ovary weight changes<br>- Annex 4 – Detailed information on data for proposed estrus cycle changes |
| 48 | The studies performed with the analogue are considered relevant in a read-across approach since the read-across for reproductive toxicity from Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (source substance) to Reaction products of diphenylamine with nonene, branched (target substance) is considered acceptable with high confidence (refer to 10.10.11). Especially, in the absence of an EOGRTS performed with Reaction products of diphenylamine with nonene, the outcomes observed in the cohorts of the EOGRTS performed with the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene have been taken into consideration in a weight of evidence approach pursuant to CLP Annex I, 1.1.1. | The read-across performed by the DS is not considered reliable as described in further detail in Annex 10 – Comments on the Read-across approach. A testing proposal to fill the data gap of toxicity to reproduction with an OECD TG 443 has been submitted in 2021 by the Registrants under REACH. |

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| 49 | While, there were no test substance-related effects on pre- and the postimplantation losses, numbers of resorptions and viable fetuses, the abortion rate was twice at the highest dose compared to control (2 abortions in control, 4 at the highest doses). The increase was not statistically significant however from the provided HCD in the study report based on 14 studies (2014-2017), only 4 abortions out 350 dams (1, 1%) were reported (the incidence by study was not reported). Furthermore, 1 abortion out of 5 does was also noted in the mid and high doses of the range finding study. However, several studies on effects of caloric restriction alone during pregnancy in rabbit have shown that abortions may occur due to markedly reduced food consumption (Matsuzawa, 1981; Cappon, 2005; Matsuoka, 2006; Lopez-Tello, 2019). Therefore, the drop in food consumption may at least partly contribute to the abortions observed in high-dose group. | The data available for this study are presented in detail in Annex 7 – Detailed information on proposed information for death of the developing organism. In brief, it can be shown that the four does affected by abortions showed extreme in appetence throughout the treatment phase. As the DS already stated, the relationship between reduced food consumption and abortions in rabbits is ell established in the literature. As for the exceedance of the historical control range, this must be expected – given high standards in laboratory animal care, control animals would not be expected to experience inappetence leading to abortion. The inappetence does not reflect a biological variation, but rather a species-specific reaction to treatment with substances. |
| 49 | Two skeletal variations related to a delay in ossification reached statistical significance between the control and the treated groups, i.e. irregular ossification of interparietal (increased and outside the historical control range in the mid- and high-dose groups) and unossified talus (increased and outside the historical control range in the mid- and high-dose groups). | Slight delays of ossification were observed, which however did not affect morphology, as the underlying cartilage model was completely intact in all these cases. This assessment is supported by the fact that the mean fetal weight of all 10 fetuses showing this finding (i.e., 18.8 g) was clearly below the mean fetal weight of all fetuses in the high dose group (33.6 g), which indicates a delay in overall development going along with the delay in ossification. The association of ossification delays with maternal toxicity and fetal body weights has previously been well established. For further information, please refer to Annex 8 – Detailed discussion of effects on delays in ossification |
| 49 | At the high-dose level there was an increase of total external malformations. While not statistically significant, this increase exceeded the HCD range when both expressed as fetal incidence and incidence of affected fetuses/litter. This increase was driven by four fetuses in one single litter (high-dose doe No. 76) having multiple craniofacial malformations, i.e. domed head, cleft palate and small tongue. One of these four fetuses had also a hydrocephaly (visceral malformation) and another one severely malformed skull bone (skeletal malformation). All of them had paw hyperflexion (variation) and empty stomach (unclassified soft tissue observation). The clustered appearance in a single litter and the similar spectrum of findings in all those four fetuses may suggest a genetic origin although the HCD report only one fetus per litter for each malformation individually or multiple external malformations. | The clustered appearance in one litter and the almost identical spectrum of ontogenetically different findings in all those fetuses strongly suggests an origin of these anomalies which is unrelated to treatment (i.e., genetic origin). Consequently, the higher incidence of high-dose findings in their respective sections is also considered to be unrelated to treatment.<br>There was no statistically significant difference in the distribution of total malformations about the groups, and the incidences in the treated groups were close to the historical control mean. Therefore, the findings observed are considered incidental and not related to treatment.<br>Further information can be found in Annex 8 – Detailed discussion of effects on delays in ossification. |
| 51-52 | Discussion of data from OECD 422 study with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene | Data on Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene are discussed in detail in the comments to the CLH proposal for this substance. Further information on why the read-across is not supported can be found in Annex 10 – Comments on the Read-across approach |

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| 52 | In the OECD TG 421 study (Unpublished study report, 2020a) performed with the substance at 5000 ppm, while birth weight was not altered by treatment, a significant decrease of mean pup body weight was observed from PND 7 to termination (-19% versus controls both sexes combined on PND13) and a significant decrease of mean pup body weight changes from PND1-13 (-22.5% both sexes combined). In dams, at this dose level during the lactation period, a significant decrease of food consumption (-23.5% d1 ->13) and a significant decrease of body weight were also observed (-17% at termination compared to controls). At 1500 ppm a transitory decrease of mean pup body weight changes was observed during PND 4-7 (up to -13.2% both sexes combined compared to controls) but no significant effect was observed in terminal body weight and on BW changes during PND 1-13. | As stated by the DS, reductions of pup body weights were only observed together with pronounced food consumption and body weight effects in dams. These data are discussed in more detail in Annex 9 – Detailed information on data for proposed effects on postnatal development. |
| 53-54 | Data on nipple retention / areola anlagen:<br>In the OECD TG 421 study (Unpublished study report, 2020a) performed with the substance, the number and percentage of male pups having areolae on PND13, was not impacted by the test substance while in the OECD TG 421 study (Unpublished study report, 2020b) performed with the analogue, an increased incidence of nipple development (100% vs. 79.6% in control) and number of nipples per animal (5.2 to control 2.5) was observed in high-dose males on PND13. The study author considered that this effect could be related to the delay of general development in male pups at this dose level. This statement was further supported by the individual data where the male pups with the highest number of nipples (n=8) had the lowest body weights (19.9 to 22.2 g vs mean body weight of 24.6 g). | As stated by the DS, the nipple / areola anlagen for the substance under evaluation were not affected by treatment.<br>Data on Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene are discussed in detail in the comments to the CLH proposal for this substance. Further information on why the read-across is not supported can be found in Annex 10 – Comments on the Read-across approach |
| 54 | Table 29: Nipple retention in males pups | The numbers presented for the OECD 421 study with Reaction productes of diphenylamine with nonene, branched is incorrect. Apparently, the DS misunderstood the tables in the study report. Indeed, the % of males showing nipples on PND 13 was 27.8, 32.5, 46.7, and 32.4% in the control, low, mid and high dose, respectively. The mean nipple numbers are misleading, as the control group is presented with the exact value whereas the treated groups were rounded to 1. In general, it should be mentioned that presentation of standard deviations would be helpful for scientific interpretation of the data by the reader. |

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| 54-57 | Discussion on effects on developmental neurotoxicity and thyroid hormones. | No data on developmental neurotoxicity are available for the substance under evaluation. Thyroid hormone values were unchanged in the study available. All data discussed in this section were derived from a read-across to Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, which is not considered reliable.<br>Further information on why the read-across is not supported can be found in Annex 10 – Comments on the Read-across approach. A testing proposal to fill the data gap of OECD 443 has been submitted in 2022 by the Registrants under REACH however the permission to perform the study has not been granted.<br>Data on Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene are discussed in detail in the comments to the CLH proposal for this substance, however since no data exist on developmental neurotoxicity for Reaction products of diphenylamine with nonene, branched, the respective sections from these comments have been included in Annex 5 – Detailed information on neurohistopathology and neuromorphometrics and Annex 6 – Detailed information on data for proposed neurological functional deficits. |

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| 58-59 | Effects from the available data set relevant for development classification:<br>(1) Death of the developing organism: Post-implantation loss and foetal viability were not affected by treatment with Reaction products of diphenylamine with nonene, branched, in both rat and rabbit PNDTSs. In the main study in rabbits, increased number of abortions (4 vs. 2 in control) was observed at the high dose level (1 abortion out of 5 does was also noted in the mid and high doses of the range finding study). While a direct effect cannot be excluded, the severe drop in food consumption GD 7-23 (up to -59% in comparison to the control group) may partly be involved, as supported by studies on caloric restriction during pregnancy in rabbit (Matsuzawa, 1981; Cappon, 2005; Matsuoka, 2006; Lopez-Tello, 2019).<br>In the generational studies performed with Reaction products of diphenylamine with nonene, branched and its analogue, the decreased litter size at birth observed in all studies results from the decreased number of implantation sites; this effect is addressed in the section dedicated to effects to sexual function and fertility. Post-implantation loss and foetal viability were not affected in any of the generational studies except in the OECD TG 422 performed with the analogue where the mean post-implantation loss in the high-dose dams was increased compared to controls (14% vs 0%). It is noteworthy that the value in controls of the study was particularly low.<br>Furthermore, the viability index was significantly reduced in this OECD TG 422 at the high-dose level (88.7% at 225 mg/kg bw/d versus 100% in controls). However, no treatment-related effect was observed on live birth, viability and lactation indices in the OECD TG 421 performed with Reaction products of diphenylamine with nonene, branched up to 5000 ppm (eq. to 443 mg/kg bw/d) or in the OECD TG 421 up to 3000 ppm (eq. to 271 mg/kg bw/d) and in the EOGRTS up to 1800 ppm (eq. to 166 mg/kg bw/d) performed with the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene induces death of the developing organism.<br>→ While post-implantation viability was not affected in the PNDTSs in rats and rabbits, the increased number of abortions in rabbits provide slight evidence that Reaction products of diphenylamine with nonene, branched could induce death of the developing organism, however these abortions may be partly related to the severe drop in food consumption observed at this dose level, as demonstrated in published studies on caloric restriction during pregnancy in rabbit.<br>Based on the available data, there is slight evidence that Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene induces death of the developing organism based on reduction of pre- and postnatal viability in the high-dose animals of the OECD TG 422, but these effects were not reproduced in the other generational studies performed with the substance and its analogue. | The DS concludes that there is slight evidence that the substance induces death of the developing organism based on increased abortions in a rabbit prenatal developmental toxicity study. However, the DS also points out that there is a well-known association with reduced food consumption in rabbits and increased incidences of abortions. As shown in Annex 8 – Detailed information on death of the developing organism, all females affected by abortions showed particularly low food consumption and therefore would need to be considered showing excessive toxicity throughout the study phase.<br>The other data mentioned by the DS on death of the developing organism were not derived from data with the substance itself, but from a read-across, which is not considered adequate. Further information on why the read-across is not supported can be found in Annex 10 – Comments on the Read-across approach. |

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| 59 | (2) Structural abnormality: Reaction products of diphenylamine with nonene, branched was not teratogenic in rat. In rabbit, an increase of external malformations was observed at the top dose (four fetuses in one single litter with multiple external malformations). The clustered appearance limited to one litter with similar spectrum of findings (i.e. craniofacial malformations consisting in domed head, cleft palate and small tongue in all those four fetuses) suggest rather a genetic origin than a treatment-related effect. Statistically significant increases of two skeletal variations (i.e. irregular ossification of interparietal and unossified talus) were also observed at this dose-level as well as a general delay in ossification.<br>In the high-dose C2A animals of the EOGRTS performed with the analogue, neurohistopathological findings (increased incidence of axonal degeneration in the thoracic cord 9/10 males as well as slight increased incidence of axonal degeneration in other area in males and females) and neuromorphometric changes (decreased brain length in males and increased corpus callosum width in both males and females) were observed. Such parameters were not investigated for Reaction products of diphenylamine with nonene, branched.<br>The increased of nipple number on PND13 observed in high-dose pups in the OECD TG 421 and in high-dose F2 pups on PND13 in the EOGRTS performed with the analogue Benzenamine, Nphenyl-, reaction products with 2,4,4-trimethylpentene is considered to be rather a consequence of a general delay of pup development than a specific effect on hormonal homeostasis in the absence of alteration in other sensitive endpoints related to antiandrogenic potential.<br>→ Based on the available data, there is clear evidence that the anologue Benzenamine, Nphenyl-, reaction products with 2,4,4-trimethylpentene induces abnormalities in the central nervous system in animals exposed during the developmental period. Such parameters were not investigated for Reaction products of diphenylamine with nonene, branched.<br>In the PNDTS in rabbits performed with the substance, delay in ossification was noted in the presence of maternal toxicity. Regarding the cluster of four fetuses with multiple common malformations from a single litter of the high-dose group, a genetic origin is considered more likely than a treatment-related effect. | The increase in external malformations described by the DS was clustered in one litter. The almost identical spectrum of ontogenetically different findings in all those fetuses strongly suggests an origin of these anomalies which is unrelated to treatment (i.e., genetic origin). Consequently, the higher incidence of high-dose findings in their respective sections is also considered to be unrelated to treatment. There was no statistically significant difference in the distribution of total malformations about the groups, and the incidences in the treated groups were close to the historical control mean. Therefore, the findings observed are considered incidental and not related to treatment.<br>The delays in ossification were observed in parts of the skeleton that occur during later development and it could be shown that the mean fetal weight of those fetuses affected was clearly below the mean fetal weight of all fetuses in the high dose group. It is well-described in the literature that fetal growth retardation secondary to maternal toxicity is often associated with delays in ossification. Additional information can be found in Annex 9 – Detailed discussion of effects on delays in ossification.<br>No data on developmental neurotoxicity are available for the substance under evaluation. Thyroid hormone values were unchanged in the study available. All data discussed in this section were derived from a read-across to Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, which is not considered reliable.<br>Further information on why the read-across is not supported can be found in Annex 10 – Comments on the Read-across approach. A testing proposal to fill the data gap of OECD 443 has been submitted in 2022 by the Registrants under REACH however the permission to perform the study has not been granted.<br>Data on Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene are discussed in detail in the comments to the CLH proposal for this substance, however since no data exist on developmental neurotoxicity for Reaction products of diphenylamine with nonene, branched, the respective sections from these comments have been included in Annex 5 – Detailed information on neurohistopathology and neuromorphometrics and Annex 6 – Detailed information on data for proposed neurological functional deficits.<br>Overall, the classification criteria for Developmental toxicity, Category 1B are considered not fulfilled. For comparison of the data with classification criteria, please refer to the section 3 Toxicity to reproduction: developmental toxicity - Comparison with CLP criteria above |

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| 58- | (3) Altered growth:<br>In rabbits exposed to Reaction products of diphenylamine with nonene, branched, a significant decrease in fetus weight (-12%) was observed at the highest dose (100 mg/kg bw/d) associated with delays of ossification. At this dose level, does consumed 31% less food than the concurrent control does during the treatment period (GD6-28), showed marked reduced defecation and had slight reduced corrected body weight.<br>Postnatal growth was also altered from PND7 up to weaning in the high-dose groups of the generational studies performed with the substance (OECD TG 421) or its analogue Benzenamine, Nphenyl-, reaction products with 2,4,4-trimethylpentene EOGRTS, OECD TG 421). At these dose levels, effects on body weight of similar magnitude were observed in females at the end of the lactation period.<br>→ Based on the available data, there is evidence that both Reaction products of diphenylamine with nonene, branched and its analogue alter growth of the developing organism at dose levels also affecting maternal/parental weight. | Based on the data available, the delays in ossification were observed in parts of the skeleton that occur during later development and it could be shown that the mean fetal weight of those fetuses affected was clearly below the mean fetal weight of all fetuses in the high dose group. It is well-described in the literature that fetal growth retardation secondary to maternal toxicity is often associated with delays in ossification. Additional information can be found in Annex 9 – Detailed discussion of effects on delays in ossification.<br>Further evidence for effects of the substance on growth of the developing organism is claimed based on reduction of postnatal weights in high dose pups of the OECD 421 study (BASF SE, 2020). Importantly, the reductions in fetal postnatal weights were limited to the high dose group and only occurred together with pronounced maternal toxicity. It is well-known that maternal toxicity can impact several developmental parameters, including fetal weights as secondary unspecific mechanism. For further information on the data available for Reaction products of diphenylamine with nonene, branched please refer to Annex 10 – Detailed information on effects on postnatal development.<br>For comparison of the data with classification criteria, please refer to the section 3 Toxicity to reproduction: developmental toxicity - Comparison with CLP criteria above |
| | (4) Functional deficiency:<br>In the EOGRTS performed with the analogue, despite limitations of the auditory startle response test, effects from the mid-dose level on mean maximal amplitude in males as well as decreased habituation from the mid-dose in males and in males and females combined are considered biologically relevant in the absence of appropriate statistical analysis (testing for interactions of sex, trial blocks and treatment) and positive controls.<br>→ Based on the available, there is some evidence that Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene induces functional deficiency. However, the deficiencies of the test limit the reliability on the effects observed in auditory startle response. Such parameters were not investigated for Reaction products of diphenylamine with nonene, branched. | No data on developmental neurotoxicity are available for the substance under evaluation. Thyroid hormone values were unchanged in the study available. All data discussed in this section were derived from a read-across to Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, which is not considered reliable.<br>Further information on why the read-across is not supported can be found in Annex 10 – Comments on the Read-across approach. A testing proposal to fill the data gap of OECD 443 has been submitted in 2022 by the Registrants under REACH however the permission to perform the study has not been granted.<br>Data on Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene are discussed in detail in the comments to the CLH proposal for this substance, however since no data exist on developmental neurotoxicity for Reaction products of diphenylamine with nonene, branched, the respective sections from these comments have been included in Annex 5 – Detailed information on neurohistopathology and neuromorphometrics and Annex 6 – Detailed information on data for proposed neurological functional deficits.<br>Overall, the classification criteria for Developmental toxicity, Category 1B are considered not fulfilled. For comparison of the data with classification criteria, please refer to the section 3 Toxicity to reproduction: developmental toxicity - Comparison with CLP criteria above |

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| 60-61 | Overall, the main critical effects are those linked to neurodevelopmental toxicity observed in C2A animals (especially in males) of the EORGTS performed with the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. In high-dose C2A animals, clear severe neurohistopathological findings (increased incidence of axonal degeneration in the thoracic cord 9/10 males as well as slight increased incidence of axonal degeneration in other area in males and females) and neuromorphometric changes (decreased brain length in males and increased corpus callosum width in both males and females) were observed. No axonal degeneration was found in C2B males (sacrificed on PND22).<br>However, exposure during the developmental period could have contributed to the delayed effects observed in C2A animals on PND77 even if not observed at an earlier time point (PND22). Furthermore, in the available OECD TG 422, no axonal degeneration was observed in males or females (not exposed during developmental phases) which further supports the involvement of developmental exposure in the occurrence of this lesion.<br>The reliability of the neurohistopathological findings from the EOGRTS compliant to GLP and to current OECD TG, make the quality of evidence convincing for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene to induce developmental neurotoxicity.<br>Despite limitations of the auditory startle response test, effects from the mid-dose level on mean maximal amplitude in males as well as decreased habituation from the mid-dose in males and in males and females combined are considered biologically relevant and further support neurodevelopmental toxicity.<br>In the absence of specific neurodevelopmental testing with Reaction products of diphenylamine with nonene, branched and considering the read-across for reproductive toxicity from Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (source substance) to Reaction products of diphenylamine with nonene, branched (target substance) acceptable with high confidence as detailed in section 10.10.11, the evidence coming from the source Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene substance is also considered convincing for Reaction products of diphenylamine with nonene.<br>The available data suggest that neurodevelopment effects could be linked to decreased thyroid hormones (THs). The similarity of thyroid effects induced by both substances further support a read-across approach for neurodevelopmental effects.<br>With respect to the severity of the above mentioned neurodevelopmental effects category 1B is considered triggered. | No data on developmental neurotoxicity are available for the substance under evaluation. All data discussed in this section were derived from a read-across to Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, which is not considered reliable.<br>Further information on why the read-across is not supported can be found in Annex 10 – Comments on the Read-across approach. A testing proposal to fill the data gap of OECD 443 has been submitted in 2022 by the Registrants under REACH however the permission to perform the study has not been granted.<br>Data on Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene are discussed in detail in the comments to the CLH proposal for this substance, however since no data exist on developmental neurotoxicity for Reaction products of diphenylamine with nonene, branched, the respective sections from these comments have been included in Annex 5 – Detailed information on neurohistopathology and neuromorphometrics and Annex 6 – Detailed information on data for proposed neurological functional deficits.<br>Overall, the classification criteria for Developmental toxicity, Category 1B are considered not fulfilled. For comparison of the data with classification criteria, please refer to the section 3 Toxicity to reproduction: developmental toxicity - Comparison with CLP criteria above<br>As regards the proposed mode of action by the DS, thyroid hormone values were unchanged in the study available, rendering this line of argumentation unlikely. |
| 63-75 | Assessment of the reliability of read-across | The read-across to Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, is considered not reliable due to lack of supporting data and differences in data quality and quantity between both substances.<br>Further information on why the read-across is not supported can be found in Annex 10 – Comments on the Read-across approach. |

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| 82 | Although some elements deviated from the standard OECD TG 305 (use of surfactant and dissolvent, measurements were made for a group of 2 fish instead of individually), the study is well conducted, follows GLP principles and is reliable for use under CLP in the absence of data on the UVCB substance. | The bioaccumulation study in fish with the test substance Mono-Nonyl diphenylamine (EC248-295-7) at the MITI institute was performed in 2000 to meet the requirements of the Japanese authorities. The performed study was not designed to meet the requirements of the updated OECD 305 guideline or the criteria required to evaluate the 'B' criterion of the PBT assessment under REACH.<br>When considered against the current criteria, the 2000 study reveals several shortcomings for deriving a revised BCF, as performed by the DS. The additional information requested by ECHA and/or evaluating member states cannot be derived from this study with sufficient robustness to draw conclusions on the bioaccumulation potential of the tested substance. |
| 82 | Although some elements deviated from the standard OECD TG 305 (use of surfactant and dissolvent, measurements were made for a group of 2 fish instead of individually), the study is well conducted, follows GLP principles and is reliable for use under CLP in the absence of data on the UVCB substance. | The registrants consider that the shortcomings in the performed fish study to derive a new BCF contribute to an unrealistic inaccurate evaluation by the DS which cannot be used to robustly assess bioaccumulation. The registrants suggest that a new OECD 305 study is performed to meet the requirements and criteria of the current guideline as well as to clarify the uncertainty in the assessment caused by the identified shortcomings. |
| 82 | Finally, based on Catalogic and BCFBAF predictions, C9DPA indicates a BCF value ≥500, thus the substance is considered to meet the criterion for a potential for bioaccumulation in aquatic organisms. | The European Chemical Agency (ECHA) considers DMAX as a potential indicator of the likelihood of bioaccumulation. The DMAX for MNDPA was calculated as 1.69 nm. The ECHA guidance Chapter R.11: PBT/vPvB assessment states "From one study of a diverse set of substances it appeared that for compounds with a DMAXaver larger than 1.7 nm the BCF value will be less than 2000". In this case provided by ECHA, the indicator value of 1.7 nm. However, this same guidance acknowledges that this is not an absolute cut-off and different models will calculate slightly different values. Therefore, it can be considered that MNDPA meets this threshold.<br>The threshold of 1.7 nm was based on the work of Dimitrov et al. (2002, 2003), cited in Environment Agency (2009). In this analysis, it was determined that there was an inverse relationship between DMAX and log BCF, with the BCF decreasing exponentially as DMAX increases. Dimitrov et al. (2003) determined that a DMAX of 14.7 nm, a BCF of 5500 would be calculated, which would meet the criteria for very bioaccumulative (vB) under REACH. However, molecules with a DMAX >1.5 nm had a log BCF of <3.3 (<2000), and therefore, do not meet the criteria for bioaccumulative (B) under REACH. As such, based on a DMAX value of 1.69 nm, MNDPA can be considered unlikely to bioaccumulate. |

| page | Citation from CLH report, proposal for Harmonized Classification and Labelling for Reaction products of diphenylamine with nonene, branched | Comments on the respective section of the CLH proposal |
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| | The bioconcentration factors at steady state (BCFSS) were used to evaluate the potential of bioaccumulation. At a concentration of 10 µg/L, a BCF for the whole body of 1730 L/kg w.w. was calculated by the authors (BCF=411 L/kg w.w. for high exposure dose). The DS revised the calculation using the R-package "bcmfR" to estimate the BCF of the low exposure dose (10µg/L) using kinetic approach and taking into account lipid normalisation. The new BCF calculated was BCFKLip=2219 L/kg. | The registrants disagree with the evaluation of the DS since an old study designs (2000) with several shortcoming was evaluated against more recently criteria (2012) to deviate the BCFkin of 2219 L/kg.. However, if the evaluation is based on the old evaluation criteria in accordance with the old study design, there is no bioaccumulation potential according to the PBT criteria (BCF 1720L/kg).<br>The experimental study was performed to meet the requirements of the Japanese authorities but was not designed to meet the requirements of the updated OECD 305 guideline or the criteria required to evaluate the 'B' criterion of the PBT assessment under REACH. There are therefore a number of shortcomings in the study, as recognized by the DS, however despite this the DS has reassessed this data against these new criteria and has produced an unrealistic worst-case evaluation which they use to assess bioaccumulation. The DS has reached the conclusion that the BCF in the fish is not 1720 but 2219 L/kg.<br>There are many issues when reassessing a study under new criteria and inherent uncertainty in these estimations. The registrants consider that the shortcomings in the performed fish study contribute to an unrealistic inaccurate evaluation which cannot be used to robustly assess bioaccumulation. Since the data are close to the threshold of 2000, and a valid assessment of "B" is crucial to the PBT assessment so the Registrants strongly recommend a re-performing the bioaccumulation study in fish - as per the current testing proposal. |
| 82 | Considering the estimated log Know ≥4 for the constituents and the measured BCF≥500 for the C9DPA, it is therefore concluded that the substance has a potential for bioaccumulation in aquatic species. | The UVCB substance is expected to be bioaccumulative (e.g. BCF > 500, KOW> 4), but not bioaccumulative in terms of PBT criteria with the B or vB criterion met, e.g with BCFvalues above 2000 or 5000, respectively. |
| 89 | The chemical specific analysis showed that only one of the two main constituents (the C9DPA) could be determined, suggesting that C9DPA is the most water-soluble and bioavailable constituent. | testing laboratory: the analytics were confined to the detection of the mono-alkylated Isomers. Given<br>concentrations refer to µg/L test item (UVCB) as for the mono alkylated isomers no analytical reference was available. Analytical system: LC-MS/MS |
| 89 | Taking into account the measured concentrations of the constituent C9DPA, the effects on reproduction occurred at concentrations below the water solubility limit of this constituent, which is < 5 µg/L (OECD TG 105). Thus, based on this study, the LOEC for the substance (according to the C9DPA measured concentrations) should be based on the LOQ of 1 µg/L (which is lower than water solubility of the substance <5 µg/L). | CLH proposal p. 92) suggests that the value of 1.28 µg/L would validly represent the whole UVCB - while in fact the DS derived a NOEC value which is solely based on measured C9DPA. In contrast, the conducting laboratory correctly evaluated the study based on nominal values (loading rates) for the whole substance, while the monosubstituted constituent group (C9DPA) was the only constituent that could be measured to demonstrate that organisms were exposed to the UVCB substance. This does not mean that other constituents were absent, nor does not mean that they did not contribute to toxicity.Due to the low detection and lack of analytical certainty of the MNDPA constituent and the potential contribution of other constituents to aquatic toxicity, the proposed classification of chronic aquatic category 1 with M factor 10is not appropriate. |

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| | "*There seems to be a slight trend to have higher dissolved concentrations of C9DPA in the test media with higher loading rates when calculating the average based on the 3 measurements from fresh solutions*", | In the course of reviewing the measured data using ANOVA, it was noticed that the standard deviations of all nominal concentrations based on their measured values at the time points day 0-1; 7-8,14-15 overlap to such an extent that no significant trend for the dissolved concentrations can be derived, and thus also no dose-response relationship can be inferred. This confirms that the measured concentrations can be used to demonstrate that the organisms were exposed to a concentration at the beginning of the water change, but are not suitable for quantification and subsequent derivation of a NOEC for the test.<br>It should be noted that the approach of using the measured concentration if the measured concentration differs by more than 20 % from the nominal concentration does not apply to UVCBs in the low concentration ranges, which is why OECD 23 also allows reference to the nominal loading rates if the analysis is inadequate. This is the case for two reasons here: a) only monosubstituted constituent group (C9DPA) has been measured in lieu of quantification of whole substance, and b) no dose-response relationship based on measured values. |
| 90 | In addition, the DS recalculated the treatment dose, based on the available measured concentration (Day 0, Day 7 and Day 14), and following the OECD TG 211 for the calculation of the time-weighted mean concentration. | When applying the LOQ/2 approach to derive mean concentrations, the registrants understand the wording of R.7b ("***detected but not quantified***") as a stipulation. However, the study report does not provide that values below LOQ were detected. In addition, the concentrations may have been only slightly below LOQ as some measured values after 24 hours suggest. Thus applying LOQ/2=0.5µg/L would lead to inaccurate estimates of aquatic toxicity in this case. However, this would only be relevant if derivation of a NOEC based on measured concentrations was a valid evaluation method for this UVCB - which is not the case. |
| 91 | Table 45: EC10 estimations and confidence intervals (REGTOX_EV7.0.7) | The DS used REGTOX_EV7.0.7 to calculate an EC10 value based on the time-weighted mean concentration of the C9DPA constituent to subsequently relate the EC10 value to the NOEC derived, concluding to use the derived NOEC.<br>According to the registrants' information, REGTOX is neither mentioned in the current versions of the OECD 211 or ECHA Guidance R.7b nor is it an established statistical tool used by laboratories. The DS is requested to justify utilization of REGTOX and to clarify the results from this model compared to established and accepted statistical tools. |
| 91 | The EC10 1.69 µg/L (CI95 1.21-1.74) is considered by DS. Confronting the data obtained from these different methods, it can be assumed that the values for LOEC, EC10 or NOEC are in the same range of concentration. This EC10 value (1.69 µg/L) being close to NOEC 1.28 µg/L, and considering that the statistical analysis from the study is adequate, the DS determined that a NOEC of 1.28 µg/L and a LOEC of 1.73 µg/L based on measured concentrations for the C9DPA will be used for further considerations. | While the testing laboratory derived valid effect values based on the nominal exposure rates (WAF, whole UVCB), the DS determined the effect values based on the measured concentrations for only one component group (C9DPA) of the UVCB substance. Considering the predicted toxicity of other constituents in the UVCB besides this one measured constituent, Registrants are of the opinion that it is unjustified to base aquatic toxicity of this substance solely on quantification of C9DPA as this will lead to an inaccurate assessment of toxicity, when daphnia are exposed to the whole UVCB (with unquantified concentrations of other constituent groups). |

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| 93 | Two studies showed adverse effects in regards to the substance.<br>The study on *Daphnia magna* indicated toxic effects of the substance (OECD TG 211, GLP compliant, 2020). The DS revised the NOEC value based on measured concentration of C9DPA constituent, which was been detected and measured in this test with the UVCB substance, and determined a NOEC of 1.28 µg/L. | There is only one valid study which is considered to be the key study. The second study is an old study with low Klimisch rating performed without analytics and without GLP. Effects were seen far above the water solubility.<br>The DS derived value of 1.28 µg/l is inaccurate and not in line with OECD 23 or the CLP guidance. The correct interpretation of effect values for the whole UVCB is based on the nominal loading rate. Based on this evaluation there is no toxicity in the range of water solubility and therefore no classification and labelling is required. |
| 93 | Long-term aquatic hazard 11.6.2 (Tab.) and<br>“Based on the CLP regulation, a classification in category 1 - H410 for aquatic chronic hazards is justified for the substance according to the criteria given in Table 4.1.0(b)(i) and considering the chronic data on toxicity<br>for *Daphnia magna* NOEC(21d) 1.28µg/L. A Mfactor=10 should apply.” | Bioaccumulation OECD 305: The registrants disagree with the evaluation of the DS since an old study designs (2000) with several shortcoming is evaluated against more recently criteria (2012). Based on the old evaluation criteria the substance is not B or vB in terms of PBT assessment (BCF < 2000).<br>Chronic daphnia (OECD 211): In line with OECD 23 the relation of effect values should be the nominal loading rate if measurement of concentrations is not feasible. Based on this evaluation there is no toxicity in the range of water solubility and therefore no classification and labelling is required. |

# Annex 2 – Detailed information on effects on implantation sites, litter size and systemic toxicity

In the present CLH proposal, the DS claims that the substance Reaction products of diphenylamine with nonene, branched causes changes in implantation sites:

> ***Lower mean number of implantation sites and consequently smaller litter sizes (main critical effect).*** *The litter size (mean number of delivered pups) was significantly reduced by 19% and 31% in the mid-dose (1500 ppm eq. 133 mg/kg bw/d) and high-dose dose (5000 ppm eq. 443 mg/kg bw/d) dams exposed to Reaction products of diphenylamine with nonene, branched.[…] This effect was dose-related and observed from dose levels (mid-dose groups) where no effects on body-weight and food consumption were observed during the premating periods.*

Based on the data available, ATC consider that this assessment and the data presentation by the DS does not adequately reflect the available data. Therefore, additional information is provided in this section and these further details should be considered for evaluation of the hazard potential for classification. The data provided in this section are limited to those data available with the substance under evaluation for harmonized classification and labeling (Reaction products of diphenylamine with nonene, branched). Additional data has been suggested as read-across information by the DS, however this is not supported by the Registrants (further information on rejection of the read-across can be found in Annex 10 – Comments on the Read-across approach) and the information available for the substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene can be found in the comments on this respective proposal for harmonized classification and labeling.

## 1. OECD TG 421

A study was conducted according to OECD TG 421 and under GLP (BASF SE, 2020a). The test substance was administered in the diet at concentrations of 0, 500, 1500 and 5000 ppm. The duration of treatment covered a 10-week premating period and a 2-week mating period in both sexes as well as the gestation period and 13 days of lactation in females. Additionally, recovery animals were included, which were treated with either 0 or 5000 ppm, but not mated, and subsequently maintained for 14 additional days without substance administration to investigate the reversibility of the relevant findings.

No treatment-related adverse effects or changes were observed as regards the mating index, fertility index, estrous cycle, gestation index, parturition/maternal care, post-implantation loss, early post-natal pup development, sex ratio and nipple/areola anlagen on post-natal day (PND) 13.

### *Implantation sites*

Mid- and high-dose group females displayed mean decreased implantation sites of 10.9 and 9.9, respectively, as compared to a mean of 14.4 in the concurrent control group (Table 1). Importantly, the numbers of implantation sites were within the historical control data (HCD) of the laboratory for all treated groups (Table 2). Given that the historical control data reflect the biological variation normally observed for this parameter in this rat strain, they are a key

consideration when determining the significance of an effect observed in a biological system. Therefore, these findings must be considered of limited biological relevance.

*Litter sizes*

The mean litter size was statistically significantly reduced in the mid and high dose groups (12.6, 12.9, 10.2 and 8.7 in control, low, mid and high dose groups, respectively, Table 1). The relevant historical control range of the laboratory was 9.0-14.0, reflecting the biological variation for this study type and rat strain. In terms of litter sizes, the DS state that there is a 19% drop in litter size in the mid dose group. However, the reduction seen in this dose group is again within the HCD for the laboratory. Therefore, the finding in the mid dose group, while statistically significant, must be considered of limited biological relevance. The reduction in litter size observed in the high dose group is only marginally outside the HCD range, so whilst it is statistically significant, there is uncertainty with regards to its biological relevance. The effects are therefore neither clear nor definitive for this endpoint, and the use of the 19% and 31% decrease statistics by the DS to support their classification proposal is simplistic; the full picture needs to be considered.

The lower number of delivered F1 pups at 5000 ppm does not appear to be the result of embryo-fetal loss or effects on in utero survival, and post-implantation loss was unaffected at all exposure levels (Table 1).

*Table 1: Summary table of mean implantation sites, litter sizes and post-implantation loss observed in OECD TG 421 study (BASF SE, 2020a)*

| Concentration in diet | ppm | **0** | **500** | **1500** | **5000** |
|---|---|---|---|---|---|
| Approx. Dose (females) | mg/kg bw/d | 0 | 44 | 133 | 419 |
| **Pregnant females [N]** | N | 9 | 10 | 9 | 10 |
| **Litters [N]** | N | 9 | 10 | 9 | 10 |
| **Implantation sites** | N | 130 | 131 | 98 | 99 |
| | Mean | 14.4 | 13.1 | 10.9** | 9.9** |
| | SD | 1.9 | 2.1 | 1.6 | 2.3 |
| **Pups delivered** | N | 113 | 129 | 92 | 87 |
| | Mean | 12.6 | 12.9 | 10.2* | 8.7** |
| | SD | 2.2 | 2.1 | 1.7 | 2.1 |
| **Post-implantation loss** | Mean [%] | 12.1 | 1.6 | 5.7 | 10.4 |
| | SD | 15.7 | 3.5 | 11.1 | 16.1 |

Wilcoxon with Bonferroni-Holm (one-sided) *p ≤ 0.05, ** p ≤ 0.01

*Historical control data*

One key argument of the DS regarding reliability of the available data was that the historical control data (HCD) provided in the study reports was unreliable:

“However, the range of HCD for the number of implantation sites from 2008 to 2018 period provided in the study report of the OECD TG 443 (Unpublished study report, 2021) performed with Benzenamine, N-phenyl-, reaction products with 2,4,4- trimethylpentene by the same laboratory, was 11.1 - 15.3 sites and 11.2-15.3 when considering a more appropriate timeframe (2015-2018). When considering the later HCD, the value of the concurrent control is well within the HCD range while the values of the mid-dose and high-dose groups are outside […]”

It should be pointed out that historical control data from one study protocol can only be adapted to another study protocol under the considerations of e.g., animal age, treatment protocol,

group size, etc. Therefore, historical control data of OECD 421/422 and OECD 443 studies are not necessarily compatible and expert judgement on the study conduct is required to assess which data are adequate for consideration as historical control data. Given that study reports are finalized as soon as possible, only data from past years can be considered as historical control data at the time of report generation. The study in-life phase was in 2019, therefore new historical control data from OECD 421/422 studies were compiled which were conducted between 2015 and 2022. These include 79 studies, with the following exposure routes: diet (11), gavage (58), drinking water (6), inhalation (4). The results are presented in Table 2.

*Table 2: Historical control data from 79 OECD 421/422 studies performed at the testing laboratory, compiled on March 6, 2024*

| | Runtime | Litters | Implantation sites [mean] | Pups delivered [mean] | Post-implantation loss [mean%] |
|---|---|---|---|---|---|
| Mean of means | | | 12.7 | 12.0 | 6.4 |
| S.D. | | | 0.90 | 0.83 | 3.90 |
| Min. | 2015 | 8 | 9.8 | 9.0 | 0.9 |
| Max. | 2022 | 10 | 14.7 | 14.0 | 16.8 |

S.D., standard deviation

*Ovary weights*

An additional finding at 5000 ppm was a significant decrease in absolute and relative ovary weights (60% and 69% of concurrent control value, respectively). Histopathological investigation showed no correlating findings; therefore, this was considered a test substance-related but not adverse effect. Importantly, the effects on ovarian weights were reversible, and the relative ovarian weights returned to normal following the 2-week recovery period (98% of concurrent control), while absolute organ weights were only slightly decreased at the end of recovery (86% of concurrent control). Due to this complete recovery in a short time frame, it is reasonable to conclude that the effect on the ovary weights is a secondary toxicity effect rather than substance-specific damage, as that would be expected to present with both concurrent histopathological findings as well as a longer recovery time.

*Systemic toxicity*

The DS states that at the top dose levels in the study, the systemic toxicity was not marked, however they define marked toxicity as “no lethality, no dramatic reduction in bodyweight and no coma”. It should be clearly pointed out that while those might be the criteria to justify non-classification based on systemic toxicity, these are also the criteria to justify humane sacrifice during study conduct according to OECD Guidance document 19 (OECD, 2000). Therefore, it should not be the goal to achieve this level of toxicity during an OECD guideline study, given that the OECD guidelines specifically state that the high dose level should be chosen to induce some toxicity, but not severe suffering or death (OECD, 2018). Therefore, these above-mentioned effects should not be assessed as “marked” toxicity, but would rather be considered “excessive” toxicity in the context of an OECD guideline study.

Main study animals of the high dose group (5000 ppm in diet, approx. 397 mg/kg bw/d in males, 419 mg/kg bw/d in females) showed decreased food consumption, decreased body weights and decreased body weight change. Main study animals of the mid dose group (1500 ppm, approx. 122 mg/kg bw/d in males and 133 mg/kg bw/d in females) showed similar signs of systemic toxicity, albeit to a lesser degree. Female food consumption, body weights and body

weight change were significantly decreased during gestation; body weights were also decreased during the lactation phase (Table 3).

*Table 3: Data on body weights, body weight change and food consumption in OECD TG 421 study (BASF SE, 2020a)*

| | | Main study groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration in diet | [ppm] | 0 | | 500 | | 1500 | | 5000 | |
| Approx. dose (♂/♀) | [mg/kg bw/d] | 0 | | 40/44 | | 122/133 | | 397/419 | |
| Body weights<br>♂ d91 / ♀ d70 | Mean (♂ / ♀)<br>SD<br>Dev. vs. control [%] | 412.9<br>29.1 | 217.9<br>11.9 | 403.1<br>15.5<br>-2.4 | 224.6<br>9.3<br>3.1 | 396.0<br>28.4<br>-4.1 | 212.3<br>13.4<br>-2.5 | 363.4**<br>21.7<br>-12.0 | 194.3**<br>14.5<br>-10.8 |
| Body weights gestation (GD20) | Mean<br>SD<br>Dev. vs. control [%] | 343.4<br>23.4 | | 339.4<br>20.2<br>-1.2 | | 313.3*<br>22.0<br>-8.8 | | 279.8**<br>20.2<br>-18.5 | |
| Body weights lactation (LD13) | Mean<br>SD<br>Dev. vs. control [%] | 294.3<br>16.2 | | 290.4<br>16.0<br>-1.3 | | 273.5*<br>18.8<br>-7.1 | | 244.6**<br>17.0<br>-16.9 | |
| Body weight change<br>♂ d 0-91 / ♀ d 0-70 | Mean (♂ / ♀)<br>SD | 296.2<br>28.6 | 122.1<br>8.5 | 285.9<br>13.4 | 128.9<br>9.9 | 279.7<br>23.0 | 116.1<br>112. | 247.3**<br>19.9 | 97.8**<br>12.5 |
| Body weight change gestation (GD 0-20) | Mean<br>SD | 118.8<br>12.4 | | 107.3<br>12.5 | | 95.7**<br>14.3 | | 82.0**<br>16.6 | |
| Body weight change lactation (LD 1-13) | Mean<br>SD | 36.5<br>9.6 | | 39.0<br>7.1 | | 31.4<br>7.7 | | 32.2<br>11.2 | |
| Food consumption d0 – d70 | Mean (♂ / ♀)<br>SD<br>Dev. vs. control [%] | 21.0<br>0.4 | 15.7<br>0.5 | 21.4<br>1.0<br>1.9 | 15.9<br>0.4<br>0.9 | 21.3<br>1.4<br>1.1 | 15.9<br>1.0<br>0.9 | 19.7<br>1.1<br>-6.4 | 13.9**<br>0.3<br>-11.6 |
| Food consumption gestation (GD0-20) | Mean<br>SD<br>Dev. vs. control [%] | 21.0<br>1.2 | | 20.1<br>1.0<br>-4.1 | | 19.2**<br>1.0<br>-8.8 | | 16.8**<br>1.6<br>-20.1 | |
| Food consumption lactation (LD1-13) | Mean<br>SD<br>Dev. vs. control [%] | 47.2<br>3.7 | | 46.0<br>3.5<br>-2.6 | | 43.9<br>3.7<br>-7.1 | | 36.1**<br>3.3<br>-23.5 | |

Dunnett test (two-sided), * $p \leq 0.05$, ** $p \leq 0.01$

As mentioned above, maternal systemic toxicity included reduced body weights and reduced food consumption. Maternal toxicity is known to adversely affect female fertility in rats including implantation, thus this pattern of effects clearly suggests a link between maternal systemic toxicity and the observed slight reduction in implantation sites. In more detail, the impact of reduced food intake leading to reduced body weight (gain) on female fertility was systemically studied in Sprague Dawley rats in an FDA regulatory study by Terry et al. (Terry et al., 2005). In this study, a restricted feeding regime (*ad libitum* as concurrent control, 20, 15, 10, or 7.5 g/rat/day) was applied to groups of 20 female rats during the 14-days pre-mating phase, throughout the mating phase, and continued until GD7, whereas male rats were fed *ad libitum* throughout the study. Females were terminated on GD14. Control rats consumed ca. 20 and ca. 28 g/day during pre-mating and gestation phases, respectively, and all measured fertility parameters in the group receiving 20 g/day were comparable to concurrent control animals. A slight reduction of the daily amount of food to 15 g/day caused a 16% reduction in the mean body weight at the end of the pre-mating phase. There were no clinical signs observed in these rats, and all animals successfully mated. However, a slight, non-significant increase in mean estrous cycle length (by 6%) and an increased incidence of prolonged diestrus was observed only when totaled over 2 weeks. The mean body weight in the 15 g/day group was reduced by 26% on GD8, after which the food was provided *ad libitum*. In this group, the fertility parameters time to mating, copulation and pregnancy rates were unaffected, however the numbers of corpora lutea, implants, and the number of viable implants were significantly reduced, yielding a mean number of implants of 12.2 ± 3.4 in the groups receiving 15 g/day vs. 15.7 ± 1.6 (i.e., -22.3%) in the concurrent control group. Consequently, the mean number of viable implants was also statistically significantly reduced to 11.5 ± 3.3 vs. 14.2 ± 2.4 (i.e., -19.0%) in the concurrent control group. The study authors concluded that the reduced number of live implants at 15 g/day was the consequence of the reduced number of corpora lutea (13.2 ± 1.9 vs. 15.9 ± 1.4) indicating reduced nidation as pre-implantation loss in this group was not abnormal.

In conclusion, this study found that body weight changes in the range of ca. 16% affected female fertility and seem to be near the threshold of subtle effects on estrous cycle parameters. Considering the reduced food consumption (-11.6% and -20.1% during the pre-mating and gestation phases, respectively) and reduced body weights (-10.8% and -18.5% during the pre-mating and gestation phases, respectively) observed in the OECD TG 421 study, and the striking similarity in the observed pattern of subtle changes in female fertility, i.e. slight reduction of the mean number of implantation sites and related lower mean number of implants in a similar order of magnitude without effect on any other reproductive parameter, strongly suggest a similar mechanistic link in the OECD TG 421 study. Comparability of the exact numbers may be hampered by unclear transferability of the data between the different rat strains; however the conclusion is supported by earlier studies which also found a relation between reduced food consumption, reduced body weights and affected fertility parameters in female rats (see references cited in (Terry et al., 2005)).

Based on this, the slight reductions in the mean numbers of implantation sites and litter size seem to be a secondary consequence to systemic maternal toxicity of the substance Reaction products of diphenylamine with nonene, branched at the highest dose level tested.

*Summary*

A reduction of implantation sites was reported in the extended screening study according to OECD TG 421 with ten weeks pre-treatment, as well as a reduction in litter size. However, the significance of these effects is uncertain, because:

1. Values for implantation sites are within the historical control data of the laboratory and therefore considered of limited biological relevance.
2. Values for litter size are only slightly below the HCD of the laboratory in the high dose group and within the HCD range for the mid dose group and therefore the effect significance and apparent dose response is uncertain.
3. Concurrent with these effects, the animals showed pronounced systemic toxicity, which has in the literature been reported to lead to similar effects on fertility as those observed in this study.

# Annex 3 – Detailed information on ovary weight changes

In the present CLH proposal, the DS claims that the substance Reaction products of diphenylamine with nonene, branched causes changes in ovary weights:

> ***Decreased ovary weights**. Dose-related and significant decrease in absolute ovarian weights was observed in dams exposed to Reaction products of diphenylamine with nonene, branched (by 18% and 40% at mid- and high dose level respectively) in the OECD TG 421 study. The relative weight was also impacted from the mid-dose level but statistically significantly only at the high dose level.*
> *While a statistically significant decrease (-14%) in absolute ovarian weight was also observed in the recovery group of this study (not mated females exposed to 5000 ppm during 10 weeks with a 2-week recovery period) no effect on ovary weight was noted in females up to 1000 mg/kg bw/d of Reaction products of diphenylamine with nonene, branched by gavage in corn oil in a GLP-compliant 90-day toxicity study (Unpublished study report, 2013- **Study 7** ).*

1. OECD TG 421

In the OECD TG 421 study, organ weights for ovaries were evaluated for the parental females of the main study groups. In addition, a 14-day treatment-free recovery period was included for control and high dose groups, after which organ weights and histopathology were also analyzed (BASF SE, 2020a). Both absolute and relative organ weights were determined. While absolute ovary weights represent the measured values upon sectioning, the relative weights encompass the ratio of organ vs. body weight for each animal. In case a treatment affects the body weights of animals as a sign of generalized toxicity (Ghasemi et al., 2021), both parameters should be considered for data interpretation. Depending on the time of treatment initiation with respect to age and duration of treatment, relative organ weights might provide a better parameter for interpretation as compared to absolute organ weights.

In the main study groups, the mean absolute ovary weights were significantly lower in mid and high dose females at the end of the treatment period. Relative ovary weights of the high dose group females were also significantly lower than those of the concurrent controls; however, the relative ovary weights of the mid dose group were not significantly different from the controls at the end of the main study period (Table 4).

At the end of the 14-day treatment-free recovery phase, absolute ovary weights of high dose females were still slightly but significantly lower than those of the concurrent controls, however relative organ weights of treated females were comparable to concurrent controls (Table 5). Therefore, the relative ovary weights had completely recovered within two weeks while absolute ovary weights had mostly recovered.

In the main study group animals, only in test group 3, the decreased absolute (absolute 66.9 mg) and relative (0.033%) weights were below the minimal values recorded for HCD (absolute 82.7 – 142.0 mg; relative 0.035 – 0.062%). No relevant histopathological findings were noted that could explain the organ weight decreases. Additionally, the findings on absolute ovary weights were mostly reversible within a 14-day recovery period, whereas no changes were observed in relative ovary weights following a 14-day recovery period. Together, these findings suggest the transient decrease in ovary weights was treatment-related but not adverse. In addition, studies in SD rats using an experimental ovarectomy model showed that removal of less than 50% of the ovaries did not affect fertility of rats and offspring development (Yang et al., 2023).

*Table 4: absolute and relative ovary weights observed in females of main groups of OECD 421 study (BASF SE, 2020a)*

| Concentration [ppm] | | 0 | 500 | 1500 | 5000 | HCD |
|---|---|---|---|---|---|---|
| Approx. dose females [mg/kg bw/d] | | 0 | 44 | 133 | 419 | |
| Absolute ovary weights [mg] | Mean | 110.8 | 104.4 | 91.0* | 66.9** | Mean 106.327<br>Min 82.7<br>Max 142.0 |
| | Deviation (% of control) | 100 | 94 | 82 | 60 | |
| | SD | 21.65 | 10.83 | 18.36 | 13.79 | |
| | n | 10 | 10 | 10 | 10 | |
| Relative ovary weights [%] | mean | 0.048 | 0.045 | 0.041 | 0.033** | Mean 0.044<br>Min 0.035<br>Max 0.062 |
| | Deviation (% of control) | 100 | 93 | 85 | 69 | |
| | SD | 0.008 | 0.007 | 0.009 | 0.006 | |
| | n | 10 | 10 | 10 | 10 | |

*p ≤ 0.05, **p ≤ 0.01

*Table 5: absolute and relative ovary weights observed in females of recovery group of OECD 421 study (BASF SE, 2020a)*

| Concentration [ppm] | | 0 | 5000 |
|---|---|---|---|
| Approx. dose females [mg/kg bw/d] | | 0 | 419 |
| Absolute ovary weights [mg] | Mean | 120.4 | 104.0** |
| | Deviation (% of control) | 100 | 86 |
| | SD | 13.81 | 6.0 |
| | n | 10 | 10 |
| Relative ovary weights [%] | mean | 0.052 | 0.051 |
| | Deviation (% of control) | 100 | 98 |
| | SD | 0.005 | 0.004 |
| | n | 10 | 10 |

*p ≤ 0.05, **p ≤ 0.01

Furthermore, Figure 1 shows that the ovary weights were in the same range as control animals, provided the body weight was similar (i.e., three animals of the 5000 ppm group with the highest body weights). Only those females with lower body weights were below the range of the concurrent control animal ovary weights.

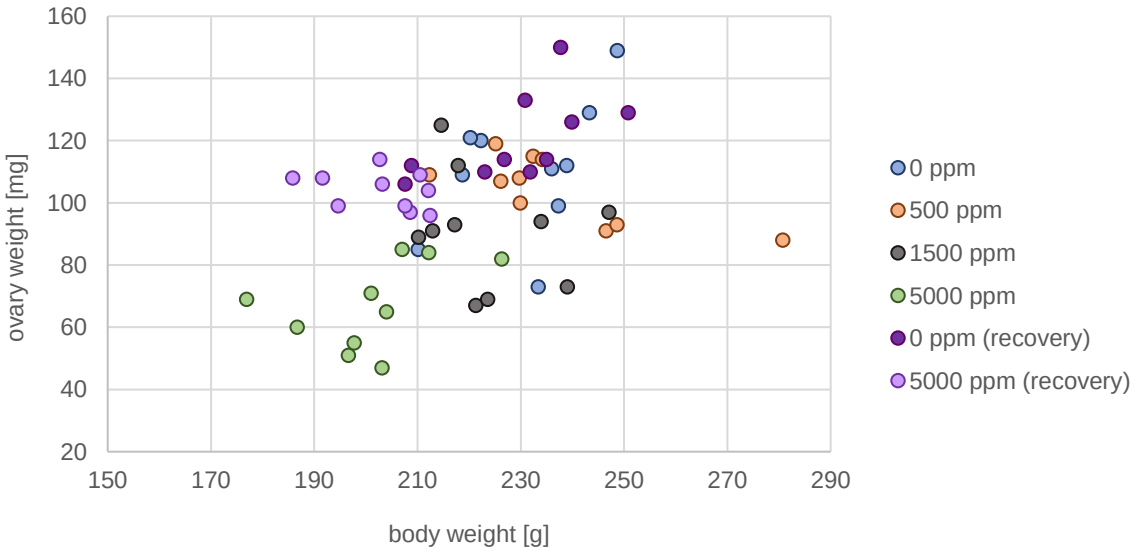



*Figure 1: comparison of absolute ovary weights with respective body weights of individual main study female animals observed in OECD 421 study (BASF SE, 2020a)*

Since the effect on ovarian weight is considered secondary to the test substance-related effect on female body weight and general systemic toxicity, was reversible after the 2-week recovery

period, and not associated with histopathological changes, this finding was considered a non-specific effect rather than direct treatment-related reproductive toxicity.

2. OECD TG 408

Additional evidence that the ovary weights findings in the OECD 421 study may be non-specific is supported by a 90-day toxicity study in rats conducted according to OECD 408 and under GLP (BASF SE, 2013). The substance was administered by gavage at dose levels of 0, 100, 300, or 1000 mg/kg bw/d. Treatment produced no adverse macroscopic or microscopic effects on male or female reproductive organs, including the ovaries, despite 90 days of dosing at a much higher exposure level (Table 6).

*Table 6: Absolute and relative ovary weights observed in OECD 408 study (BASF SE, 2013)*

| Dose level [mg/kg bw/d] | | **0** | **100** | **300** | **1000** |
|---|---|---|---|---|---|
| Absolute ovary weights [mg] | Mean | 105.8 | 99.9 | 105.0 | 102.7 |
| | Deviation (% of control) | 100 | 94 | 99 | 97 |
| | SD | 16.75 | 16.07 | 15.94 | 16.43 |
| | n | 10 | 10 | 10 | 10 |
| Relative ovary weights [%] | mean | 0.049 | 0.046 | 0.051 | 0.049 |
| | Deviation (% of control) | 100 | 94 | 104 | 101 |
| | SD | 0.008 | 0.006 | 0.008 | 0.007 |
| | n | 10 | 10 | 10 | 10 |

# Annex 4 – Detailed information on data for proposed estrus cycle changes

In the present CLH proposal, the DS claims that the substance Reaction products of diphenylamine with nonene, branched causes effects on estrus cyclicity:

> ***Effects on cyclicity.*** *Increase of estrous cycles length associated with an increased mean percentage of days in diestrous stage was observed in high-dose dams exposed to Reaction products of diphenylamine with nonene, branched or Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. Statistical significance was reached only with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene in in high-dose females of the OECD TG 421 and in high-dose P1 females of the EOGRTS.*

The authors would like to point out, that all data available for this argument were derived from a study conducted with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. This read-across conducted by the DS is not considered reliable; further information on the reasoning can be found in Annex 10 – Comments on the Read-across approach. Therefore, only the data available on the substance under evaluation should be considered.

On the data available on estrus cyclicity for Reaction products of diphenylamine with nonene, branched, the DS writes:

“At the high dose level, a slight non-statistically significant increase of estrous cycles length (4.3 days vs. control 4.0 days) associated with a slight increased in the mean percentage of days in diestrous stage (35% vs 28% in control).”

A disruption in cycling is characterized by persistent estrus, diestrus, or an extended duration of irregular cycles (Goldman et al., 2007).

In the OECD 421 study with Reaction products of diphenylamine with nonene, branched, the estrous cycle duration reported was 4.0, 4.0, 4.0 and 4.3 days in control, low, mid and high dose animals, respectively (BASF SE, 2020a). The slight increase in the high dose group was not statistically significant and due to only one animal showing one cycle with long diestrus duration. All other animals were in the range of the control group for estrus cycle length and number of days in diestrus. The one animal with one long diestrus cycle showed normal cycles with 1-2 days in diestrus for the remainder of the observation period. Thus, this finding is considered incidental and not treatment related.

Generally, the rat estrus cycle is divided into proestrus, estrous, metestrus and diestrus. Average duration of diestrus is around 55-57 hours (Chaitra et al., 2020; Cora et al., 2015; Paccola et al., 2013; Westwood, 2008), thus with daily staging during study conduct, a 1-3 day-long detection of diestrus is considered normal.

Overall, the findings on estrus cyclicity of the substance were neither statistically significant nor biologically relevant. Moreover, as described in more detail in Annex 2, subtle non-significant changes in mean estrus cycle length can be a secondary consequence of the observed maternal systemic toxicity, i.e. reduced food consumption and lower body weights. Therefore, the effects on cyclicity should not be taken into consideration for a sound scientific decision on classification.

# Annex 5 – Detailed information on neurohistopathology and neuromorphometrics

In the present CLH proposal, the DS claims that:

> *Based on the available data,* ***there is clear evidence that the anologue Benzenamine, Nphenyl-, reaction products with 2,4,4-trimethylpentene induces abnormalities in the central nervous system*** *in animals exposed during the developmental period. Such parameters were not investigated for Reaction products of diphenylamine with nonene, branched.*

The authors would like to point out, that all data available on this section of the document were derived from a study conducted with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. As even the DS mentioned, no data are available on these effects for the substance under evaluation for harmonized classification and labelling (Reaction products of diphenylamine with nonene, branched). It should be highlighted though that this is not due to a lack of willingness to generate these data from industry side, as the registrants for Reaction products of diphenylamine with nonene, branched have submitted a testing proposal for an adequate study on October 6th, 2021.

The data available for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene were addressed in the comments on the CLH proposal for that substance, but are also included below to facilitate assessment for the reader.

1. OECD TG 443

An extended one-generation reproduction toxicity study was performed according to OECD TG 443 and under GLP (BASF SE, 2021b). Wistar rats (25 per sex and dose) were treated with doses of 0, 200, 600 or 1800 ppm Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene via diet. F0 animals were treated for at least 10 weeks prior to mating to produce a litter (F1 generation). Pups of the F1 litter were selected (F1 rearing animals) and assigned to 4 different cohorts (1A, 1B, 2A and 2B) which were subjected to specific postweaning examinations. Cohort 1B (F1 generation parental animals) was selected to produce F2 pups; Cohort 1A animals were analyzed for specific reproductive parameters. Cohorts 2A (C2A) and 2B (C2B) were employed to investigate developmental neurotoxicity.

*Neurohistopathology*

As noted by the DS, a slightly higher incidence of axonal degeneration in the thoracic cord was observed in 9 of 10 F1 adult C2A male rats of the highest exposure group (i.e. 1800 ppm) tested with the substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. However, it is our opinion that the CLH dossier fails to put this finding into proper context from a weight-of-evidence perspective. Furthermore, the claim that a slight increase in the incidence of axonal degeneration occurred in “other” areas in males and females does not accurately reflect the data or report. Other than the thoracic cord, there are no data to support the presence of axonal degeneration in any other areas in male or female rats at any dose level in the OECD TG 443 study with the substance. Therefore, the following paragraphs will focus on the CLH claims that the substance induces axonal degeneration in the thoracic cord and neuromorphometric changes in the brain.

There are several direct lines of evidence that indicate that the slightly higher incidence of axonal degeneration in the thoracic cord of the high dose C2A males are either an age- or rat-specific artifact, or at worst, a secondary response to chronic high dose exposure. There is no evidence in this study of a developmental origin for this finding or that this change produced functional effects on the central nervous system. Firstly, the slightly higher incidence of this finding in high dose C2A males occurred against a high background of axonal degeneration in the thoracic cord of both males and females across all dose groups including healthy concurrent control animals. As shown in Table 7, 20% of C2A control males and 40% of C2A control females exhibited this same finding, with an equal incidence and severity in control and high dose females. There is no evidence of a dose-response pattern in C2A females, and no clear indication that severity worsened with increasing dose for either sex. The severity reported by the study pathologist across all dose groups including controls was graded as “minimal” (lowest severity grade), with only a single study animal showing a slightly higher grade of “slight to mild” at the highest dose tested of 1800 ppm, which is a dose that also produced overt signs of systemic toxicity in both F0 and F1 animals.

While the DS states that axonal degenerations were also slightly increased in other areas “(2 vs 0 tibial nerve degeneration in high dose males; lumbar cord axonal degeneration and sciatic nerve degeneration in 2 high dose females vs 0 in controls)” (CLH dossier, p.57), it should also be pointed out that axonal degeneration is commonly observed in all age groups of rats as an occasional spontaneous finding (Blankenship et al., 2016; Kaufmann et al., 2012). These findings mentioned by the DS were either made in single animals or are comparable to control. Based on the common observation of axonal degeneration in rats as incidental finding in this type of investigations, no toxicological weight can be given to single appearances of these findings without corroborating information.

*Table 7. Incidence and severity of axonal degeneration in the thoracic cord of F1 generation Cohort 2A (PND 77) male and female rats following chronic exposure with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene in an OECD TG 443 study (BASF SE, 2021b)*

| Thoracic cord | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Concentration in diet [ppm] | 0 | 200 | 600 | 1800 | 0 | 200 | 600 | 1800 |
| No. of animals | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Degeneration, axonal | 2 | 5 | 4 | 9 | 4 | 3 | 1 | 4 |
| Grade 1 (minimal) | 2 | 5 | 4 | 8 | 4 | 3 | 1 | 4 |
| Grade 2 (slight, mild) | | | | 1 | | | | |
| Gitter cells present | 1 | 2 | 1 | 5 | 2 | 1 | 0 | 0 |

In addition to the high background rate and minimal severity observed in adult animals across all dose groups, including healthy controls, this finding did not correlate with any functional neuromuscular or neurobehavioral deficits in these same C2A animals. The results of the functional observations batteries (FOBs) and neurobehavioral testing in these animals were normal (for further information, see also Annex 6 – Detailed information on data for proposed neurological functional deficits).

One weakness of the OECD TG 443 study is its inability to distinguish between chronic neurotoxicity effects of exposure and latent neurodevelopmental effects in Cohort 2 animals. Unlike the OECD TG 426 test guideline for developmental neurotoxicity (DNT) studies, exposure to the test substance in an OECD TG 443 study continues beyond PND 21 until the time of scheduled necropsy at 12 weeks of age for C2A (adult) animals. Importantly, neurohistopathological evaluation of the C2B adolescent animals showed no evidence of axonal degeneration in control or high dose animals earlier in development on PND 22,

suggesting that the presence of this finding in C2A adult animals, including controls, was either age-related or the result of chronic exposure.

The DS provides the argument that the neurohistopathological changes observed are likely developmental, since a previous OECD TG 422 study performed with the substance showed no histopathological findings on spinal cord and sciatic nerve. However, it should be taken into consideration that during an OECD TG 422 study the fixation procedure of tissues is suboptimal for detection of these effects when compared to the neuropathological investigations performed for the DNT cohort of the OECD TG 443 (immersion fixation vs. perfusion fixation). In addition, the sectioning in which this finding was detected in the neurohistopathology of the OECD TG 443 study DNT module were longitudinal sections whereas an OECD TG 422 study only includes cross-sections of the spinal cord. Therefore, a very slight finding in the neuropathological investigation of the OECD TG 443 study DNT cohort might not have been detected in the OECD TG 422 study. Additionally, no findings in the thoracic cord were noted in generation F0 and F1A in the OECD TG 443 study. In these cohorts, the thoracic cord samples were immersion fixed and examined as cross sections comparable to the OECD TG 422 study.

*Neuromorphometrics*

The DS claims that neuromorphometric changes were observed in high dose F1 animals in the OECD TG 443 study with the substance. Specifically, the DS noted a decrease in brain length in C2A males and an increase in the width of the corpus callosum in both C2A males and females.

Figure 2 shows brain length and width data for individual C2A adult males across all dose groups. Mean brain length x width measurements for C2A adult males were 21.28 mm x 15.81 mm for control males versus 20.59 mm x 15.56 mm for high dose males, which represents mean differences in brain size of less than 1 mm, i.e., 0.69 mm difference for length (-3.2%) and 0.25 mm difference for width; the changes observed for brain width did not reach statistical significance. As shown in Figure 2, part of this difference may be attributed to two control males (No. 602 and 604) with higher-than-average brain length and width measurements (as compared to HCD, Table 8). Individual brain length and width measurements for all C2A males in the high dose group fell within the range of values in the concurrent control group. In addition, the absolute brain weights of C2A males were 95% of controls, whereas relative brain weights were 102%. The reduced brain length of -3.2% was in the same range as the reduced absolute brain weights and thus is more likely to be a consequence of the animals being smaller rather than a substance-specific effect. In the absence of any corresponding effects on brain width, neurobehavioral or FOB endpoints in males or females (see also Annex 6 – Detailed information on data for proposed neurological functional deficits), these data do not support the claim of a neuromorphometric effect on brain length in C2A adult males. This is especially true given that literature measuring differences in brain size found no direct correlation with overall cognitive performance (Schoenemann et al., 2000).

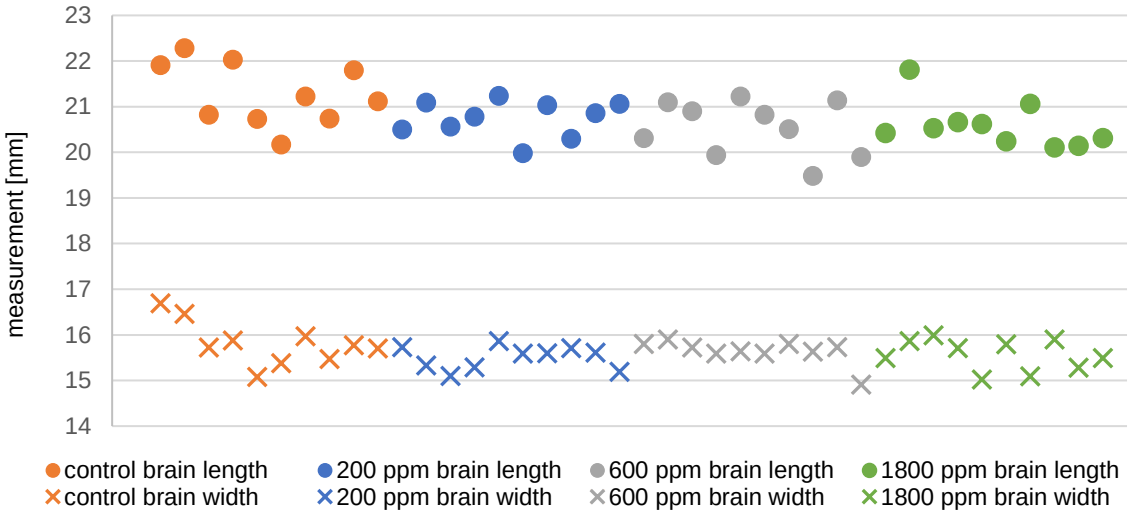



*Figure 2: Individual animal brain length and width of F1 generation Cohort 2A (adult) male rats in OECD TG 443 study (BASF SE, 2021b).*

The DS claims that brain morphometric analysis shows an increased width of the corpus callosum in high dose C2A adult males and females. Mean width of the male and female corpus callosum in the high dose group was 0.69 mm and 0.65 mm, respectively, compared to 0.59 mm and 0.56 mm in the concurrent control males and females. Of note, there is considerable variability across individual animals. Using statistical analysis suitable also for non-normally distributed data, no statistical significance could be found for either sex. Only if males and females are considered together in a statistical test assuming normal distribution is statistical significance attained.

*Table 8: Historical control data compiled for neuromorophometrics and brain weights in male animals based on five OECD TG 443 studies with DNT cohort performed between 2014 and 2024 (analysis at PND 77)*

| | Brain | | | |
|---|---|---|---|---|
| | Length [mm] | Width [mm] | Absolute weights | Relative weights |
| Mean | 21.116 | 15.859 | 2.048 | 0.650 |
| Min. | 20.760 | 15.580 | 1.955 | 0.636 |
| Max. | 21.700 | 16.600 | 2.132 | 0.670 |

Historical control data was compiled on corpus callosum width based on five OECD TG 443 studies with DNT cohorts conducted between 2014 and 2024 (Table 9). Three studies were conducted via diet, one via gavage and one via drinking water. The HCD are depicted for each, males and females, given that there is generally a sex-dependent difference in measurements. Based on the historical control data available, the values reported for high dose animals are well within the normal biological variation observed for this parameter in this rat strain. For the control group males, the values were unusually low with 8/10 animals below the HCD for this parameter. In addition, the group mean value for Corpus callosum width for control males was below the HCD of the laboratory (Table 9). In females, 4/10 animals of the control group were below the HCD range of the laboratory, while the group mean value was just within.

In addition, a coefficient of variation was determined for this parameter by the laboratory, to provide further orientation for normal biological variation. Based on the variations commonly observed for this parameter, the change of high dose group as compared to controls of +16% in males and females is within the commonly observed biological variation range for this parameter.

Overall, the changes presumed by the DS are of limited biological relevance.

*Table 9: Historical control data compiled for neuromorophometrics (corpus callosum width) based on five OECD TG 443 studies with DNT cohort performed between 2014 and 2024 (analysis at PND 77)*

| | Corpus callosum width [mm] | | Corpus callosum coefficient of variation [%] | |
|---|---|---|---|---|
| | Females | Males | Females | Males |
| Mean | 0.68 | 0.74 | 18.89 | 16.53 |
| Min. | 0.54 | 0.68 | 12.56 | 13.44 |
| Max. | 0.76 | 0.8 | 23.89 | 21.6 |

Moreover, the direction of change (increase in width) does not correlate with other parameters that would be considered of toxicological concern. The width of the corpus callosum is one of 10 morphometric measurements collected on the brains of the C2A animals in an OECD TG 443 study. There was no indication of corresponding effects in any of the other nine brain regions analyzed by morphometry in these same C2A males or females. An additional assessment of the data available with the statistical method conducted by the DS (two-way ANOVA accounting for treatment and sex) did not show statistically significant changes in any other neuromorphometric parameter (Table 10). The DS found it noteworthy that *"the corpus callosum is the principal inter-hemispheric myelinated tract (white matter) and histopathological findings linked to myelin degeneration in the cord white matter were observed in C2A animals."* (CHL dossier, page 56). However, neurohistopathological findings in the study were limited to the thoracic part of the spinal cord, no relevant findings were obtained in other sections. Many studies have been published on the detrimental effects of a smaller or missing corpus callosum in rats and humans, but there is no evidence in studies of rats that shows a small increase in the corpus callosum without other physiological effects causes any differences in overall development.

*Table 10: Analysis of neuromorphometric parameters obtained in OECD TG 443 study (BASF SE, 2021b) using two-way ANOVA (sex,treatment) to compare control and high dose groups.*

| Neuromorphometric parameter | p-value |
|---|---|
| Base of lobus vermis cerebelli | 0.65044 |
| Corpus callosum width | 0.03675 |
| Frontal cortex left | 0.94775 |
| Fronal cortex right | 0.99080 |
| Hippocampus left | 0.82395 |
| Hippocampus right | 0.23663 |
| Nucleus caudatus width left | 0.11406 |
| Nucleus caudatus width right | 0.73170 |
| Parietal cortex left | 0.21128 |
| Parietal cortex right | 0.20159 |

In addition, studies that reviewed a thickened corpus callosum in humans indicate that clinical manifestation of this change was never seen in isolation but "invariably associated with additional brain abnormalities" and "part of a neurogenetic syndrome in most cases" (Lerman-Sagie et al., 2009). Therefore, a slight increase in one of 10 measured brain morphometric parameters (i.e., width of corpus callosum), in the absence of corresponding effects on any of the other nine brain morphometric parameters or neurobehavioral or FOB endpoints in males or females (see Annex 6 – Detailed information on data for proposed neurological functional deficits), does not support the DS claims of a neuromorphometric effect in C2A animals. Contrary to the DS claims that a few isolated findings represent *"clear evidence that Substance [2] induces abnormalities in the central nervous system in animals exposed during the developmental period"*, the weight-of-evidence presented here demonstrates otherwise; the findings were either present in healthy control animals at high background rates, are known to occur

spontaneously, showed minimal severity, lacked clear dose-response patterns, represented small and sometimes non-significant differences, were not consistently seen at other developmental timepoints examined, and/or occurred in isolation with no correlation to other neuromorphometric parameters or functional signs of neurotoxicity or neurobehavioral effects.

*Discussion of proposed developmental neurotoxicity due to thyroid imbalances*

None of the studies available with the substance show significant effects on thyroid hormones in pups prior to weaning. The DS suggests that a relevant effect might have occurred in the OECD TG 443 study even in the absence of statistical significance. Looking at the absolute values of thyroid hormones, this must be clearly contradicted. Well-known within the toxicological community, thyroid hormone values show a large intragroup variation to begin with, making data interpretation very challenging (Beekhuijzen et al., 2019; European Chemicals Agency., 2023; Li et al., 2019). Given that control males were below the HCD values already, a decrease from 16 to 14.1 nmol/L must be regarded as minimal, especially looking at the standard deviation of above 5 for controls. As the values were low to begin with, every minimal decrease seems large in relative numbers, but without corroborating findings such as statistical significance or increases in TSH, which were clearly not observed, no toxicological relevance can be attributed. On PND 22, statistically significant increases in TSH were found in both males and females, however all values were within the HCD of the laboratory and therefore within the biological variation commonly observed for rats of this age and strain. No concurrent change in T4 values was found. Therefore, these findings must be considered of limited toxicological relevance.

The DS mentions that *"T4 and TSH measurements were not performed in F2 pups which is not in line with OECD TG 443 requirements"* (CLH dossier, p. 58). However, it should be clearly pointed out that the OECD TG 443 guideline does not specify thyroid hormone measurements in the F2 pups. While it is commonly done following ECHA's recommendations on OECD TG 443 studies (European Chemicals Agency., 2023), this publication was not available at the time of study conduct and could therefore not be taken into consideration. Therefore, the statement by the DS is incorrect and the study was conducted in full compliance with the OECD Guideline 443.

2. Assessment of available data in the context of CLP

RAC-62 guidance (RAC-62, 2022) on addressing developmental neurotoxicity and neurotoxicity under the current CLP hazard classes concludes the following:

*"Adverse effects on the nervous system investigated or detected at any point in the life span of the organism exposed during the developmental period, covering both prenatal and postnatal development until sexual maturation (determined by preputial separation and vaginal opening), should be addressed under developmental toxicity (DNT), even if the exposure had also continued after sexual maturation."*

This guidance references CLP 3.7.1.4 in further defining developmental toxicity as "any effect which interferes with normal development." Such effects can manifest at any point in the life span of the organism and includes death of the developing organism, structural malformations, altered growth, and functional deficiency. While we do not disagree with the scientific rationale for these guidance criteria, we do question the broad application of such criteria particularly for categorizing specific findings in an EOGRTS as Category 1B or Category 2. The sole basis for this guidance according to RAC-62 is due to the fact that:

*"It is generally not possible to distinguish the precise origin or timing of the toxicological insult when the exposure has continued after the developmental period."*

This inability to precisely distinguish between developmental toxicity versus chronic toxicity in adult animals within the scope of a given study design seems to be a reasonable basis for further investigation, not a basis for automatic classification of any observable change as a definitive developmental toxicant (i.e., Category 1B). At worst, such a finding in F1 C2A adult animals may warrant ultraconservative classification as a suspected developmental finding (i.e., Category 2) until further data become available for more precise classification. However, the DS has taken the extreme approach of broadly applying this RAC guidance to automatically categorize an already questionable isolated finding without functional impairment (axonal degeneration) in F1 C2A adult males of the OECD TG 443 study as a definitive Category 1B development neurotoxicant with little consideration for the scientific evidence. As previously noted, axonal degeneration was observed in F1 C2A males and females across all dose groups including 20-40% of healthy control animals, and the severity was the same across all groups with no evidence of a dose response pattern, i.e., minimal to slight (lowest severity grades). Even more importantly, there is absolutely no evidence that this change "interfered with normal development" in any of these animals, which is the foremost property of a development toxicant. Specifically, this change did not manifest in any of the classic conditions associated with developmental toxicity, including death of the developing organism, structural malformations, altered growth or functional deficiency. No functional deficits were observed in any of these same animals during the functional observational batteries or neurobehavioral assessments. This specific change has also not been confirmed or reproduced in other studies to date. Based on the weight of evidence, there is currently no sound scientific basis for classifying this specific change, which was observed in both treated and control animals with minimal severity and not associated with any functional deficits, as a definitive Category 1B developmental toxicant as proposed by the DS, especially without consideration of further studies or investigation to better distinguish the origin of this change in treated and control animals.

CLP Annex I, section 3.7.1.4 specifies that "[…] for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure." Therefore, in the spirit of the legislation, a clear distinction should be made between effects arising from exposure during development as compared to subsequent adult life.

RAC further notes that a similar approach for considering findings in F1 C2A animals as evidence for developmental neurotoxicity should be broadly applied to other target organ toxicities investigated at any point in the lifespan of the offspring. In the broadest sense of this application, all findings observed in any of F1 or F2 cohorts in an EOGRTS would by default be considered developmental toxicity since it is not possible to distinguish the precise origin or timing of the toxicological insult within the scope of the OECD 443. While we understand the importance of not missing potential signals of developmental toxicity, we must agree as scientists that not all observed changes in F1 adult animals should by default be grounds for classification as a definitive Category 1B developmental toxicant, especially on the sole basis of being unable to distinguish the origin within the scope of the study design. At worst, follow-up studies or investigation would be warranted for more accurate hazard identification and classification. This is particularly true in this case where the finding in question was of minimal/slight severity across all groups, including an atypically high incidence of the same change in control animals, and not associated with any functional or neurobehavioral deficits.

# Annex 6 – Detailed information on data for proposed neurological functional deficits

In the CLH dossier, neurological functional impairment was reported as effects on the auditory startle response as well as decreased habituation:

> *In the EOGRTS, despite limitations of the auditory startle response test, effects from the mid-dose level on mean maximal amplitude in males as well as decreased habituation from the mid-dose in males and in males and females combined are considered biologically relevant in the absence of appropriate statistical analysis (testing for interactions of sex, trial blocks and treatment) and positive controls.*
> *→ Based on the available,* ***there is some evidence that Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene induces functional deficiency****. However, the deficiencies of the test limit the reliability on the effects observed in auditory startle response. Such parameters were not investigated for its analogue.*

The authors would like to point out, that all data available on this section of the document were derived from a study conducted with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. As even the DS mentioned, no data are available on these effects for the substance under evaluation for harmonized classification and labelling (Reaction products of diphenylamine with nonene, branched). It should be highlighted though that this is not due to a lack of willingness to generate these data from industry side, as the registrants for Reaction products of diphenylamine with nonene, branched have submitted a testing proposal for an adequate study on October 6th, 2021.

The data available for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene were addressed in the comments on the CLH proposal for that substance, but are also included below to facilitate assessment for the reader.

1. OECD TG 443

An extended one-generation reproduction toxicity study was performed according to OECD TG 443 and under GLP (BASF SE, 2021b). Wistar rats (25 per sex and dose) were treated with doses of 0, 200, 600 or 1800 ppm Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene via diet. F0 animals were treated for at least 10 weeks prior to mating to produce a litter (F1 generation). Pups of the F1 litter were selected (F1 rearing animals) and assigned to 4 different cohorts (1A, 1B, 2A and 2B) which were subjected to specific postweaning examinations. Cohort 1B (= F1 generation parental animals) was selected to produce F2 pups; Cohort 1A animals were analyzed for specific reproductive parameters. Cohorts 2A (C2A) and 2B (C2B) were employed to investigate developmental neurotoxicity.

*Auditory startle response*

As part of the developmental neurotoxicity investigations of the OECD TG 443 DNT module conducted with the substance, an auditory startle response test was carried out on post-natal day (PND) 24 in all animals of cohort 2A. The DS had the following comments on the ASR:

*"The ASR test presented some limitations: poor reporting of the apparatus used, statistical analysis not in line with the NAFTA guidance (i.e. no mention, or results presented for testing for interactions of sex, trial blocks and treatment) for maximal amplitude and latency as well as a complete absence of any statistical testing for habituation (a variable required under OECD 443). Furthermore, the lack of HCD and positive control increases the possibility the risk of false negative findings taking into account the low statistical power in DNT investigations."*

The following is a detailed description of the process:

On PND 24, the auditory startle response test was carried out in all animals of cohort 2A using the SR-LAB; STARTLE RESPONSE SYSTEM (San Diego Instruments, San Diego, CA, U.S.A.). For all animals, the examinations started in the morning at their respective test date. If, at a given test date, several animals were tested, the trials were conducted in a randomized sequence. Age-appropriately sized, tube shaped, transparent acrylic enclosures were used to accommodate the animals during the test. Each enclosure has an attached motion sensor and is mounted on a solid enclosure base. The entire unit is placed in a heavy wooden, plastic-laminated, isolation cabinet, which minimizes extraneous noise and vibrations. Each cabinet is equipped with internal light and fan, and contains, in a separate compartment, a complete sound generation system able to produce background noise and white noise stimuli, the level of both is adjustable. The response of the animal to the sound stimulus is issued by the motion sensor as voltage which is automatically recorded over the entire response window. The highest voltage during the response window is labeled "maximum amplitude", the time in milliseconds from the start of the response to the maximum amplitude is labeled "latency to the peak of the response".

The animals were given a 5-minute acclimation period in the enclosure with a 70 dBA background noise. Then the startle response was recorded in 50 trials at a startle stimulus sound level of 120 dBA with a 5 - 10 second variable interval between the trials. Response was recorded for 50 milliseconds. Measurement was carried out with the light and ventilator switched on in the isolation cabinets; no food or water was provided during the test. Data (maximum amplitude, latency to the peak of the response) were analyzed in 5 blocks of 10 trials each.

All records were printed, signed and are part of the raw data.

No influence of the test substance on auditory startle habituation (maximum amplitude and latency) was observed in any male or female animal in all treated groups. The DS claims to have observed a decrease in the auditory startle response (ASR) relative to concurrent control animals. This perceived relative decrease in ASR of mid and high dose group animals stems from one animal in the control group, which showed an extremely high maximum amplitude compared to the other animals (Figure 3). Therefore, this one animal is considered an outlier. Similarly, three animals in the low dose group showed a mean maximum amplitude that was more than twice as high as those of the rest of the group. With data as variable as this, analysis of mean values can be error-prone due to outliers. Therefore, data can be either (a) reanalyzed excluding outliers or (b) analyzed using a median instead of a mean value for the group. If the data are analyzed without these outliers, the mid and high dose group males are within the same range as the concurrent controls (Table 11). Similarly, the median was calculated in addition to the mean value and also this calculation resulted in no relevant change of control vs. high dose group values (high dose group as 99.1% of control, Table 11).

*Table 11: Mean maximum amplitude of startle response in PND24 males (Cohort 2A) in OECD TG 443 study (blocks 1-5) (BASF SE, 2021b)*

| Concentration in diet [ppm] | | 0 | 200 | 600 | 1800 |
|---|---|---|---|---|---|
| Full dataset | N | 10 | 10 | 10 | 10 |
| | Mean | 398.5 | 477.1 | 350.8 | 324.7 |
| | SD | 227.4 | 253.6 | 85.1 | 63 |
| | % of control [based on mean] | 100 | 119.7 | 88.03 | 81.5 |
| | Median | 316.5 | 381 | 359 | 313.5 |
| | % of control [based on median] | 100 | 120.4 | 113.4 | 99.1 |
| Without outliers | Mean | 330.1 | 329.7 | 350.8 | 324.7 |
| | SD | 75.2 | 66.4 | 85.1 | 63 |
| | N | 9 | 7 | 10 | 10 |
| | % of control | 100 | 99.9 | 106.3 | 98.4 |

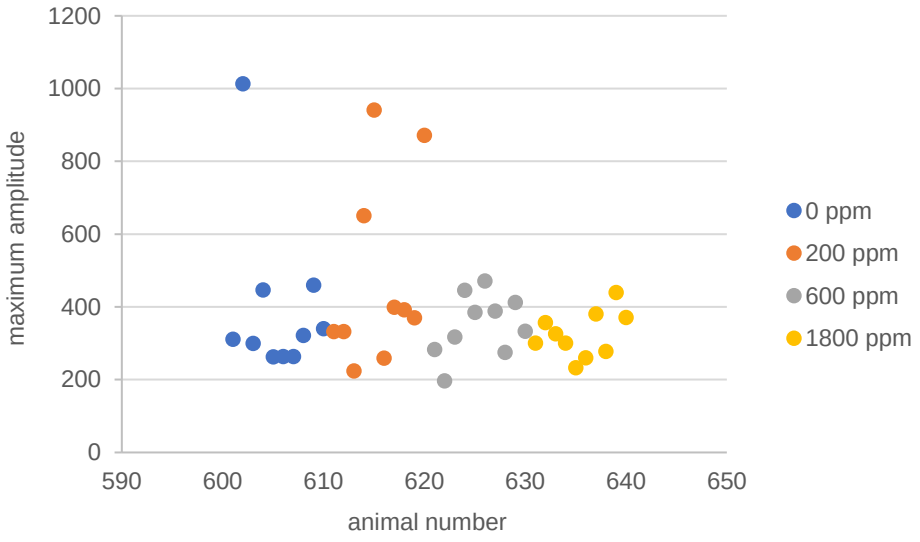



*Figure 3: Individual animal data for auditory startle response in males at PND24 (mean maximum amplitude values) observed in OECD TG 443 (BASF SE, 2021b)*

In addition, historical control data were compiled for mean maximum amplitude parameter of auditory startle response. Data from eight studies containing ASR investigations were available between 2014 and 2022, and the control values observed were collected and are presented in Table 12. The mean maximum amplitudes of all groups were well within the historical control data of the laboratory and any changes are therefore considered not biologically relevant.

*Table 12: Historical control data for maximum amplitude of ASR, obtained from 8 studies between 2014 and 2022.*

| | Block 1-5 | |
|---|---|---|
| | ♂ | ♀ |
| Mean max. amplitude | 443.5 | 404.9 |
| SD | 87.4 | 66.4 |
| Min. | 292.1 | 295.9 |
| Max. | 535.1 | 513.0 |

*Habituation*

Further, the DS analyzed the habituation response of the animals. Habituation of rats to auditory stimuli can be used as an approximation of learning. Generally, it should be emphasized that due to the low animal numbers and high individuality of the response, these results can only be considered indicative and definitive learning and/or memory experiments are required to investigate effects of a substance on learning/memory. While the DS calculated the difference between mean values of block 1 and 5 and then normalized the values to the concurrent control, looking at the single animal values, shows that this is difficult due to the spread of values and high standard deviations. Therefore, the startle response data were re-analyzed with mean values for each block and plotting those for the control and high dose groups (blue and orange icons in Figure 4, respectively). Based on these data, no relevant change in habituation can be identified based on the data available. Statistical analysis of the data (Kruskal-Wallis and Wilcoxon (two-sided)) did not provide any statistical significance of the data.

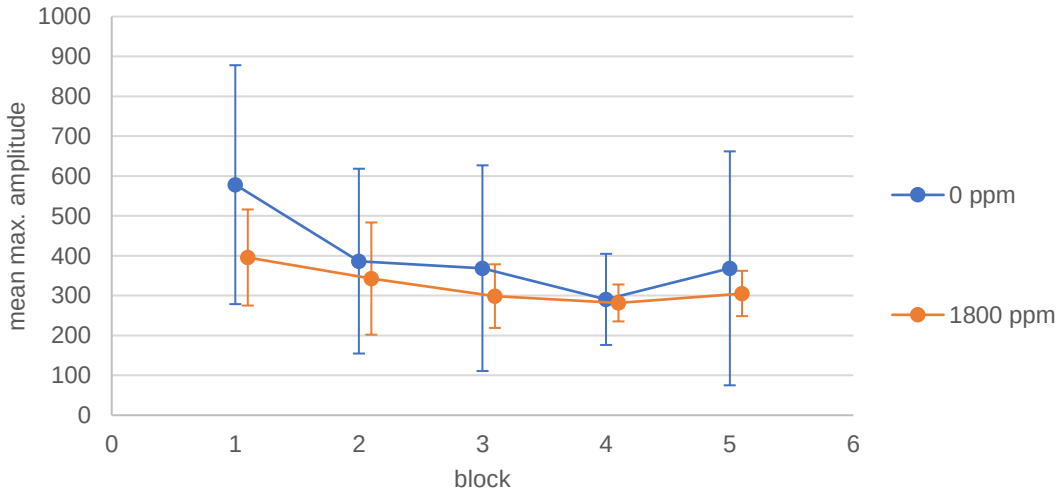



*Figure 4: Mean max. amplitude of control (blue) and high dose (orange) C2A males over five blocks in response to auditory startle as observed in DNT module of OECD TG 443 study (BASF SE, 2021b).*

Based on these data as well as the lack of findings in the functional observational battery, it can be concluded that exposure of animals with the substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene during developmental stages does not lead to a functional impairment.

# Annex 7 – Detailed information on proposed information for death of the developing organism

In the present CLH proposal, the DS claims that there is slight evidence that Reaction products of diphenylamine with nonene, branched induces death of the developing organism:

> *While post-implantation viability was not affected in the PNDTSs in rats and rabbits, the increased number of abortions in rabbits provide* ***slight evidence that Reaction products of diphenylamine with nonene, branched could induce death of the developing organism****, however these abortions may be partly related to the severe drop in food consumption observed at this dose level, as demonstrated in published studies on caloric restriction during pregnancy in rabbit.*
> *Based on the available data, there is* ***slight evidence that Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene*** *induces death of the developing organism based on reduction of pre- and postnatal viability in the high-dose animals of the OECD TG 422, but these effects were not reproduced in the other generational studies performed with the substance and its analogue.*

The data available on Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene is discussed in detail in the respective comments on CLH proposal for this substance. Only the data available for Reaction products of diphenylamine with nonene, branched will be addressed in the subsequent sections.

1. OECD TG 421

In the OECD TG 421 study available with Reaction products of diphenylamine with nonene, branched, no impact of treatment was observed on pre- or postnatal survival (BASF SE, 2020a). The post-implantation loss was 12.1% in control, 1.6% in low dose group, 5.7% in mid dose group, and 10.4% in high dose group. No difference was observed between treated animals and controls and the values reflected the normal range of biological variation inherent in the strain of rats used for this study as all respective values were within the range of the historical control data 0.9-16.8% (Table 13).

*Table 13: post-implantation loss observed in OECD 421 study (BASF SE, 2020a)*

| Dose levels | | Control | Low dose | Mid dose | High dose | HCD |
|---|---|---|---|---|---|---|
| Post-implantation loss | Mean [%] | 12.1 | 1.6 | 5.7 | 10.4 | 0.9 – 16.8 |
| | SD | 15.7 | 3.5 | 11.1 | 16.1 | |
| | N | 9 | 10 | 9 | 10 | |

2. OECD TG 414 (rabbit)

In a prenatal developmental toxicity study conducted according to OECD TG 414 and under GLP, groups of 25 inseminated rabbits were administered Reaction products of diphenylamine with nonene, branched at doses of 0, 10, 30, or 100 mg/kg bw/d in 0.5% CMC via gavage from gestation days (GD) 6 through 28 (BASF SE, 2019). The dams of the high dose group showed clear signs of systemic toxicity with reduced food consumption (mean -31% as compared to controls on GD6-28) and reduced mean body weights and body weight loss (-24 g vs. +104.4 g in controls on GD6-28). Fetuses in the high dose group showed reduced fetal weights and

delays in ossification. No relevant changes were observed in the low and mid dose groups. The DS points out that while not statistically significant, a higher number of abortions was observed in the high dose group as compared to the concurrent controls (four vs. two dams with complete abortions). Importantly, the DS also points out that in rabbits a link between reduced food intake and increased abortions is well established (Cappon et al., 2005; Lopez-Tello et al., 2019; Matsuoka et al., 2006; Matsuzawa et al., 1981). Looking at food consumption of the affected four dams of the high dose group, it is apparent that these females showed a particularly low food intake over large parts of the study, even compared to the high dose group mean values (Figure 5). Taken together with the lack of statistical significance, the increased incidences of abortions are interpreted by the registrants as secondary to severely reduced food consumption. The DS further pointed out that in the historical control data of the laboratory, only four of 350 females showed abortions. However, it should be kept in mind that severe reductions of food consumption are rarely observed in the control groups but are frequently a sign of systemic toxicity in rabbits. In fact, this species is especially prone to feed refusal upon administration of substances (Moxon et al., 2023). Therefore, the reduced feed intake would not be considered a biological variation inherent to this species, but rather a species-specific reaction to substance exposure, which cannot be demonstrated with HCD.

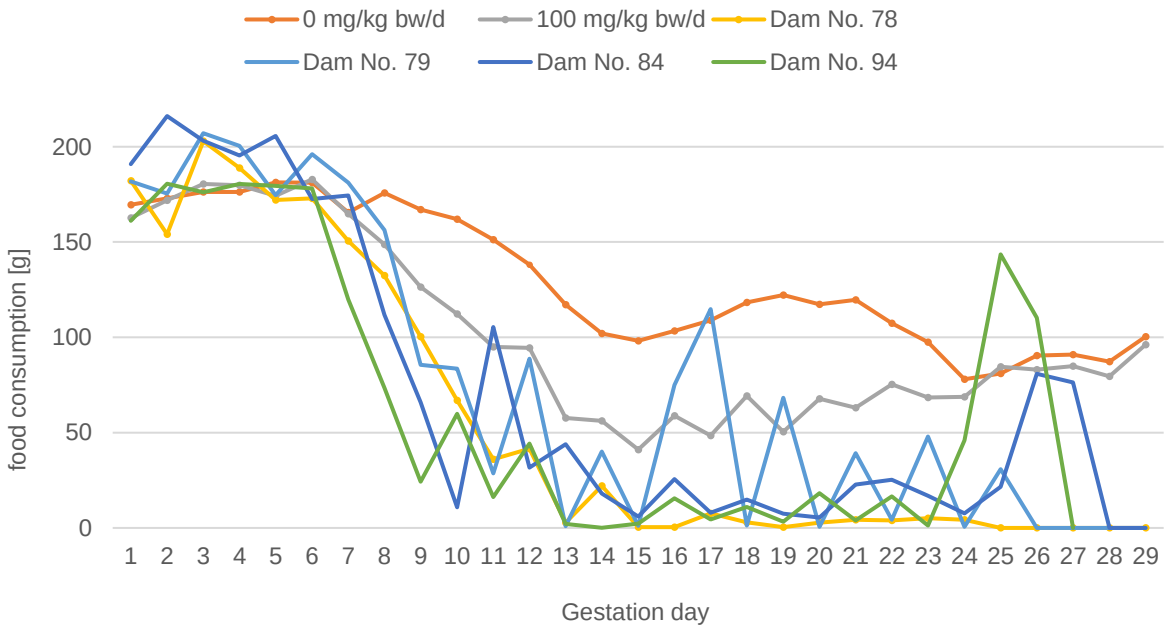



*Figure 5: Food intake over time in dams of OECD 414 study with Reaction products of diphenylamine with nonene, branched (BASF SE, 2019). Orange line, mean food intake of control group. Grey line, mean food intake of high dose group (100 mg/kg bw/d). Yellow, blue, green lines, food intake of single high dose females that had complete abortions. Substance administration started on Gestation day 6*

# Annex 8 – Detailed discussion of effects on delays in ossification and malformations

In the present proposal for harmonized classification and labelling, the DS claims that

> *In the PNDTS in rabbits performed with **the substance, delay in ossification was noted in the presence of maternal toxicity**. Regarding the cluster of four fetuses with multiple common malformations from a single litter of the high-dose group, a genetic origin is considered more likely than a treatment-related effect.*

1. OECD TG 414 (rabbit)

The substance Reaction products of benzeneamine, N-phenyl with nonene (branched) was tested for its prenatal developmental toxicity according to OECD TG 414 in New Zealand White rabbits (BASF SE, 2019). The test substance was administered as an aqueous suspension to groups of 25 inseminated female New Zealand White rabbits orally by gavage in doses of 0, 10, 30 and 100 mg/kg bw/d in 0.5% CMC on gestation days (GD) 6 through 28.

The study reported increased incidences of no or reduced defecation along with reduced food consumption in mid and high dose does. The mean food consumption in the high dose group was significantly reduced during GD7-23 (up to -59% compared to controls). Overall, high dose does consumed 31% less food than the concurrent control animals during the treatment period.

The mean body weights (BW) and the average body weight gain (BWC) of the high-dose rabbits were distinctly reduced. Overall, the high-dose rabbits lost weight (-24.0 g vs. +104.4 g in control) during the treatment period (GD6-28, Figure 6), up to -102 g on GD21.

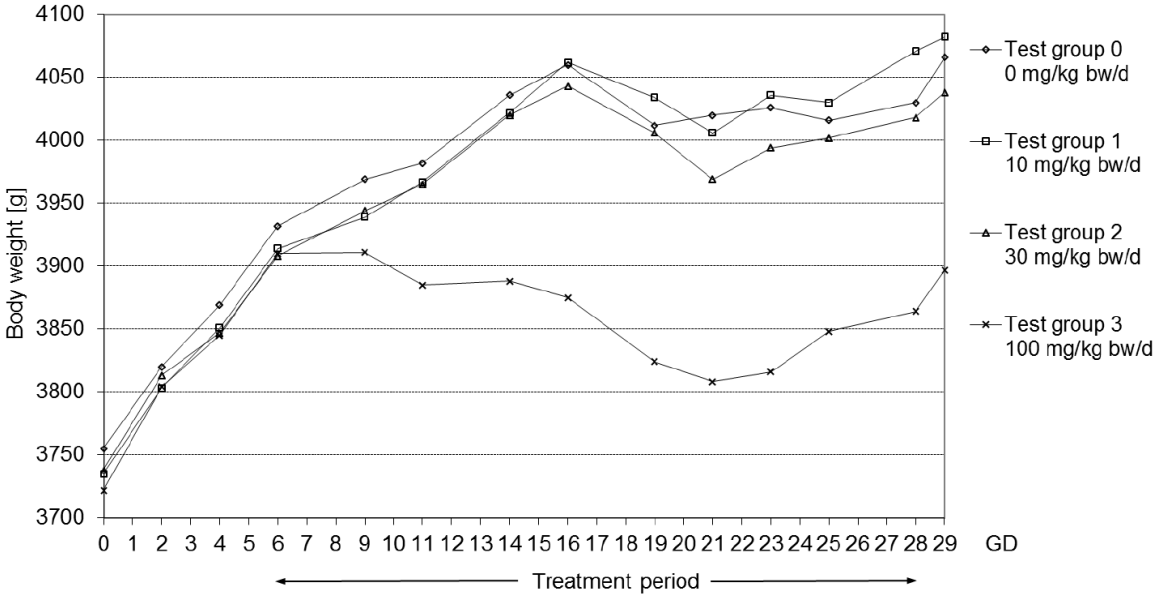



*Figure 6: Mean body weights of pregnant animals observed in OECD 414 study in rabbits (BASF SE, 2019)*

The mean fetal weight of the high dose group was statistically significantly lower than control in male fetuses and when both sexes were combined (-12% in comparison to the concurrent

control). The mean weight of the female high-dose fetuses was also slightly lower but without attaining statistical significance. The mean fetal weights of low and mid dose groups were not influenced by the test substance and did not show any biologically relevant differences in comparison to the control group.

*Table 14: Rabbit fetal weights observed in OECD 414 study*

| Dose [mg/kg bw/d] | 0 | 10 | 30 | 100 |
|---|---|---|---|---|
| All viable fetuses | | | | |
| Mean | 38.3 | 38.6 | 36.3 | 33.6* |
| SD | 5.23 | 6.72 | 5.07 | 5.93 |
| N | 20 | 24 | 24 | 20 |
| Male Fetuses | | | | |
| Mean | 39.1 | 39.2 | 36.0 | 33.9* |
| SD | 5.76 | 5.48 | 5.31 | 5.91 |
| N | 20 | 23 | 23 | 20 |
| Female Fetuses | | | | |
| Mean | 37.6 | 37.3 | 36.0 | 33.6 |
| SD | 5.15 | 6.83 | 5.49 | 6.30 |
| N | 20 | 23 | 24 | 20 |

Statistics: Dunnett-test (two-sided), * $p \leq 0.05$

Reduced rabbit fetal weights were observed concurrent with severe maternal toxicity only. For the rabbit, the literature describes that in case of severe reduction of feed intake for does, the fetal weights are affected as well (Beyer et al., 2011; Cappon et al., 2005; Danielsson, 2013; Tyl, 2012). These findings are in quality and quantity comparable to the effects observed in the prenatal developmental toxicity study in rabbits.

*Table 15. Total external malformations observed in OECD 414 with rabbits (BASF SE, 2019)*

| Dose [mg/kg bw/] | | 0 | 10 | 30 | 300 |
|---|---|---|---|---|---|
| Litter | N | 20 | 24 | 24 | 20 |
| Fetuses | N | 174 | 189 | 205 | 179 |
| Fetal incidence | N (%) | 1 (0.6) | 1 (0.5) | 0.0 | 5 (2.8) |
| Litter incidence | N (%) | 1 (5.0 | 1 (4.2) | 0.0 | 2 (10) |
| Affected fetuses/litter | Mean % | 0.5 | 0.4 | 0.0 | 2.4 |

mg/kg bw/d = milligram per kilogram body weight per day; N = number; % = per cent

*Table 16: Occurrence of statistically significantly increased fetal skeletal variations (expressed as mean percentage of affected fetuses per litter) observed in OECD with rabbits (BASF SE, 2019)*

| Finding | Dose level [mg/kg bw/d] | | | | HCD Mean (%) (range) |
|---|---|---|---|---|---|
| | 0 | 10 | 30 | 100 | |
| Irregular ossification of interparietal | 0.6 | 1.6 | 2.9* | 2.6* | 0.8 (0.0 – 1.7) |
| Misshapen sacral vertebra | 2.4 | 3.8 | 8.7** | 5.9 | 4.4 (1.9 – 8.6) |
| Unossified sternebra; unchanged cartilage | 9.3 | 10.3 | 24.5* | 11.7 | 13.5 (7.7 – 23.5) |
| Unilateral ossification of sternebra; unchanged cartilage | 0.7 | 1.6 | 3.2* | 0.9 | 2.7 (0.5 – 5.4) |
| Unossified talus; cartilage present | 0.0 | 0.8 | 0.0 | 4.4** | 1.0 (0.0 – 2.6) |

Wilcoxon-test [one-sided], * = $p \leq 0.05$, ** = $p \leq 0.01$

Irregular ossification of interparietal was increased and outside the historical control range in the mid- and high-dose groups (Table 16). This finding represents small irregularities in the

shape of the ossification nuclei in the interparietal. As desmal ossification of the neurocranium continues during later development, and interparietal membrane as well as surrounding bones were intact, a completely regular ossification of this bone can be expected to occur postnatally. Thus, this finding was considered of negligible toxicological relevance.

The finding 'unossified talus (with present cartilage)' was statistically significantly increased and outside the historical control range in the high dose group (Table 16). This finding may represent slight delays of ossification which did not affect morphology, as the underlying cartilage model was completely intact in all these cases. This assessment is supported by the fact that the mean fetal weight of all 10 fetuses showing this finding (i.e., 18.8 g) was clearly below the mean fetal weight of all fetuses in the high dose group (33.6 g), which indicates a delay in overall development going along with the delay in ossification. The association of ossification delays with maternal toxicity and fetal body weights has previously been well established (DeSesso and Scialli, 2018). Therefore, the delays in ossification observed are considered secondary to reduced fetal weights due to maternal toxicity rather than a substance-specific effect.

There were noted external, soft tissue, and skeletal malformations in all test groups including the control. The distribution of total malformations about the groups was not related to dose. One fetus of the control, two fetuses of the low-dose, four fetuses of the mid-dose and four fetuses of the high-dose group had more than one malformation or were multiple-malformed across the different examination areas. For a better overview, all those malformations were listed in Table 17.

*Table 17: Fetuses with more than one malformation found in OECD 414 study with rabbits (BASF SE, 2019)*

| Dose [mg/kg bw/d] | Doe No.-Fetus No., Sex | Finding |
|---|---|---|
| 0 | 25-10 F | domed head, hydrocephaly |
| 10 | 26-10 M | thoracic hemivertebra, misshapen thoracic vertebra |
| | 44-01 F | malpositioned kidney, short ureter |
| 30 | 68-07 F | exoccipital fused with 1st cervical arch, cervical hemivertebra |
| | 69-03 M | multiple malformations of the great vessels (persistent truncus arteriosus, aortic arch atresia, malpositioned subclavian origin) |
| | 75-02 F | aortic arch atresia, malpositioned kidney |
| | 75-06 F | thoracic hemivertebra, branched rib |
| 100 | 76-04 M | multiple external malformations (domed head, cleft palate, small tongue), hydrocephaly |
| | 76-06 F | multiple external malformations (domed head, cleft palate, small tongue) |
| | 76-11 F | multiple external malformations (domed head, cleft palate, small tongue), severely malformed skull bones |
| | 76-12 M | multiple external malformations (domed head, cleft palate, small tongue) |

mg/kg bw/d = milligram per kilogram body weight per day; No. = number; M = male; F = female

Most noticeable was a cluster of four fetuses in litter No.76 which showed a broad spectrum of malformations, and several less severe findings such as (among others) paw hyperflexion and empty stomach (devoid of amniotic fluid). All these findings contributed to higher rates of external malformations and variations as well as unclassified soft tissue observations in the high-dose group. The clustered appearance in one litter and the almost identical spectrum of ontogenetically different findings in all those fetuses strongly suggests an origin of these anomalies which is unrelated to treatment (i.e., genetic origin). Consequently, the higher incidence of high-dose findings in their respective sections is also considered to be unrelated to treatment.

Other malformations, such as umbilical hernia, open eye and absent subclavian were scattered observations in individual fetuses of low or high dose groups. No ontogenetic pattern is recognizable for the individual malformations, nor was there any cluster of any of these individual malformations seen in the other offspring of these test groups. They were not dose-related and all of them can be found in the historical control data at comparable or higher frequency.

There was no statistically significant difference in the distribution of total malformations about the groups, and the incidences in the treated groups were close to the historical control mean. Therefore, the findings observed are considered incidental and not related to treatment.

# Annex 9 – Detailed information on data for proposed effects on postnatal development

In the present CLH proposal, the DS concludes that:

> *In rabbits exposed to Reaction products of diphenylamine with nonene, branched, a significant decrease in fetus weight (-12%) was observed at the highest dose (100 mg/kg bw/d) associated with delays of ossification. At this dose level, does consumed 31% less food than the concurrent control does during the treatment period (GD6-28), showed marked reduced defecation and had slight reduced corrected body weight.*
>
> *Postnatal growth was also altered from PND7 up to weaning in the high-dose groups of the generational studies performed with the substance (OECD TG 421) […]. At these dose levels, effects on body weight of similar magnitude were observed in females at the end of the lactation period.*
>
> *→ Based on the available data, there is evidence that both Reaction products of diphenylamine with nonene, branched and its analogue* ***alter growth of the developing organism*** *at dose levels also affecting maternal/parental weight.*

1. OECD TG 414 (rabbit)

The substance Reaction products of benzeneamine, N-phenyl with nonene (branched) was tested for its prenatal developmental toxicity in New Zealand White rabbits (BASF SE, 2019). The test substance was administered as an aqueous suspension to groups of 25 inseminated female New Zealand White rabbits orally by gavage in doses of 0, 10, 30 and 100 mg/kg bw/d in 0.5% CMC on gestation days (GD) 6 through 28.

Clinical symptoms found in mid and high dose does comprised increased incidence of no or reduced defecation together with reduced food consumption. The mean food consumption in the high dose group was significantly reduced during GD7-23 (up to -59% compared to controls). Overall, high dose does consumed 31% less food than the concurrent control animals during the treatment period.

The mean body weights (BW) and the average body weight gain (BWC) of the high-dose rabbits were distinctly reduced. Overall, the high-dose rabbits lost weight (-24.0 g vs. +104.4 g in control) during the treatment period (GD 6-28, Figure 6).

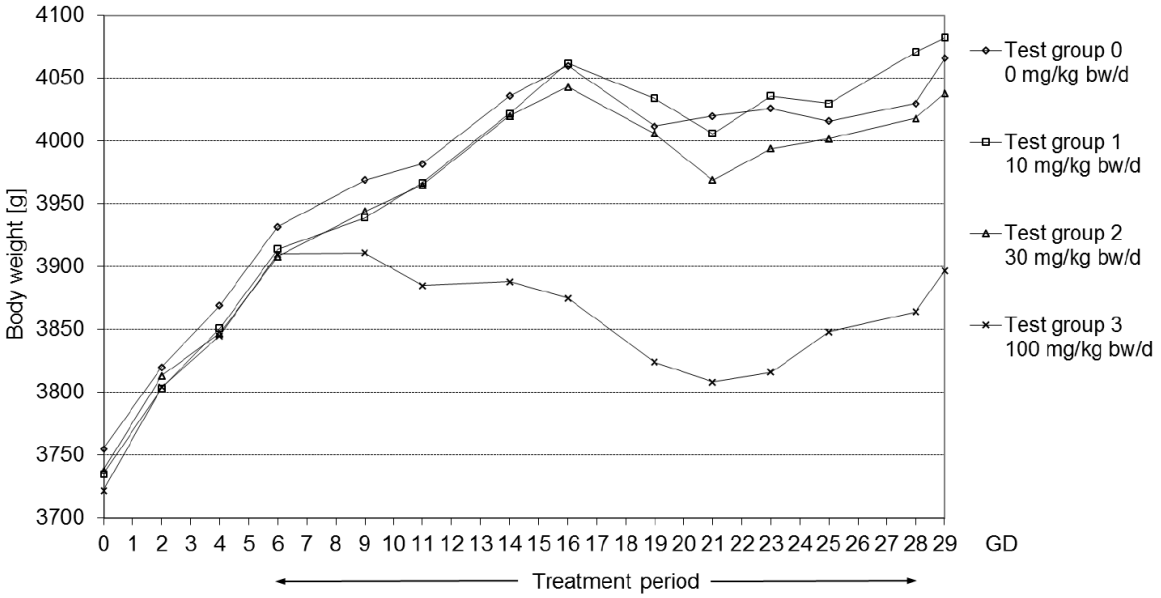



*Figure 7: Mean body weights of pregnant animals observed in OECD 414 study in rabbits (BASF SE, 2019)*

The mean fetal weight of the high dose group was statistically significantly lower than control in male fetuses and when both sexes were combined (-12% in comparison to the concurrent control). The mean weight of the female high-dose fetuses was also slightly lower but without attaining statistical significance. The mean fetal weights of low and mid dose groups were not influenced by the test substance and did not show any biologically relevant differences in comparison to the control group.

*Table 18: Rabbit fetal weights observed in OECD 414 study (BASF SE, 2019)*

| Dose [mg/kg bw/d] | 0 | 10 | 30 | 100 |
|---|---|---|---|---|
| All viable fetuses | | | | |
| Mean | 38.3 | 38.6 | 36.3 | 33.6* |
| SD | 5.23 | 6.72 | 5.07 | 5.93 |
| N | 20 | 24 | 24 | 20 |
| Male Fetuses | | | | |
| Mean | 39.1 | 39.2 | 36.0 | 33.9* |
| SD | 5.76 | 5.48 | 5.31 | 5.91 |
| N | 20 | 23 | 23 | 20 |
| Female Fetuses | | | | |
| Mean | 37.6 | 37.3 | 36.0 | 33.6 |
| SD | 5.15 | 6.83 | 5.49 | 6.30 |
| N | 20 | 23 | 24 | 20 |

Statistics: Dunnett-test (two-sided), * $p \leq 0.05$

Reduced rabbit fetal weights were observed concurrent with severe maternal toxicity only. For the rabbit, the literature describes that in case of severe reduction of feed intake for does, the fetal weights are affected as well (Beyer et al., 2011; Cappon et al., 2005; Danielsson, 2013; Tyl, 2012). These findings are in quality and quantity comparable to the effects observed in the prenatal developmental toxicity study in rabbits.

Based on the correlation of maternal toxicity and reduced fetal weights as well as the literature available on this topic, this finding is considered secondary to maternal toxicity and not a substance-specific developmental effect.

2. OECD TG 421

Reaction products of benzeneamine, N-phenyl with nonene, branched, was administered via diet to groups of 10 male and 10 female Wistar rats at concentrations of 0, 500, 1500 and 5000 ppm (BASF SE, 2020a). The duration of treatment covered a 10-week premating followed by a 2-week mating period in both sexes. Females were further treated throughout gestation as well as up to 13 days of lactation period.

Clinical examinations showed reduced food consumption in high dose females throughout the premating period, the gestation phase and during lactation (-11.6%, -20.1% and -23.5% vs. controls, respectively). In the mid dose group, the food consumption was reduced during gestation (-8.8% vs. control).

Body weights of high dose females were reduced during the premating phase. At the end of premating on study day 70, the decrease in body weight was -10.8% as compared to the concurrent controls (Table 19).

During gestation, a decreased body weight was observed for high dose and mid dose females (-18.5% and -8.8% on GD20 as compared to controls). During lactation period, decreased body weight in female animals of the high dose group was observed from lactation days 1 to 13 (-16.9% as compared to controls) and in the mid dose group (-7.1%).

*Table 19: Data on body weights, body weight change and food consumption in OECD 421 study (BASF SE, 2020a)*

| | | Main study groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration in diet | [ppm] | 0 | | 500 | | 1500 | | 5000 | |
| Approx. dose (♂/♀) | [mg/kg bw/d] | 0 | | 40/44 | | 122/133 | | 397/419 | |
| Body weights<br>♂ d91 / ♀ d70 | Mean (♂ / ♀)<br>SD<br>Dev. vs. control [%] | 412.9<br>29.1 | 217.9<br>11.9 | 403.1<br>15.5<br>-2.4 | 224.6<br>9.3<br>3.1 | 396.0<br>28.4<br>-4.1 | 212.3<br>13.4<br>-2.5 | 363.4**<br>21.7<br>-12.0 | 194.3**<br>14.5<br>-10.8 |
| Body weights<br>gestation (GD20) | Mean<br>SD<br>Dev. vs. control [%] | 343.4<br>23.4 | | 339.4<br>20.2<br>-1.2 | | 313.3*<br>22.0<br>-8.8 | | 279.8**<br>20.2<br>-18.5 | |
| Body weights<br>lactation (LD13) | Mean<br>SD<br>Dev. vs. control [%] | 294.3<br>16.2 | | 290.4<br>16.0<br>-1.3 | | 273.5*<br>18.8<br>-7.1 | | 244.6**<br>17.0<br>-16.9 | |
| Body weight change<br>♂ d 0-91 / ♀ d 0-70 | Mean (♂ / ♀)<br>SD | 296.2<br>28.6 | 122.1<br>8.5 | 285.9<br>13.4 | 128.9<br>9.9 | 279.7<br>23.0 | 116.1<br>112. | 247.3**<br>19.9 | 97.8**<br>12.5 |
| Body weight change<br>gestation (GD 0-20) | Mean<br>SD | 118.8<br>12.4 | | 107.3<br>12.5 | | 95.7**<br>14.3 | | 82.0**<br>16.6 | |
| Body weight change<br>lactation (LD 1-13) | Mean<br>SD | 36.5<br>9.6 | | 39.0<br>7.1 | | 31.4<br>7.7 | | 32.2<br>11.2 | |
| Food consumption<br>d0 – d70 | Mean (♂ / ♀)<br>SD<br>Dev. vs. control [%] | 21.0<br>0.4 | 15.7<br>0.5 | 21.4<br>1.0<br>1.9 | 15.9<br>0.4<br>0.9 | 21.3<br>1.4<br>1.1 | 15.9<br>1.0<br>0.9 | 19.7<br>1.1<br>-6.4 | 13.9**<br>0.3<br>-11.6 |
| Food consumption<br>gestation (GD0-20) | Mean<br>SD<br>Dev. vs. control [%] | 21.0<br>1.2 | | 20.1<br>1.0<br>-4.1 | | 19.2**<br>1.0<br>-8.8 | | 16.8**<br>1.6<br>-20.1 | |
| Food consumption<br>lactation (LD1-13) | Mean<br>SD<br>Dev. vs. control [%] | 47.2<br>3.7 | | 46.0<br>3.5<br>-2.6 | | 43.9<br>3.7<br>-7.1 | | 36.1**<br>3.3<br>-23.5 | |

Dunnett test (two-sided), * $p \leq 0.05$, ** $p \leq 0.01$

Mean body weights of the male and female pups of test group 3 (5000 ppm) were statistically significantly reduced from PND 7 (-15.0% in male pups, -15.4% in female pups, and -15.7% both sexes combined) onwards until scheduled sacrifice on PND 13 (-18.8% in male pups, -18.4% in female pups, and -18.8% below control, both sexes combined, Table 20).

Decreases in pup weights were observed only in dose groups that also showed significant maternal toxicity. Body weight reductions in dams were of similar magnitude as compared to those in pups (-16.9% vs. -18.8%, respectively). No other growth delays were observed for the offspring.

*Table 20: pup weights on post-natal day 13 observed in OECD 421 study (BASF SE, 2019)*

| Concentration in diet | [ppm] | 0 | 500 | 1500 | 5000 |
|---|---|---|---|---|---|
| Males PND 13 | Mean | 33.0 | 31.8 | 30.4 | 26.8** |
| | SD | 2.7 | 2.4 | 3.0 | 2.0 |
| | Dev. Vs. control [%] | | -3.7 | -7.8 | -18.8 |
| Females PND 13 | Mean | 32.2 | 30.9 | 29.9 | 26.2** |
| | SD | 2.2 | 2.3 | 2.4 | 2.1 |
| | Dev. Vs. control [%] | | -3.8 | -7.2 | -18.4 |
| Males + Females PND 13 | Mean | 32.5 | 31.3 | 30.2 | 26.4** |
| | SD | 2.3 | 2.3 | 2.7 | 2.1 |
| | Dev. Vs. control [%] | | -3.7 | -7.3 | -18.8 |

Dunnett test (two-sided), *p ≤ 0.05, ** p ≤ 0.01

Maternal toxicity is well-known to influence developmental parameters, including pup weights (Beyer et al., 2011; Danielsson, 2013; Tyl, 2012). Based on the concurrent incidence, the similar magnitude and the well-established causal relationship, the reduction in pup weights are considered secondary to unspecific maternal toxicity and not a direct substance-specific developmental toxicity.

# Annex 10 – Comments on the Read-across approach

Over a decade ago, the industry had sought to adapt information requirements for the substance under evaluation by applying a read-across approach broadly for toxicological and ecotoxicological properties. The read-across hypothesis is constantly under refinement as more data are generated. The COLLA pilot project on substituted diphenylamines started in 2017. In June 2017, France and Slovenia provided a Substituted Diphenylamines (SDPAs) draft screening report, which "asked the registrants to explore the possibility of applying the OECD subgrouping approach used for the repeated dose toxicity endpoint also to the other endpoints of concern". However, in ECHA's notes from the kickoff meeting on 20 June 2017, it states regarding the Hazard assessment for human health "ECHA also stressed that to confirm the applicability of read-across for [human health] endpoints Registrants might need more bridging studies."

This Annex will describe why read across is not applicable for reproductive and developmental toxicity based on differing modes of action.

1. Assessment of the read-across according to Read-across assessment framework (RAAF)

Several new lines of evidence prove that read-across between Reaction products of diphenylamine with nonene, branched (the target substance) and Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (the source substance) is no longer applicable based on the principles presented in ECHA's Read-Across Assessment Framework (RAAF) for human health endpoints (European Chemicals Agency., 2017a, 2017b).

*RAAF Read-across scenario chosen*

The DS chose the following read-across scenario according to the read-across assessment framework (European Chemicals Agency., 2017b):

*"An analogue approach has been selected by the DS since this read-across concerns two structurally similar substances. Furthermore, the read-across hypothesis is based on different compounds with qualitatively similar properties (RAAF scenario 2)"*

As the substances under evaluation are UVCB substances, additionally the Considerations on multi-constituent substances and UVCBs must be taken into consideration (European Chemicals Agency., 2017a). The DS failed to communicate the applicable scenario from this guidance, however based on the data available, it is assumed that it would be model case 6 (section 5.3.4 of the document, "Prediction from a UVCB to a UVCB").

The ECHA RAAF guidance specifies that each scenario consists of a pre-defined set of assessment elements. The following assessment elements apply to analogue approaches as the one chosen by the DS:

1. Identity and characterization of the source substance
2. Link of structural similarities and differences with the proposed prediction
3. Reliability and adequacy of the source study
4. Bias that influences the prediction

Further, scenario-specific assessment elements apply:

2.1. Compounds the test organism is exposed to
2.2. Common underlying mechanism, qualitative aspects
2.3. Common underlying mechanism, quantitative aspects
2.4. Exposure to other compounds than those linked to the prediction
2.5. Occurrence of other effects than covered by the hypothesis and justification

The assessment elements will be further discussed in the following sections.

*AE 1: Identity and characterization of the source substance*

The RAAF specifies that “Structural similarity is a pre-requisite for any prediction based on read-across under REACH. To assess the structural similarity between the source and the target substances, the identity and characterisation of both substances needs to be clear. Assessment of the substance characterisation of the target substance has been addressed already at the preparatory assessment step […]. This AE investigates whether the identification and characterisation of the source substance, including its impurity profile, are sufficient for a scientific assessment of the read-across approach.” (European Chemicals Agency., 2017b) In addition, the RAAF document on consideration on multi-constituent substances must also be taken into consideration (European Chemicals Agency., 2017a).

The substances under evaluation and used in the read-across approach by the DS are UVCB substances. Therefore, no clearly defined composition is available, and a direct comparison of full substance composition is not possible. While both substances contain several main components, differences in staring materials and manufacturing processes can result in a variety of impurities which the DS has not taken into consideration.

According to the literature, the alkylation reaction occurs from the aromatic compound (Diphenylamine, DPA) over the double bond (olefine), for that reason the kinetic of the reaction is different depending on the kind of olefine used. Therefore, since reaction conditions are different in both processes, the impurities profile are not expected to be comparable, because most of them comes from completely different alkylating agents (Smith and March, 2006).

EC 701-385-4 and EC 270-128-1 are alkylated products of Diphenylamine (DPA) with two different source of alkyl chains. Based on the presence of C4 DPA derivatives, the reactivity of the alkenes is obviously different and confirmed by the presence of mono and di-tert-Butyl DPA derivatives. Shorter alkyl chains were not found in EC 701-385-4. Additionally, EC 270-128-1 employed a defined alkene source, 2,4,4- trimethylpentene, which break down partially to C4, which in turn results in a bulkier tert-butyl substitution.

In conclusion, despite the common diphenylamine core, the impurities of EC 701-385-4 and EC 270-128-1 cannot be compared, and some of their constituents result in a completely different chemical structure. The differences in manufacturing and purification process lead to different impurities.

*AE2: Link of structural similarities and differences with the proposed prediction*

The DS states that both substances have similar functional groups with varying alkyl chain length. This structural similarity assessment was copied from the justification document submitted by the registrants (without acknowledgement of intellectual property), however fails to take into consideration that the registrants have already excluded read-across for the

endpoint toxicity to reproduction, fertility. This conclusion was drawn based on the data obtained in bridging studies (BASF SE, 2020a, 2020b) conducted for both substances.

OECD 421 studies with extended pre-treatment period (at least 10 weeks) were conducted for both substances in the same laboratory around the same time. Dose levels differed between substances based on substance-specific DRF studies showing different substance-intrinsic toxicological properties to ensure both studies fulfilled the OECD Guideline criteria for high dose level selection. For both substances, the liver was identified as the target organ, however the dose levels at which effects were observed grossly differed between the substances. Further, effects on thyroid as secondary target organ were observed in the OECD 421 study for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, but not for Reaction products of diphenylamine with nonene, branched, although dose levels were higher. Similarly, slight anemia was observed for the target substance, but not for the source substance. While the liver frequently is a target organ for organic substances, this can simply be explained by the fact that this type of chemistry must be metabolized prior to excretion, a process which naturally occurs in the liver. Upon xenobiotic exposure, the liver reacts to the increased metabolization demand with swelling of liver cells and organelles required for metabolization processes in order to meet the increased demand. This process is considered adaptive and quickly reversible at the end of xenobiotic exposure. However, in case the liver cannot sufficiently adapt, adverse consequences such as single cell apoptosis, increase in liver enzymes and pronounced increases in liver weights are observed. Therefore, the liver as a target organ is a very unspecific mode-of-action and can by itself not be considered sufficient to support a read-across between UVCB substances.

The DS further states that the thyroid was a target organ for both substances. For the source substance, the thyroid was affected at low doses with increased organ weights together with histopathological changes and thyroid hormone changes. These are discussed in more detail in the respective comments document for the source substance. For the target substance, no adverse changes were observed in the thyroid in the bridging OECD 421 study, although the analysis of thyroid parameters was limited. Nevertheless, the organ weights were not significantly changed between animals and no changes in thyroid hormones were observed in F0 males at the end of treatment period or in F1 pups. In an OECD 408 study available for the target substance, animals were administered the test substance via gavage for 90 days at doses of 100, 300 and 1000 mg/kg bw/d. Thyroid weights were not significantly changed in females at any dose level, males showed increased relative thyroid weights only (absolute thyroid weights were unaffected by treatment). Histopathology showed minimal to slight hypertrophy / hyperplasia of thyroid follicular cells, however only at the highest dose level also at a high incidence. Thyroid hormones were not determined in this study. Based on the lack of thyroid effects in the bridging study and only observing them in the OECD 408 with bolus administration of the limit dose level, the comparability of the thyroid effects between both substances is highly questionable in terms of quality and not existing in terms of quantity.

A table comparing endpoint-specific similarities and differences was compiled for both UVCB substances (Table 21).

In a metabolome study conducted in 2014, data from a 28-day study with the source substance were compared with data from a 90-day study with the target substance. While metabolome analysis is a very good tool to support comparability of data, there were several confounding factors that accompanied the analysis of both substances. The data came from different study types with differences in administration regimen and also different ages of animals. The dose levels compared were significantly different (125 and 300 mg/kg bw/d for the source substance and 1000 mg/kg bw/d for the target substance). While both substances showed effects on lipid metabolism perturbations, there were some distinct differences between both profiles, which are further depicted in Figure 8. A comparison of the blue and orange lines shows several

differences both in quality and quantity of the effects, which must be taken into consideration for assessment.

Based on the data available, a mode of action has been proposed for fertility effects of the source substance, which cannot be supported with the data available for the target substance, thus precluding the use of read-across for the endpoint for which the mode of action is relevant. For details on the mode of action proposed, please refer to the comments submitted for the CLH proposal on Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene.

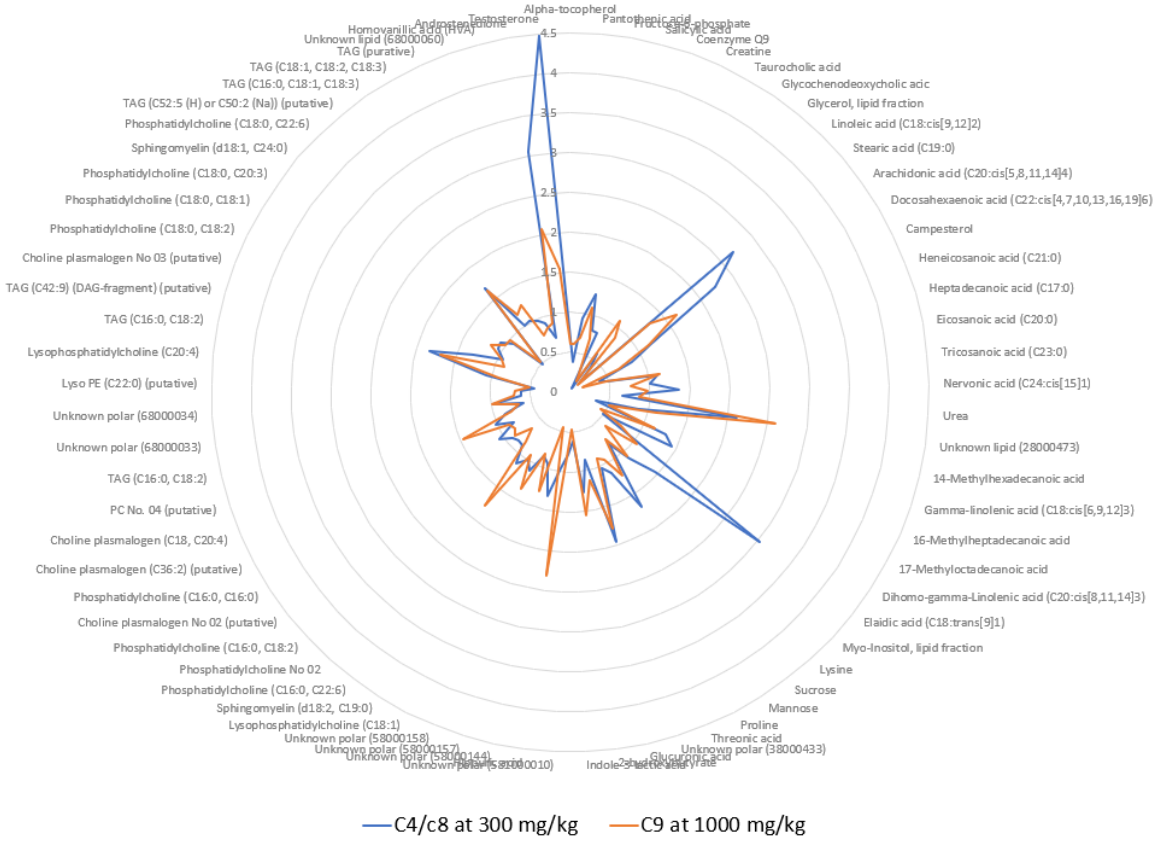



*Figure 8. Metabolite changes relative to controls after the animals exposed to Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene at 300 mg/kg/day or Reaction products of diphenylamine with nonene, branched at 1000 mg/kg/day.*

## *AE3: Reliability and adequacy of the source study*

All available studies conducted for the purposes of assessment of toxicity to reproduction were conducted according to OECD testing guidelines and under GLP. They are therefore considered reliable without restriction for assessment of the respectively tested substance.

## *AE4: Bias that influences the prediction*

Based on the UVCB nature of both source and target substance, significant bias must be expected for the prediction of a read-across. This is due to a large uncertainty arising from the structural dissimilarity, including relevant physical-chemical parameters. While the principal chemistry is similar (diphenylamine (DPA) with vaying alkyl sidechains), the two substances do not share any principal components. Despite the similarities in predicted toxicokinetic parameters by both the DS as well as existing assessments (European Chemicals Agency., 2018; Health Canada, 2017; OECD, 2016), it should be kept in mind that all previous

assessments also cautioned with regards to the uncertainties of the grouping and elaborated on the challenges of read-across between UVCB substances. The OECD Case report on integrated approaches for testing and assessment for SDPA concluded that “Since there is a variation across the subgroup with respect to relevant phys-chem properties and predicted oral bioavailability, we are proposing to apply read across between closest members of the subgroup by comparing structure/composition, relevant physicochemical properties and related change in oral bioavailability of the components.” (OECD, 2016). Thus, structural similarity supplemented with in silico data alone is considered insufficient to support the read-across. Rather, bridging data must be adequately considered to clarify the applicability of a read-across between UVCB substances. If the data of bridging studies are inconsistent both in terms of quality and quantity of effects, the need for further data generation must be considered prior to hazard conclusion.

*AE 2.1: Compounds the test organism is exposed to*

The bioavailability data available and presented by the DS was generated using the PK Explorer software by OECD in 2016 (OECD, 2016). This data was employed by the DS to conclude on toxicokinetic properties of both substances. However, the DS failed to also communicate that this analysis presented by OECD is associated with significant uncertainties, as only representative structures were analyzed and OECD states that “The PK Explorer model does not provide reliability or applicability domain information. The model training set contains 790 compounds that were compiled from reference pharmacokinetic tabulations and various articles from peer-reviewed scientific journals. A cursory examination of the training set revealed no SDPA substances. $LogK_{ow}$ observed for the training set may not cover some of the more lipophilic SDPAs. As a consequence, reliability in the quantitative values generated is considered low for the model results but the results are useful for a comparative analysis.” Based on these findings, higher weight should be given to the comparison of the data obtained in the bridging studies between both substances than the *in silico* analysis included in the read-across justification by the DS.

*AE 2.2: Common underlying mechanism, qualitative aspects*

The DS claims that both substances caused similar effects in OECD TG 421 studies, with the pattern being “lower numbers of implants with subsequently smaller litter sizes, decreased ovary weight and altered cyclicity. […] Effects observed for other properties were also highly similar with both substances, the main target organs being liver and thyroid.”

A reduction in implantation sites and subsequently also smaller litter size was observed for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene in the OECD 421 study. This effect occurred in the presence of pronounced systemic toxicity with the main target organ being the liver and secondary effects also on thyroid. Thyroid hormone disturbances were previously shown to also impact female fertility and lead to a decrease in implantation sites in rats. A detailed analysis of the proposed mode of action together with supporting evidence is presented in the comments on Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. Absolute ovary weights were decreased in the high dose group, however relative ovary weights taking into consideration the significantly smaller body weights of the animals did not show significant differences. The same is true for the data obtained in the OECD 443 study. In addition, no histopathological changes were found that would suggest an adverse effect of the test substance on the ovaries. Therefore, the decreased absolute ovary weights are considered not relevant since relative ovary weights were comparable to

controls and no histopathological correlates were found. Increased estrous cycle length was found in the OECD 421 study and in the Cohort 1B animals of the OECD 443. In the F0 and F1 Cohort 1A groups of the OECD 443 study, no changes in estrous cycle lengths have been noted. This finding was therefore not consistently observed in the available studies.

In the OECD 421 study conducted with Reaction products of diphenylamine with nonene, branched, a statistically significant reduction in implantation sites was observed for the mid and high dose groups, however both dose group values were within the range of the biological variation typically observed for this rat strain and study type. The litter size was also significantly reduced and just below the historical control data of the laboratory. Based on the low magnitude of the effect as compared to the typical biological variation, the biological relevance of this finding is uncertain. Importantly, while effects on the liver were observed, no impact on thyroid weights or hormones was found in the OECD 421 study, which is in contrast to the findings with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. Significant decreases in absolute and relative ovary weights were observed at the end of the treatment period in the OECD 421 with Reaction products of diphenylamine with nonene, branched, which however were mostly reversible within a 14 days recovery period. The ovary weights did correlate with reduced implantation sites, however the relevance of this would need to be investigated in further studies to better understand the mode of action of this substance. No changes in estrous cyclicity were observed in this study.

A detailed overview of the effects observed in the OECD 421 study and how they compare are presented in Table 21.

Based on the data available, a mode of action has been proposed for the source substance. However, this could not be provided for the target substance since the mode of action suggested for the source substance does not fit the available data for the target substance. Based on these arguments, a different mechanism is suspected for the target substance, which would in turn conclude that the read-across approach regarding qualitative aspects is of very limited reliability.

An *in silico* analysis of both substances was conducted to characterize their chemical and bioactivity descriptors that might impact their reproductive toxicity potential.

Principal Component Analysis (PCA) is a dimensionality-reduction method that is often used to reduce the dimensionality of large data sets. When applied to molecular data, such as chemical descriptors, PCA helps to reduce the dimensionality of the dataset while retaining its essential information. PCA can identify key structural features that contribute most to the variance within a set of compounds. Cluster analysis or clustering is the task of grouping a set of objects in such a way that objects in the same group (called a cluster) are more similar in some sense to each other than to those in other clusters. KNIME was used to analyze and visualize the data.

Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene was used as the benchmark substance, constituents with 345 plausible structural variability were computationally generated, and a hierarchical clustering was created through their chemical characteristics and toxicological properties. Substances 4-Nonyl-n-(4-nonylphenyl)aniline and Ar-nonyldiphenylamine (EC701-385-4) were not enumerated for all possible isomers for a better visualization, their estimates were overlaid with the benchmark in chemical and toxicological domains. A hierarchical clustering was created through their chemical characteristics (molecular volume and shape, water solubility and lipophilicity), and toxicological properties (ADME predictions, developmental and reproductive toxicity). A total of 46 different properties were studied using the ACD/Percept software (Predict Molecular

Properties | Percepta Software | ACD/Labs (acdlabs.com)), and few examples were shown below.

Physicochemical properties (4 properties total), examples:

- Solubility
- Lipophilicity

Absorption and Distribution (16 properties total), examples:

- P-gp inhibition
- Brain-plasma penetration
- Bioavailability
- Binding to human plasma protein

Metabolism (15 properties total), examples:

- Substrate for CYP450s (CYP3A4, CYP2D6, CYP1A2, CYP2C9, *etc.*)
- Inhibitor of CYP450s (CYP3A4, CYP2D6, CYP1A2, CYP2C9, *etc.*)

General toxicity (11 properties total), examples:

- Binding affinity to estrogen receptor
- hERG inhibition

This technique can visualize discrete and/or blocks of UVCB constituents with similar or dissimilar hazard properties and facilitate comparative assessment of their toxicological potential. The results manifested the correlation between physicochemical properties and chemical activity of compounds. Significant relationships were determined between the chemical structures and 16 absorption and distribution parameters (Figure 9, Figure 10). As can clearly be seen in the graphical depictions, the carbon chain lengths of the molecules strongly influenced both physicochemical parameters as well as toxicokinetic predictions.

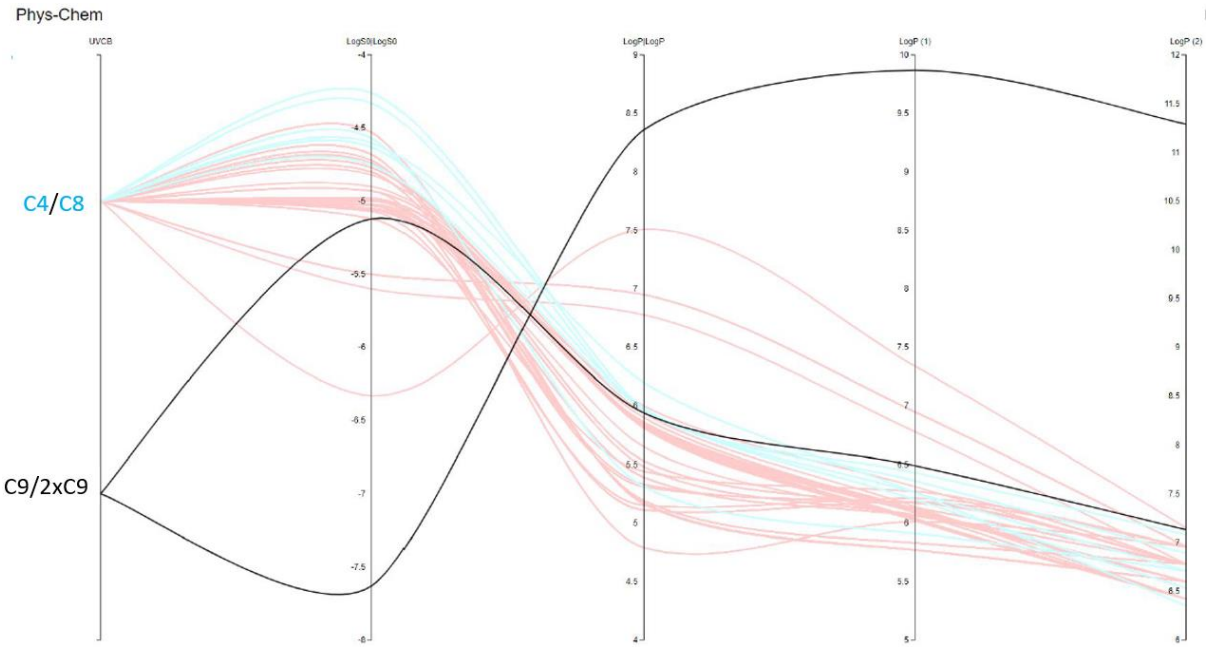



*Figure 9: Graphical depiction of in silico analysis of physicochemical properties; changes in carbon chain length and number of alkyl groups can lead to differences in molecular volume and shape.*

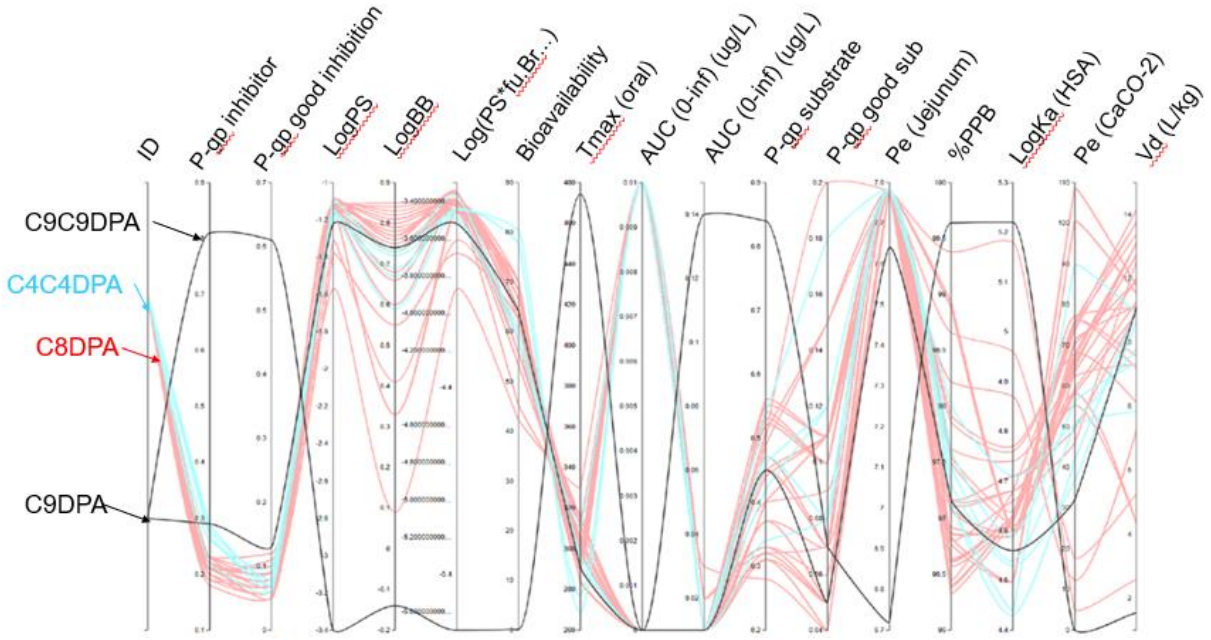



*Figure 10: Graphical depiction of in silico analysis of physicochemical properties; changes in carbon chain length and number of alkyl groups resulted in great differences in absorption and distribution.*

To better visualize the distinctions between both substances but nevertheless take into consideration the complexity of the data set, molecular fingerprinting was conducted using the RDKit software (https://www.rdkit.org). This program provides a range of functionalities for molecular fingerprinting and descriptor calculation; by leveraging RDKit's capabilities in combination with PCA, valuable insights into the structure-activity relationship of chemical compounds can be gained (Figure 11).

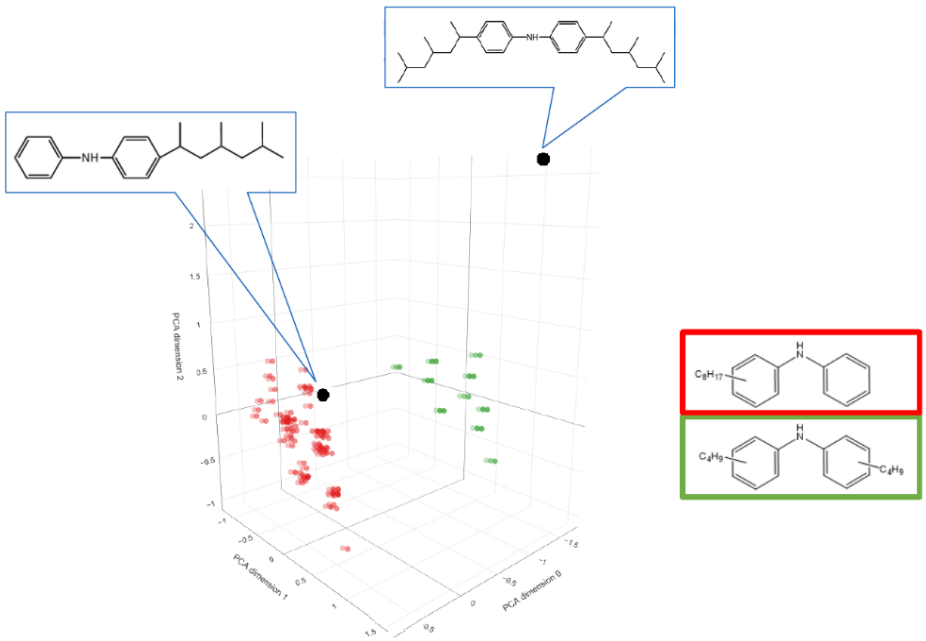



*Figure 11: PCA of RDKit molecular descriptors. A total of 345 structures were generated and compared and discrete molecular descriptors were observed.*

This work demonstrates that toxicological descriptors as well as characteristics of all structural variations indicated differences in toxicological activity between source and target substance which must be taken into consideration for assessment of read-across validity.

*AE 2.3: Common underlying mechanism, quantitative aspects*

Based on the data available for the OECD 421 studies, a lower systemic toxicity can be clearly determined for the target substance. Not only did effects on body weights and food consumption tolerate testing at higher doses, but also the organ weight parameters show that the source substance is of higher systemic toxicity. Whereas clear effects on liver and thyroid parameters were observed in the OECD 421 study with the source substance, only liver effects were found for the target substance, whereas the thyroid parameters (including organ weights and hormone analysis) remained unaffected, although higher dose levels were tested. Similarly, an effect on implantation sites was observed on the source substance, which, albeit slight was both statistically significant and outside of the biological variation range observed in the laboratory. In contrast, the data on the target substance show a statistically significant reduction, however the absolute values were within the range of biological variation typically observed for this rat strain. Based on these observations, the target substance is considered to be of lower toxicological significance than the source substance.

*AE 2.4: Exposure to other compounds than those linked to the prediction*

No impurities with known reprotoxic potential were identified for the substances. However, the DS failed to analyze metabolic transformation products and account for their role regarding potential contribution to systemic toxicity that might result in secondary effects on toxicity to reproduction. Without a thorough understanding for the mode of action of the target substance, a comparative analysis or read-across is afflicted with high uncertainty. Further, since the responsible compound(s) in the source substance are unknown, a direct correlation to structures in the target substance UVCB composition is not possible. In light of the lack of information on mode of action for the target substance as well as the high variability for substance composition, an influence of unknown structures or metabolites cannot be excluded.

*AE 2.5 Occurrence of other effects than covered by the hypothesis and justification*

For the source substance, a mode of action has been proposed which includes hepatic enzyme induction following chemical exposure. Secondary to hepatic enzyme induction, thyroid hormones are imbalanced (e.g., due to increased metabolization and excretion), thus leading to decreased levels of T4 and subsequent compensatory increase of TSH secretion. Thyroid hormone imbalance has been reported to interfere with sexual hormones and thus also impact female fertility. For detailed analysis of mode of action for the source substance, please refer to the Mode of action Annex in the comments on the CLH dossier for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene.

This mode of action cannot be supported for the target substance. While liver effects have been reported in the OECD 421 study, no changes in thyroid organ weights or thyroid hormones were observed. In an OECD 408 study conducted via gavage, thyroid follicular cell hypertrophy was observed in all treated animals, however at low severity and also the incidences observed were low compared to the dose levels administered (only at the limit dose of 1000 mg/kg bw/d were all females affected, whereas also at this dose only 8/10 males were

diagnosed with histopathological changes in the thyroid gland). Based on these findings, only very limited effects were found on the thyroid gland for the target substance and those were also only observed following bolus dosing of animals at limit dose for 90 consecutive days.

The DS considers estrous cycle changes as contributing arguments for both substances, however the target substance did not show any relevant changes in estrous cycle lengths and must therefore be considered unaffected with regards to this parameter.

*Table 21: Comparison of effects observed for target organs in OECD 421 bridging studies with Reaction products of diphenylamine with nonene, branched (target substance) and Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (source substance). Depiction of effects considers both male and female animals.*

| | Source | | | Target | | |
|---|---|---|---|---|---|---|
| Approx. Dose (♂/♀) [mg/kg bw/] | 26/28 | 87/95 | 260/271 | 40/44 | 122/133 | 397/414 |
| Liver weights | | | | | | |
| Clinical chemistry (liver parameters) | | | | | | |
| Liver histopathology | | | | | | |
| Thyroid organ weights | | | | | | |
| Thyroid hormone changes | | | | | | |
| Thyroid histopathology | | | | | | |

No treatment-related findings
Treatment-related findings, not considered adverse
Adverse, treatment-related findings
Parameter not investigated

# Annex 11 – Detailed information on bioaccumulation in fish

In the present CLH proposal, the DS claims that

> "*Reaction products of diphenylamine with nonene, branched".*
> *The bioaccumulation potential in aquatic species of one constituent of the UVCB substance (C9DPA) was examined in a study. The study follows the guideline of the test methods designated for New Chemical Substances (1974, amended 1998) under Chemical Substances Control Law of Japan (MITI). The study was realised on Cyprinus carpio in continuous flow-through system for 42 days of exposure followed by additional 42 days of depuration duration. The C9DPA constituent was prepared by addition of test substance to HCO-30 surfactant which was then dissolved in 2-methoxyethanol and fish were exposed at two nominal concentrations: 100 µg/L and 10 µg/L. The higher concentration was well above the water solubility of the test item (11.3 µg/L) and was not considered in this assessment. Although some elements deviated from the standard OECD TG 305 (use of surfactant and dissolvent, measurements were made for a group of 2 fish instead of individually), the study is well conducted, follows GLP principles and is reliable for use under CLP in the absence of data on the UVCB substance.*
> *The bioconcentration factors at steady state (BCFss) were used to evaluate the potential of bioaccumulation. At a concentration of 10 µg/L, a BCF for the whole body of 1730 L/kg w.w. was calculated by the authors (BCF=411 L/kg w.w. for high exposure dose). The DS revised the calculation using the R-package "bcmfR" to estimate the BCF of the low exposure dose (10µg/L) using kinetic approach and taking into account lipid normalisation. The new BCF calculated was* $BCF_{KLip}$*=2219 L/kg.*
> *Considering the estimated log Kow≥4 for the constituents and the measured BCF≥500 for the C9DPA, it is therefore concluded that the substance has a potential for bioaccumulation in aquatic species.*"

Bioaccumulation potential was assessed using experimental data, with support of QSAR modelling. An OECD 305 bioaccumulation study in fish was performed with the test substance Mono-Nonyl diphenylamine (MNDPA, EC248-295-7) at the MITI institute in 2000. Different modelling approaches identified the constituent MNDPA as the most critical component with regard to bioaccumulation for the registered UVCB. Considering the available experimental data and the QSAR data of the different models, the BCF based on experimental data is used to conservatively assess the bioaccumulation potential and used for the chemical safety assessment. This value as calculated by the study directors is 1730 L/kg. The position of the Registrants is that the substance is not bioaccumulative, as per Annex XIII of regulation 1907/2006/EC and the Guidance on information requirements and chemical safety assessment Chapter R.11 (PBT assessment, December 2023).

However, the experimental study was performed to meet the requirements of the Japanese authorities but was not designed to meet the requirements of the updated OECD 305 guideline or the criteria required to evaluate the 'B' criterion of the PBT assessment under REACH. There are therefore shortcomings in the study, as recognized by the DS, however despite this the DS has reassessed this data against these new criteria and has produced an unrealistic inaccurate evaluation which they use to assess bioaccumulation. The DS has reached the conclusion that the BCF in the fish is not 1720 but 2219 L/kg.

There are many issues when reassessing a study under new criteria and there is inherent uncertainty in these estimations as described in OECD 305. In addition, the registrants consider that the shortcomings in the performed fish study contribute to an unrealistic worst-case evaluation which cannot be used to robustly assess bioaccumulation. With all this in mind, it is also true that the data are close to the threshold of 2000, and a valid assessment of "B" is

crucial to the PBT assessment, so the Registrants consider that the multiple uncertainties would best be dealt with by re-performing the bioaccumulation study in fish - as per the current testing proposal.

The following information details the assessment as performed by the Registrants looking at both modelling and also the fish BCF study and the issues therein.

1. QSAR prediction of bioaccumulation

Two QSAR predictions are provided in the REACH dossier. Of particular note is the prediction using CATALOGIC v5.11.19 BCF base-line model v02.09. This model follows the OECD principles for the Validation, for Regulatory Purpose, of (Q)SAR Models. The prediction falls within the model domain and indicates that this substance is not bioaccumulative. Using the latest version of the model (CATALOGIC v5.16.1.10, BCF-baseline model v.06.13), the substance is also not considered to be bioaccumulative. The model predicts a log BCF of 2.86 (BCF = 724) (Figure 12) and is within the domain range of the model and follows the OECD principles for QSAR predictions.

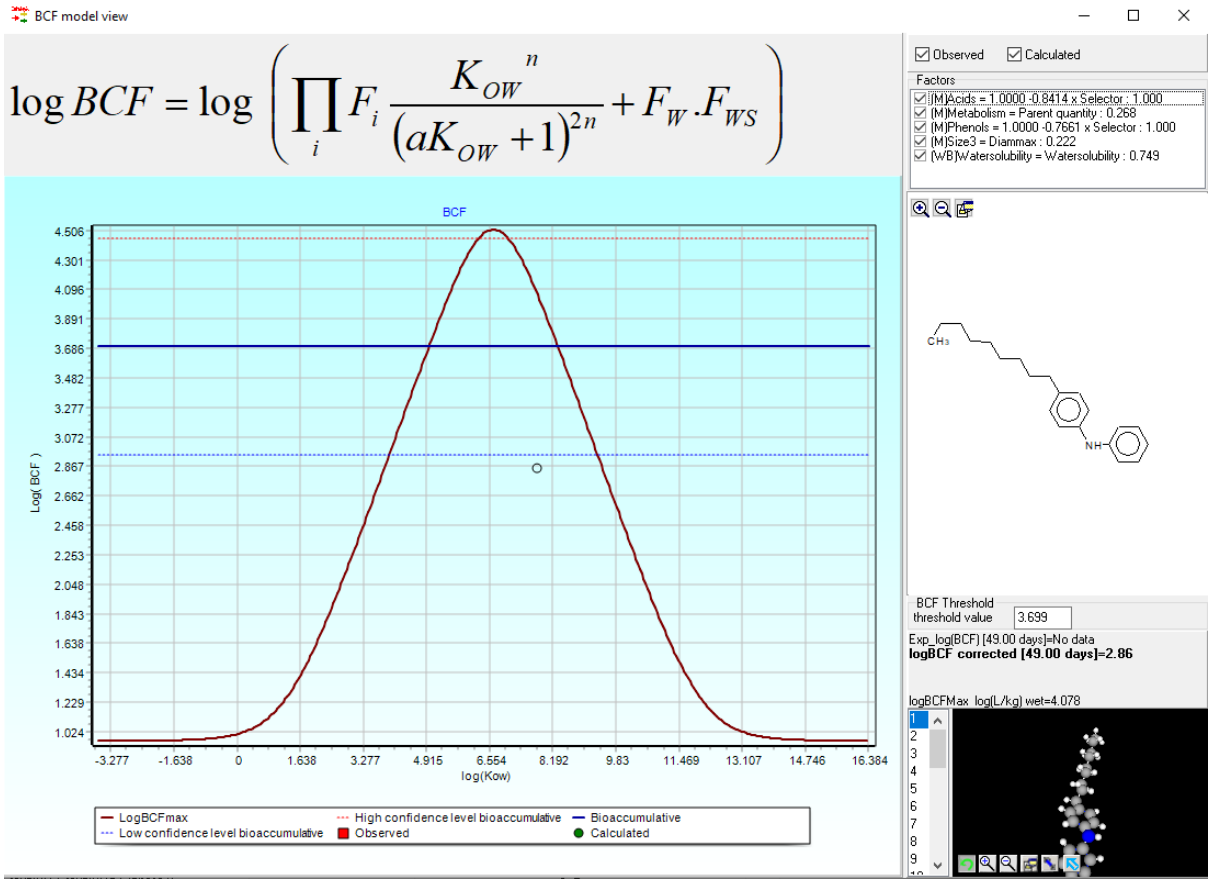



*Figure 12 Predicted BCF of MNDPA*

This model applies mitigating factors to account for factors such as metabolism and steric hinderances, and as such, provides a robust prediction of realistic bioaccumulation. Therefore, this prediction indicates that the substance is unlikely to be bioaccumulative. This is also supported by a range of substance located in the training set for the model, which are similar to this substance, and also do not meet the criteria for bioaccumulation. As such, based on these data, there is an argument that this substance is not bioaccumulative.

Given the borderline nature of the experimental data (see below information) and the limitations of this study described above, these results further demonstrate the need to clarify the bioaccumulative properties of this substance. As such, a new bioaccumulation study, conducted in accordance with the updated OECD 305 guideline, may be justified.

| **Substance** | **Log BCF** |
|---|---|
| Target substance (MNDPA; EC248-295-7) | 2.86 (predicted) |
| **Analogues identified in the training set** | **Experimental Log BCF** |
| Diphenylamine | 2.18 |
| N,N-Bis(octylphenyl)amine | 1.00 |
| N,N'-Diphenyl-p-phenylenediamine | 3.12 |
| 1-(N-Phenylamino)naphthalene | 3.23 |
| 2-(N-Phenylamino)naphthalene | 2.23 |
| p,p'-dioctyldiphenylamine | 0.000 |
| 4,4'-bis(alpha,alpha-dimethylbenzyl)diphenylamine | 2.00 |

2. Hindrance of bioaccumulation based on molecular parameters

In further consideration of bioaccumulation, the European Chemical Agency (ECHA) considers $D_{MAX}$ as a potential indicator of the likelihood of bioaccumulation. The $D_{MAX}$ for MNDPA was calculated as 1.69 nm. The ECHA guidance Chapter R.11: PBT/vPvB assessment states "*From one study of a diverse set of substances it appeared that for compounds with a* $DMAX_{aver}$ *larger than 1.7 nm the BCF value will be less than 2000*". In this case provided by ECHA, the indicator value of 1.7 nm. However, this same guidance acknowledges that this is not an absolute cut-off and different models will calculate slightly different values. Therefore, it can be considered that MNDPA meets this threshold.

The threshold of 1.7 nm was based on the work of Dimitrov *et al.* (Dimitrov et al., 2003, 2002), cited in Environment Agency (*Calculation of molecular dimensions related to indicators for low bioaccumulation potential*, 2009). In this analysis, it was determined that there was an inverse relationship between $D_{MAX}$ and log BCF, with the BCF decreasing exponentially as $D_{MAX}$ increases. Dimitrov *et al.* (2003) determined that a $D_{MAX}$ of 14.7 nm, a BCF of 5500 would be calculated, which would meet the criteria for very bioaccumulative (vB) under REACH. However, molecules with a $D_{MAX}$ >1.5 nm had a log BCF of <3.3 (<2000), and therefore, do not meet the criteria for bioaccumulative (B) under REACH. As such, based on a $D_{MAX}$ value of 1.69 nm, MNDPA can be considered unlikely to bioaccumulate.

This lack of bioaccumulation above a $D_{MAX}$ of 1.5 nm can be explained by comparison with the cell membrane architecture; this threshold for maximum diameter is approximately the same as half the thickness of one of the two lipid layers that constituent a cell membrane (*Calculation of molecular dimensions related to indicators for low bioaccumulation potential*, 2009; Dimitrov et al., 2003, 2002). Therefore, at a $D_{MAX}$ of >1.5 nm (15 Å), molecular size is such that passive diffusion across the cell membrane does not readily occur, and therefore, substances above this threshold are not bioavailable. As such, the adoption of a threshold of 1.7 nm (17 Å) as a 'limit' for bioavailability in the scientific literature is likely to be highly conservative for the assessment of bioaccumulation potential.

A 2009 study by Nichols et al. (Nichols et al., 2009) further supports that MNDPA will poorly diffuse across cell membranes and therefore will not bioaccumulate. As shown in Figure 13 (taken from (Nichols et al., 2009)), the probability of a substance to cross the cell membrane decreases as the $D_{MAX}$ increases. In this study, it was suggested that the probability of a substance crossing cell membranes decreases by 50 % when the $D_{MAX}$ is 1.33 nm (Nichols et al., 2009). Based on this graph, it would appear that at a $D_{MAX}$ of 1.69 nm, the probability of a substance crossing the cell membrane is ~10 %.

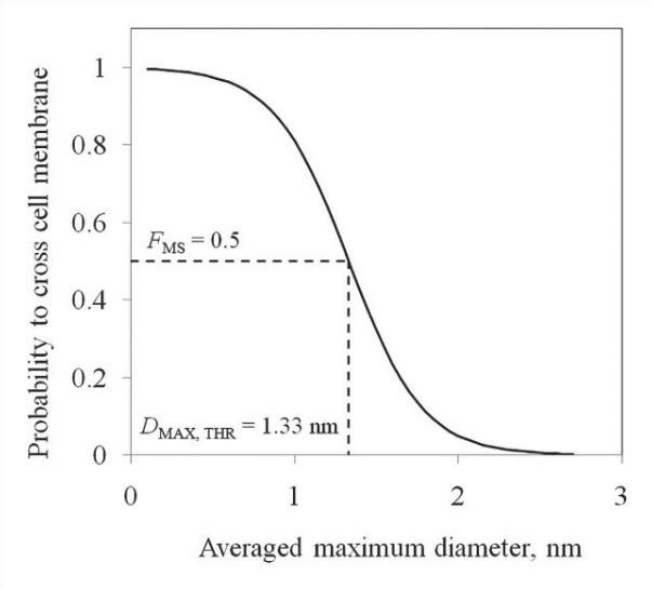



*Figure 13 Relationship between the probability that a substance will cross a cell membrane and $D_{MAX}$ (Nichols et al., 2009)*

A recent study on the fish intestinal membrane provides further support to indicate that MNDPA cannot readily pass through cell membranes (Miyata et al., 2022). This study assessed the absorption of nine chemicals across the intestinal tract of *Cyprinus carpio* using an everted gut sac method. The first four chemicals had $D_{MAXaver}$ values ranging from 1.16 to 1.51 nm, and the remaining five chemicals had $D_{MAXaver}$ values ranging from 1.72 to 3.49 nm. These results are summarized in Table 22.

For those chemicals where the $D_{MAXaver}$ were $\leq$1.51 nm ($\leq$15.1 Å), concentrations were higher in the inner solution than in the outer solution, indicating significant migration across the membrane, and therefore, the substance can be considered bioavailable. However, for chemicals with $D_{MAXaver}$ >1.51 nm (>15.1 Å), there was barely any passage through the intestinal membrane. Whilst there may be some differences in absorption characteristics between the fish intestine and gills, this study provides further supporting information on the use of $D_{MAXaver}$ for the assessment of bioaccumulation.

*Table 22 Absorption of nine chemicals across a fish intestinal tract in an everted gut sac assay (Miyata et al., 2022)*

| **Test chemical** | **$D_{MAXaver}$ (nm)** | **Concentration in:** | |
|---|---|---|---|
| | | **Outer solution (mg/l)** | **Inner solution (mg/l)** |
| 1 | 1.16 | 122 ± 5.8 | 3350 ± 639 |
| 2 | 1.21 | 24.8 ± 6.2 | 305 ± 120 |
| 3 | 1.4 | 50.4 ± 5.4 | 303 ± 134 |
| 4 | 1.51 | 57.6 ± 4.0 | 140 ± 49 |
| 5 | 1.72 | 85.8 ± 4.2 | <0.06 (limit of quantification) |
| 6 | 2.33 | 25.9 ± 1.8 | <0.1 (limit of quantification) |
| 7 | 2.09 | 63.7 ± 5.1 | <0.08 (limit of quantification) |
| 8 | 3.49 | 97.7 ± 1.5 | <0.1 (limit of quantification) |
| 9 | 2.38 | 75.7 ± 5.1 | 0.3 ± 0.2 |

Therefore, based on a review of the literature information, it can be concluded that the DMAX of MNDPA is sufficiently high that bioaccumulation will not occur.

There are then further questions which arise regarding the experimental study. As such, it is considered appropriate to conduct a new OECD 305 study to address these shortcomings and derive a more reliable value.

3. Experimental data - Bioaccumulation study in fish

The bioaccumulation study in fish was performed for Mono-Nonyl diphenylamine (EC 248-295-7, MNDPA, in 2000 according to the protocol for New Chemical Substances (1974, amended 1998), which prescribes the procedure of testing new chemical substances as required by the Chemical Substances Control Law of Japan. Modelling approaches identified the constituent MNDPA as the most critical component with regard to bioaccumulation for the registered UVCB ”Reaction products of diphenylamine with nonene, branched (EC 701-385-4)”. The bioaccumulation study in fish was performed according to the protocol for New Chemical Substances (1974, amended 1998), which prescribes the procedure of testing new chemical substances as required by the Chemical Substances Control Law of Japan. The applied protocol is similar to the OECD 305 protocol of 2000.

However, the OECD 305 protocol was revised and updated in 2012 and several significant modifications were implemented e.g. determination of a BCF kinetic (BCF k). Comparing the old guideline protocol with the latest OECD 305 protocol, it becomes obvious that the modifications and deviations in the OECD 305 guideline lead to shortcomings/weak points (normalization to 5% fat, growth correction, depuration phase until 95% of the mass (TS) is removed) if a study performed in 2000 is evaluated according to the today-standards of the OECD 305 guideline. The individual modifications and resulting shortcomings might seem small, but when considering the borderline nature of the results, they can result in the test substance being either non-bioaccumulative or bioaccumulative, based on the type of analysis used. This must be taken into consideration, as the registrant believes that these shortcomings clearly contribute to a worst-case evaluation which is not scientifically justified given the inherent uncertainty in the data.

*Water solubility and solubilizer*

The water solubility was determined in two independent studies. In the first study from 2002, the solubility ranged from 9-12 µg/L at 20°C in pure water. However, no details on the test substance characteristics (i.e., chemical name, CAS no., batch No., purity, etc.) were provided. As such, this study is considered of limited use in chemical assessment.

In a second, more recent study from 2010, the maximum water solubility at 20 °C for the whole UVCB (CAS 36878-20-3; including Mono-Nonyl diphenylamine) was determined to be 5 µg/L.

The results from this latter study providing also a full description of the test material, questions the maximum solubility of the selected target compound (Mono-Nonyl diphenylamine) in the MITI BCF study. The tested concentrations in the bioaccumulation study were 10 and 100 µg/L, far above the maximum solubility of the test material. This was due to the use of solubilizers to increase the concentration of test material in solution. Although the use of solubilizer is not excluded, the OECD 305 protocol does not recommend the use of solubilizers and suggests that their concentrations should be kept to a minimum. Notably, the concentrations of solubilisers in this study exceeded the concentrations of test substance in solution (<25 mg/L 2-methoxyethanol and 0.4-4 mg/L HCO-30), and neither solubiliser are listed in the current OCED 305 guideline. It should also be noted that in bioaccumulation studies performed for REACH registration, solubilizers are generally not recommended.

As the concentration of test substance in fish directly corresponds to the concentration in water, an artificial increase of the chemical concentration above the water solubility (as has been achieved here with the use of solubilizers) will also artificially increase the concentration of the chemical in fish. Therefore, a valid BCF value can only be determined, if the test concentration is in the range of the water solubility under test conditions. In the existing bioaccumulation study, this cannot be the case; the use of solubilizers has artificially increased the availability of test substance in water, which in turn may result in an artificially high bioaccumulation factor.

*Exposure via the water phase vs dietary exposure*

According to OECD 305, exposure via the water phase is appropriate for substance with a log Kow in the range of 1.5 to 6. For substance with a higher log Kow, exposure via the water phase should only be considered, if stable and dissolved concentrations can be achieved within the solubility limits. The highest concentration tested in the 2000 bioaccumulation study was 100 µg/L, which is demonstrably above the limit of water solubility. The lower concentration used in this study (10 µg/L) was also likely above the limit of water solubility. Due to the limited details available, it is not clear if a stable concentration could be maintained without the use of a solubilizer.

The tested substance has an estimated log Kow of 7.6, a dietary exposure study based on the protocol of OECD 305 can be considered more appropriate. However, it is recognized that dietary bioaccumulation studies can provide significant challenges, notably in calculating a precise dose received by each fish, and with regards to the release of test substance from spiked food into the water phase, which may alter the type of exposure that takes place. As such, we would seek advice from the Regulator on the design of this study.

As an alternative, although not explicitly required under OECD 305, discussions with internal and external CROs indicate that from a practical point, tests with substances having a water solubility in the range of 10 µg/L and a high log Kow value should be performed with radiolabeled material in order to monitor the stable concentrations as well as uptake and loss rates of the substance in fish and to allow for a mass balance analysis. Therefore, it could be considered that running an OECD 305 study in the water-phase using $^{14}$C-labelled test material, without the use of solubilisers may be appropriate. This would address the concerns with the 2000 bioaccumulation study highlighted above, and will provide a definitive BCF value for this substance.

*Kinetic BCF*

The derivation of the kinetic BCF was introduced by the update of the guidance in 2012, however, was not required when the study was performed in 2000. Therefore, the study was designed to meet the requirements of the Japanese authorities, e.g. $BCF_{whole\ body}$. The registrants understand the demands of the authorities to provide a kinetic BCF value, a BCF value at steady state as well as normalized BCF values to 5% fat according to the current criteria of the OECD 305 guideline. These requirements cannot be reliably met using the study performed in 2000. However, an adjusted test design, considering the monitoring of individual test fish, exposure and depuration duration, the sampling intervals and the number of test fish is required:

Sixty fish were treated at each exposure level, however, only two fish were analyzed to determine the highest accumulated organs/tissues. The use of two fish is not statistically

significant nor representative of current practices, and therefore cannot be used to draw reliable conclusions of the bioaccumulation potential of a test substance.

Furthermore, to calculate an accurate kinetic constant, k2, the depuration phase should last until 95% of the mass is removed, or for a maximum of 56 days. The depuration phase was not studied completely in this Japanese testing. 82% of the substance had been removed within 42 days of depuration time. However, a partially k2 cannot be reliably extrapolated to the whole-study k2, as the k value fluctuated over the study period. For example, the k2 is about 0.037 and 0.05 in the first 10 days and second 10 days of depuration. About 50% difference in the two periods.

Based on ECHA's guidance (R.7C), the $BCF_k$ can be calculated when the first-order kinetics apply. However, due to the limitations of the MITI study, it cannot be determined if this substance follows first-order kinetics. Therefore, it is clearly inappropriate to make such extrapolations based on this 2000 study and the re-calculated BCF value proposed by the DS should not be considered reliable or relevant for classification.*BCF values and growth correction*

According to the revised protocol of OECD 305 in 2012, a kinetic and a steady state BCF should be normalized to 5 % fat. Furthermore, the individual length of fish should be monitored in order to correct the BCF values for growth effects. However, in the MITI study length and weight measurements of individual fish were not provided, and thus any growth correction based on the data provided is not possible. Therefore, the BCF values may be considered with high uncertainty as they were not lipid- and growth normalized and thus neglecting the lipid content of the used test organism and a possible dilution due to possible/likely fish growth. In addition, the determination of the test substance in a mixed sample of two pooled fish did not allow to correct values according to the fish weight accounting for the high variation of the BCF values.

*Conclusion:*

The bioaccumulation study in fish with the test substance Mono-Nonyl diphenylamine (EC248-295-7) at the MITI institute was performed in 2000 to meet the requirements of the Japanese authorities. The performed study was not designed to meet the requirements of the updated OECD 305 guideline or the criteria required to evaluate the 'B' criterion of the PBT assessment under REACH.

When considered against the current criteria, the 2000 study reveals several shortcomings for deriving a revised BCF, as performed by the DS.. The additional information requested by ECHA and/or evaluating member states cannot be derived from this study with sufficient robustness to draw conclusions on the bioaccumulation potential of the tested substance. Many relevant data like the individual fish parameters (e.g., changes in weight and length or the lipid content) were not monitored but have a great impact on the resulting BCF values. This is a particular concern, as the original analysis resulted in a BCF that was close to, but below the threshold for bioaccumulation under REACH. Re-analysis using current requirements and potentially flawed assumptions results in a BCF that is close to, but marginally above the threshold for bioaccumulation but which may be inaccurate and introduces more uncertainty. Furthermore, robust QSAR analysis, which included the identification of a range of similar substances indicates that the bioaccumulation criteria are not met. This is supported by analysis of the molecular parameter, $D_{MAX}$, which indicates that uptake, and subsequent bioaccumulation of MNDPA is likely to be hindered. As such, there is significant uncertainty regarding the use of the 2000 MITI study for PBT assessment.

The registrants consider that the shortcomings in the performed fish study to derive a new BCF contribute to an unrealistic inaccurate evaluation by the DS which cannot be used to robustly assess bioaccumulation.To overcome these shortcomings, the registrants suggest that a new OECD 305 study is performed to meet the requirements and criteria of the current guideline.

# Annex 12 - Detailed information on long term toxicity on daphnia (*Daphnia magna*)

In the present CLH proposal (p. 89-91), the DS states that:

> *"In this key study on Daphnia magna, the test was conducted as a semi-static limit test at nominal loading rates (1.98 - 2.96 - 4.45 - 6.67 - 10.0 mg/L). The chemical specific analysis showed that only one of the two main constituents (the C9DPA) could be determined, suggesting that C9DPA is the most water-soluble and bioavailable constituent. The concentration of dialkylated isomers (C9C9DPA) was below the Lowest Calibration Level (LCL) of 0.1 µg/L in every sample in the preliminary range finding, thus not determined in the final test."*

and

> *"The study on Daphnia magna indicated toxic effects of the substance (OECD TG 211, GLP compliant, 2020). The DS revised the NOEC value based on measured concentration of C9DPA constituent, which was the only constituent that could have been detected and measured in this test with the UVCB substance and determined a NOEC of 1.28 µg/L".*

They therefore propose:

> *a classification in category 1 - H410 for aquatic chronic hazards is justified for the substance [1] according to the criteria given in Table 4.1.0(b)(i) of the CLP Regulation and considering the chronic data on toxicity for Daphnia magna NOEC (21d) 1.28µg/L and M factor 10 should apply,*

A long-term daphnia study (OECD 211) based on nominal loading rates and analytics is available. While the testing laboratory derived valid effect values based on the nominal exposure rates which apply to the whole substance, the DS determined the effect values based on the measured concentrations for only one component group (C9DPA) of the UVCB substance. Considering the predicted toxicity of other constituents in the UVCB besides this one measured constituent, Registrants are of the opinion that it is unjustified to base aquatic toxicity of this substance solely on quantification of the constituent C9DPA as this will lead to an inaccurate assessment of toxicity, when the daphnia are actually being exposed to the whole UVCB (with unquantified concentrations of other constituent groups).

Currently available data and arguments provided below as well as supporting data for the main constituent (MNDPA) indicate that there is no aquatic toxicity within the range of water solubility. Therefore, no classification for aquatic chronic toxicity is warranted or justified.

The registrants proposed to re-conduct the chronic daphnia study using a more sophisticated approach (passive dosing), which has already been agreed by the Member States Committee in the relevant CORAP for this substance (see information on other regulatory processes).

1. <u>Study methods</u>

In the available long term daphnia study (OECD 211), organisms were exposed to nominal concentrations based on Water Accommodated Fractions (WAFs). All test solutions were

prepared as water accommodated fractions with the registered substance in excess of the water solubility. As defined by ECETOC, for complex substances, the concept of a single defined water solubility has no meaning, since the total amount in solution will be the equilibrium amount of all dissolved components, which may be different from the composition of the complex substance itself and will vary depending on the amount of substance added, i.e. the loading rate. Therefore, the concept of water-accommodated fractions (WAF) or loading rates (LR) was established for insoluble substances. The idea is that of a defined amount of substance, only the soluble part ends up in solution and only this part is responsible for a possible effect on organisms. The insoluble portion of the substance is removed either by filtration or centrifugation so that physical effects can be excluded. If the water solubility is exceeded, a saturated water solution of the test substance is reached.

The WAF approach has been used successfully in regulatory assessment for complex substances for many years.

The purpose of ecotoxicological studies is to evaluate the inherent toxicological property of a substance or in case of multiconstituent substances or UVCBs of the critical constituent(s) rather than physical effects e.g. caused by attachment of particles to antennas of daphnia or gills of fish or cell walls of algae. ECETOC states in the monograph: Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable Substances: “The driving force for uptake is the dissolved concentration in the aqueous medium. The presence of undissolved substance does not influence the relationship between dissolved concentration and uptake. Hence, there is no advantage in testing above the water solubility limit in order to assess the inherent toxicity..”(ECETOC, 1996)

For complex substances containing sparingly water-soluble compounds, it is appropriate to express exposure in terms of the overall loading rate used in the preparation of the WAF or water-soluble fraction (WSF) and toxicity in terms of LL/EL/NOEL values. (ECETOC,1996).

In addition, the CLP guidance recommends:

” For this reason, such complex substances are usually tested as a WSF or WAF, and the L(E)C50 recorded based on the loading or nominal concentrations. Analytical support data are not normally available since the dissolved fraction will itself be a complex mixture of components. The toxicity parameter is sometimes referred to as LL50, related to the lethal loading level. This loading level from the WSF or WAF may be used directly in the classification criteria**.”**

2. <u>OECD guideline vs ECHA expectations</u>

For poorly water-soluble substances for which adequate analytics cannot be carried out, the OECD guidelines allow reference to and use of nominal values for toxicity assessment. This approach is viewed critically under REACH, therefore, the authorities (ECHA), with reference to OECD 23, request sufficient accompanying analytics to ensure that the organisms are exposed to the intended substance concentrations or their soluble fractions. Studies with poorly soluble substances, which refer to nominal concentrations, are sometimes deemed to be unreliable and in some instances new studies are requested with a requirement for analytical measurements. It is true that at times Inadequate analytics of poorly water-soluble substances leaves room for diverse interpretation of results when assessing the toxicity of a substance. However it is not the case that reliable assessment is impossible, it requires experience and scientific judgement.

In theory, if a UVCB or multiconstituent substance is applied in excess of the water solubility, a comparable amount of soluble fraction should be dissolved even if different loadings rates are used. Effects causing oversaturation should be avoided. However, experience shows that adherence to this principle is not always possible. Therefore concentration series with poorly soluble substances do not always show a clear dose-response relationship.

In the case of this chemistry, with the request for new studies (see Further work proposed and other regulatory processes), established methods had to be modified, supplemented or newly established and validated. Furthermore, extensive efforts by the registrants were made to refine the analytics to be sufficiently sensitive to monitoring even very low concentrations. However, ultimately only limited analytical measurement was possible.

Different water- and media solubilities for the substance are reported to be between <5 µg/L for the whole UVCB substance (BASF, 2010), for the main constituent MNDPA in fish medium (OECD 305) to be 11µg/L (Lubrizol, 2000) or 2.22 µg/L in algae medium (Lubrizol, 2003). Although only limited analytics were available, the test solutions at the beginning of each media exchange can be considered as saturated solutions. The daily exchange of the media supported the exposure of organisms at or close to the water solubility of the test substance. In the 24h aged media nearly all concentrations dropped below the limit of quantification (LOQ).

The media was daily renewed exchanged and analytical monitoring was performed at day 0-1, 7-8 and 14-15 for new and aged media. Effects were related to adult mortality and reproduction.

After 21d of exposure at a nominal concentration of 10 mg/L (WAF) adult mortality reached 50 %. Therefore, according to the guideline reproduction in daphnia was based on cumulative offspring per female. A significant reduced reproduction rate was observed for a loading rate of 6.67 mg/L, the corresponding NOELR was derived to be 4.45 mg/L.

Since no effects were observed in the low concentrations of 1.98 up to 4.45 mg/L loading rate, the NOELR of 4.45 mg/L indicates that the next higher concentrations are close to oversaturation. In contrast, the 24h aged media reveal a significant drop in the concentration below the LOQ, only in solvent stabilized aliquots concentrations were measurable.

**Specific issues with the CLH proposal:**

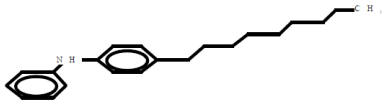
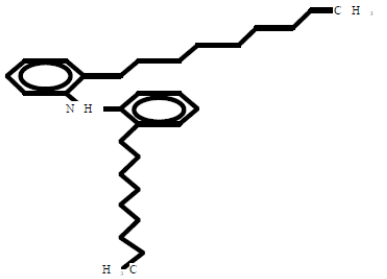
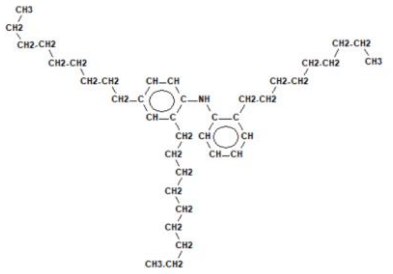
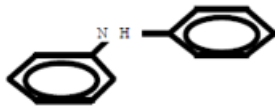
1. <u>Inadequate/inappropriate NOEC derived</u>

Derivation of NOEC by ANSES based on main constituent group C9DPA only.

Even though dialkylated isomers (C9C9DPA) could not be analytically determined, even low concentrations of C9C9DPA below LCL may contribute to the overall toxicity profile of the UVCB as based on ECOSAR predictions: chronic value daphnid for C9C9DPA = $4.34 \times 10^{-7}$ mg/L; this value is exceeding the predicted water solubility ($7.8532 \times 10^{-8}$ mg/L).

## *Predicted chronic aquatic toxicity to daphnia for selected constituents of SDPA C9*

*Table 23: Predicted chronic aquatic toxicity to daphnia for selected constituents of SDPA C9, modelled with ECOSAR v1.11*

| **Constituent** | **CAS** | **Sameness composition** | **WS [mg/L] WSKOWWin v1.43** | **Ecosar Class** | **Chronic Value (Daphnid) [mg/L]** |
|---|---|---|---|---|---|
| Diphenylamine, monononylated N-phenyl-(ar-nonyl)aniline (MNDPA, C9DPA)<br>  <br>C21H29N | 27177-41-9 | 10% < value <50 %(w/w) | 0.003035 = 3.035 µg/L (exper. value: <5 µg/L at 20°C (UVCB) | Neutral Organics | 0.000666 |
| Diphenylamine, dinonylated N-phenyl-bis(ar-nonyl)aniline (DNDPA, C9C9DPA)<br>  <br>C30H47N1 | 36878-20-3 | >50 % value <90 % (w/w) | 7.8532E-8 | Neutral Organics | 4.34E-7* |
| Diphenylamine, trinonylated N-phenyl-tris(arnonyl)aniline (TNDPA, C9C9C9DPA)<br>  <br>C39H65N | - | Sum Constituents C(12+n)H(11+2n)N:<br><br>< 10%(w/w) | 1.85E-12 | Neutral Organics | 2.57E-10* |
| Diphenylamine (DPA):<br>  <br>C12H11N | 122-39-4 | (depends) | 53 (exper. value: 40) | Neutral Organics | 0.837 |

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

The Table 23 above illustrates that other constituents could contribute to the chronic aquatic toxicity of this substance and therefore toxicity cannot be based solely on measured values of C9DPA. Overall, considering the experimental or predicted toxicity of other constituents in the UVCB SDPA C9 besides C9DPA, it is unjustified to base aquatic invertebrate toxicity of the substance solely on the low amount of detectable of C9DPA as this clearly leads to an inaccurate evaluation of toxicity when daphnia are exposed to the whole UVCB (with unquantified concentrations of other constituent groups). Furthermore, the concentrations of C9DPA, measured were extremely low and generally below the limit of quantification.
In the present CLH proposal (p. 92), the DS states that:

“For long-term aquatic hazard the NOELR of 1.28 µg/L used for classification is lower than, although in the same range as, the water solubility of the substance (<5 µg/L OECD TG 105).”

“NOELR of 1.28 µg/L used for classification” (CLH proposal p. 92) suggests that the value of 1.28 µg/L would validly represent the whole UVCB - while in fact the DS derived a NOEC value which is solely based on measured C9DPA. In contrast, the conducting laboratory correctly evaluated the study based on nominal values (loading rates) for the whole substance, while the monosubstituted constituent group (C9DPA) was the only constituent that could be measured to demonstrate that organisms were exposed to the UVCB substance. Due to the low detection and lack of analytical certainty of the C9DPA, constituent and the potential contribution of other constituents to aquatic toxicity, the proposed classification of chronic aquatic category 1 with M factor 10is not appropriate.

2. <u>LOQ/2 approach to derive mean concentrations</u>

In the present CLH proposal (p. 90), the DS explains that:

“In addition, the DS recalculated the treatment dose, based on the available measured concentration (Day 0, Day 7 and Day 14), and following the OECD TG 211 for the calculation of the time-weighted mean concentration. Where measurements indicated <LOQ, the DS considered the approach mentioned in the Guidance on the Biocidal Products Regulation Vol IV B+C (ECHA, 2017d, p. 109 & p.183)[11] and used a value corresponding to LOQ/2=0.5µg/L. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b: Endpoint specific guidance (p.76) also mentioned that “when the substance is detected but not quantified, it is good practice to use half of the limit of quantification” (ECHA, 2017c). There seems to be a slight trend to have higher dissolved concentrations of C9DPA in the test media with higher loading rates when calculating the average based on the 3 measurements from fresh solutions. The corresponding treatment doses (measured) are indicated in the table below.”

When applying the LOQ/2 approach to derive mean concentrations, the registrants understand the wording of R.7b (“detected but not quantified”) as a stipulation. However, the study report does not provide that values below LOQ were detected. In addition, the concentrations may have been only slightly below LOQ as some measured values after 24 hours suggest. Thus applying LOQ/2=0.5µg/L would lead to inaccurate estimates of aquatic toxicity in this case. However, this would only be relevant if derivation of a NOEC based on measured concentrations was a valid evaluation method for this UVCB - which is not the case.

3. (No) Correlation of loading rates and mean measured concentrations

Re ”There seems to be a slight trend to have higher dissolved concentrations of C9DPA in the test media with higher loading rates when calculating the average based on the 3 measurements from fresh solutions”, the registrants would like provide the following additional information: In the course of reviewing the measured data using ANOVA, it was noticed that the standard deviations of all nominal concentrations based on their measured values at the time points day 0-1; 7-8,14-15 overlap to such an extent that no significant trend for the dissolved concentrations can be derived, and thus also no dose-response relationship can be recorded. This confirms that the measured concentrations can be used to validate that the organisms were exposed to a concentration at the beginning of the water change, but are not suitable for quantification and subsequent derivation of a NOEC for the test.

It should be noted that the approach of using the measured concentration if the measured concentration differs by more than 20 % from the nominal concentration does not apply to UVCBs in the low concentration ranges, which is why OECD 23 also allows reference to the nominal loading rates if the analysis is inadequate. This is the case for two reasons here: a) only monosubstituted constituent group (C9DPA) measured in lieu of quantification of whole substance, and b) no dose-response relationship based on measured values.

4. Statistical tool used to derive EC10 value

In the present CLH proposal (p. 91), the DS further states that:

“The DS also used REGTOX_EV7.0.7 to calculated an EC10 value based on the time-weighted mean concentration of the C9DPA constituent.”

The DS used REGTOX_EV7.0.7 to calculate an EC10 value based on the time-weighted mean concentration of the C9DPA constituent to subsequently relate the EC10 value to the NOEC derived, concluding to use the derived NOEC.

According to the registrants’ information, REGTOX is neither mentioned in the current versions of the OECD 211 and ECHA Guidance R.7b nor an established statistical tool used by laboratories. The DS is requested to justify utilization of REGTOX and to clarify the results from this model compared to established and accepted statistical tools.

5. Conclusion on classification and labelling for environmental hazards

A long-term daphnia study (OECD 211) based on nominal loading rates and analytics is available. While the testing laboratory derived valid effect values for the whole substance based on the nominal exposure rates, the DS determined the effect values based on the measured concentrations for only one component group (C9DPA) of the UVCB substance. This is not considered to be an accurate, or even reliable worst case, assessment of the hazard, and the data indicate that there is no aquatic toxicity within the range of water solubility. Therefore, no classification for aquatic chronic toxicity is warranted.

# Annex 13 - Further work proposed and other regulatory processes

Other regulatory processes are driving new work which may be highly beneficial to this CLH process. For the benefit of non-regulatory personnel (who may evaluate this information under a public consultation process) we try to put the evaluation of dossier data in a regulatory context, and to this end ECHA has published the following scheme in the Registrant's guide - How to act in substance evaluation, November 2022.

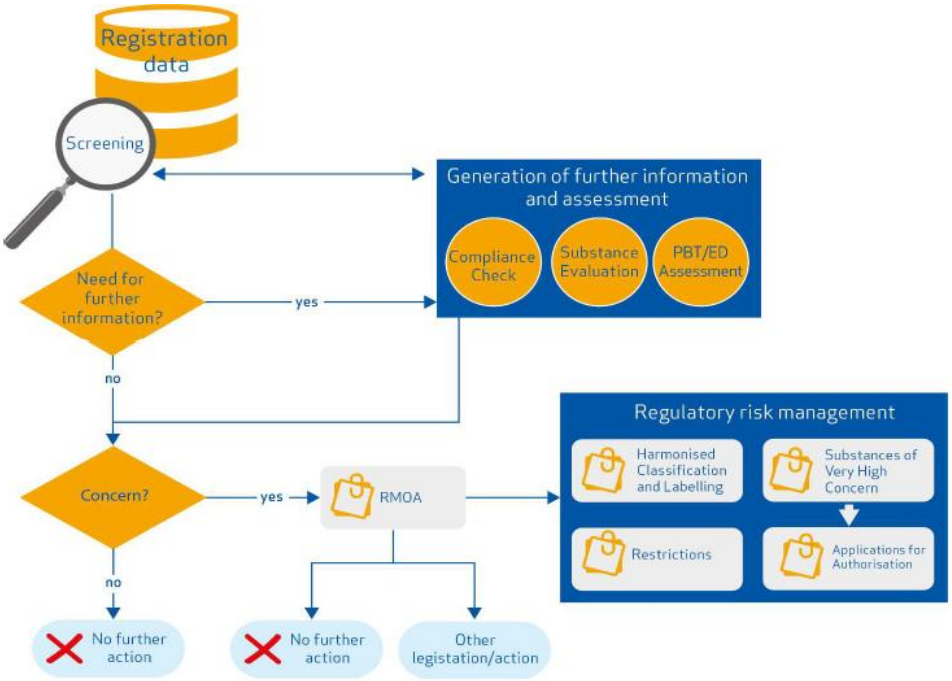



**Figure 1: Substance evaluation in the regulatory context**

Accordingly, data are assessed as to whether all relevant data are available and whether the data are consistent and sufficiently valid to carry out an assessment of the CLH dossier. If the member states in the member state committee (MSC) are of the opinion that further tests are needed, these can be requested as part of a CORAP process. Finally, the data are evaluated and in case of a concern, a harmonized classification and labeling is derived.

According to ECHA's guidelines and figure 1 "substance evaluation in the regulatory context", a CLH process carried out in parallel to a CORAP process in principle is not foreseen, if dossier data are considered as insufficiently valid or inadequate. Clearly while not foreseen, this is happening now. This substance is part of a CORAP process where the MSC has requested further studies, and these are ongoing. Even though the SeV is only based on a constituent of the UVCB registered substance (4,4'-Di-Tert-Butyldiphenylamine CAS 4627-22-9) the results of the CORAP process will affect the whole registered substance, in terms of PBT assessment. If one constituent is PBT, the entire UVCB substance will be considered to fulfill the PBT criteria.

1. Studies requested under CORAP

The substance is part of a CORAP process in which the ECHA requested a further study. The requested studies are already initiated by the lead registrant and first results are expected in Q3/4, 2024. A summary of regulatory activities in the EU is given below.

Even though the study requested in the SeV of C9 SPDA is only based on a constituent of the UVCB, i.e. the registered substance Mono-nonyl Diphenylamine, related CAS 27177-41-9, the results of the CORAP process will affect the whole registered substance, in terms of PBT assessment.

The registrants intend to perform a new study on this endpoint which will use a recently agreed passive dosing technique designed to maximize the levels of substance in the medium and to enable reliable analytical measurements. This study is required under CORAP and the methods have been agreed with the Member State Committee and ECHA. In the context of the SeV concerning the substance MNDPA, the proposal has already been adopted by the ECHA in order to provide a sound scientific basis for the ecotoxicological assessment of the substance. Therefore the registrants request the RAC to await the results of this new study prior to moving forward in the CLH process and concluding on hazard.

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