EUROPEAN CHEMICALS AGENCY

Helsinki, 22 November 2017

Addressee: [redacted]

Decision number: TPE-D-2114373433-50-01/F
Substance name: MELAMINE
EC number: 203-615-4
CAS number: 108-78-1
Registration number: [redacted]
Submission number: [redacted]
Submission date: 29.01.2016
Registered tonnage band: 1000+T

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), ECHA examined your testing proposal(s) and decided as follows.

Your testing proposal is modified and you are requested to carry out:

1. **Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: EU B.56./OECD TG 443) in rats, oral route with the registered substance specified as follows:**
   - **At least two weeks premating exposure duration for the parental (P0) generation;**
   - **Dose level setting shall aim to induce some toxicity at the highest dose level;**
   - **Cohort 1A (Reproductive toxicity);**
   - **Cohort 1B (Reproductive toxicity) with extension to mate the Cohort 1B animals to produce the F2 generation;**
   - **Cohorts 2A and 2B (Developmental neurotoxicity) with the inclusion of the investigations on learning and memory function as described in paragraph 37 of the OECD TG 426 on animals of cohort 2A subsequent to PND 63; and**
   - **Cohort 3 (Developmental immunotoxicity)**

You are additionally requested to perform:

2. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a second species (rabbit), oral route using the registered substance

You may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI of the REACH Regulation. In order to ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring and conforming to the appropriate rules in the respective Annex, and an adequate and reliable documentation.

You are required to submit the requested information in an updated registration dossier by **29 May 2020**. You shall also update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Advice and further observations are provided in Appendix 3.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals.

Authorised[1] by Ofelia Bercaru, Head of Unit, Evaluation E3

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons

The decision of ECHA is based on the examination of the testing proposal(s) submitted by you.

1. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.)

Pursuant to Article 40(3)(b) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test under modified conditions.

The basic test design of an extended one-generation reproductive toxicity study (Cohorts 1A and 1B, without extension of Cohort 1B to include a F2 generation, and without Cohorts 2A, 2B and 3) is a standard information requirement as laid down in column 1 of 8.7.3., Annex X of the REACH Regulation. If the conditions described in column 2 of Annex X are met, the study design needs to be expanded to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3. Further detailed guidance on study design and triggers is provided in in ECHA *Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.6 (version 6.0, July 2017).

The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Reproductive toxicity (extended one-generation reproductive toxicity study). ECHA notes that you provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

You have submitted a testing proposal for an extended one-generation reproductive toxicity study according to EU B.56./OECD TG 443 by the oral route, to be performed in mice with the following justification and specification of the study design: "*Indications (of doubtful relevance) of testicular effects were reported in the mouse in the last years: Wang 2013, Yin 2013, Chang 2014, Zhang 2011*" and that the proposed study should be performed in mice in order to "*clarify the reported testicular changes using a standard OECD method, and by this to confirm or to reject the results reported in the recent papers*". You further indicated that "*there is no justification for an extension to produce a F2 generation and no justification for the use of additional animals*", that "*there is no particular concern on (developmental) neurotoxicity*" and that "there is no particular concern on (developmental) immunotoxicity" and therefore concluded that "*there is no justification for the production and assessment of the cohorts 2A/2B (developmental neurotoxicity) and/or cohort 3 (developmental immunotoxicity) and no justification for the use of additional animals*". Further details of your justification for this study design are presented and addressed below.

ECHA considers that the proposed study design requires modification to fulfil the information requirement of Annex X, Section 8.7.3. of the REACH Regulation. Specifically, ECHA is of the opinion that the proposed study should be conducted in rats and that, on the basis of the available information on the substance subject to this decision, the cohorts 2A/2B and 3 should be included in the study design. Further justification for these modifications of the proposed study design are provided below.

Species and route selection

You proposed testing in mice with the following justification:"*The main argument in favour of selecting the mouse is: Indications (of doubtful relevance) of testicular effects were reported in the mouse in the last years: Wang 2013, Yin 2013, Chang 2014, Zhang 2011; see section 7.8.3. Testicular toxicity was reported in the mouse, caused by melamine at doses that are in some cases even below the NOAEL of the rat. It should be noticed that up to then the mouse was generally considered to be less susceptible to melamine than the rat, with a NOAEL much more than 10 times higher*" and specified that "*It is the main purpose of this testing proposal for an EOGRTS to clarify the reported testicular changes using a standard OECD method, and by this to confirm or to reject the results reported in the recent papers*". You also provided arguments in favour of performing the proposed study in the rat, indicating that it is the species recommended in the OECD 443 guideline, that it was recognised as the more susceptible species in repeated dose toxicity studies and that NOAELs are available not only for repeated dose toxicity but also for developmental toxicity in this species. You also highlighted concerns on the availability of testing facilities with the experience in conducting this study in the mouse.

According to the test method EU B.56./ OECD TG 443, the rat is the preferred species for conducting an extended one-generation reproductive toxicity study. Transferring this study protocol in another species may require considerable deviations from the recommendations of the OECD TG 443 to accommodate for the species differences. Moreover, as you higlighted in your testing proposal justification, testing facilities with experience in conducting this study with the design required in this decision in mice may be limited. On the basis of these considerations, ECHA considers that testing should be performed in rats.

ECHA understands that you have intended to further investigate a potential concern on testicular toxicity arising from data generated in mice by means of this study. However, ECHA considers that this concern may be better followed up with a different study than an extended one-generation reproductive toxicity. A screening study according to the OECD test guideline 421/422, modified as appropriate to address your specific concern, may for example provide useful information.

To conclude on the choice of species, ECHA considers that the proposed extended one-generation reproductive toxicity study should be performed in rats.

You proposed testing by the oral route. ECHA agrees that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) R.7a, chapter R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

Premating exposure duration and dose-level setting

You proposed that "*Pre-mating exposure for males should be adapted to the length of spermatogenesis of mice (or rats) to cover the possible testicular toxicity and effects on sperm integrity, indicated in recent publications. Otherwise: according to the basic test design of the OECD 443 EOGRT study*".

For the reasons above ECHA considers that the proposed study should be performed in rats rather than in mice. To ensure that the study design adequately addresses the fertility endpoint, the duration of the premating exposure period and the selection of the highest dose level are key aspects to be considered. According to ECHA Guidance, and in agreement with the information provided in your justification for the duration of the pre-mating exposure, the starting point for deciding on the length of premating exposure period should be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Ten weeks premating exposure duration is required if there is no substance specific information in the dossier supporting shorter premating exposure duration as advised in the ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.6 (version 6.0, July 2017). In this specific case, animals of Cohort 1B are mated to produce the F2 generation and, thus, the premating exposure duration will be 10 weeks for these Cohort 1B animals and the fertility parameters will be covered allowing an evaluation of the full spectrum of effects on fertility in these animals. Thus, shorter premating exposure duration for parental (P) animals may be considered. However, the premating period shall not be shorter than two weeks and must be sufficiently long to reach a steady-state in reproductive organs as advised in the ECHA Guidance. The consideration should take into account whether the findings from P animals after a longer premating exposure duration would provide important information for interpretation of the findings in F1 animals, e.g. when considering the potential developmental origin of such findings as explained in ECHA guidance.

The highest dose level shall aim to induce some toxicity to allow comparison of effect levels and effects of reproductive toxicity with those of systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels.

If there is no relevant data to be used for dose level setting, it is recommended that results from a range-finding study (or range finding studies) are reported with the main study. This will support the justifications of the dose level selections and interpretation of the results.

Extension of Cohort 1B

If the column 2 conditions of 8.7.3., Annex X are met, Cohort 1B must be extended, which means that the F2 generation is produced by mating the Cohort 1B animals. This extension provides information also on the sexual function and fertility of the F1 animals.

You proposed not to include an extension of Cohort 1B and provided justifications following the criteria described in column 2 of Section 8.7.3 of Annex X and detailed in ECHA *Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.6 (version 6.0, July 2017).

A proposal for amendment requested the extension of Cohort 1B. ECHA notes that uses leading to significant exposure of the registered substance are currently indicated in the joint submission: the substance is used by consumers in form of "*White crystalline powder, used in high-performance products like wood-based panels, laminates, coatings, molding, powders and flame retardants*". ECHA is of the opinion that these consumer uses alone are sufficient to meet the exposure criterion for the extension of cohort 1B.

In addition, in your response to the proposal for amendments you acknowledge the following uses:

- *Tableware, wood-laminated floors, wood furniture and similar that are made of Melamine-formaldehyde resin;*
- *Flame-retardant added to plastics are Melamine based salts (cyanurate and phosphate)*

You have also acknowledged the use of melamine by professionals in paints and coatings; moreover in your dossier you disclose the use of the registered substance in the product class finger-paints. You have concluded there is no significant exposure from any of the uses mentioned above. [redacted] Moreover, you have explained that the substance is incorporated in paints in its free form, hence ECHA considers that significant exposure can occur, e.g. to professionals from paints.

Furthermore, as many of the articles described above appear to be widely and frequently used by professionals and consumers there is a concern with respect to melamine migrating out of such articles (either unreacted melamine which is incorporated in unbound form in the matrices, or to melamine which results from the breakdown of the polymer matrix e.g. at high temperature) which contributes to the exposure of professionals and/or consumers.

ECHA considers these elements further substantiate the fulfilment of the exposure criterion for the extension of cohort 1B.

Moreover, there are indications of one or more relevant modes of action related to endocrine disruption:

- Reduced levels of serum testosterone and reduced numbers of Leydig cells were observed in a 28-day study on juvenile male mice[2];
- Increased numbers of atretic follicles were observed in a 28-day study on juvenile female rats[3];
- Supported by decreased relative gene expression of the progesterone and the alpha-oestrogen receptor in the ovaries observed in juvenile mice in a 30-day study[4].

Therefore, ECHA concludes that Cohort 1B must be extended to include mating of the animals and production of the F2 generation because the uses of the registered substance is leading to significant exposure of professionals and consumers and the studies identified above indicate endocrine-disruption modes of action for the registered substance.

[2] Sun et al. Melamine negatively affects testosterone synthesis in mice. Research in Veterinary Science 109 (2016) 135-141
[3] Sun et al. Ovarian Toxicity in female rats after oral administration of melamine or melamine and cyanuric acid. PLOS ONE, February 11, 2016
[4] Yin et al. Effect of melamine on immunohistochemical expression of Bax/Bc1-2 protein in testis and ER-a/PR mRNA in ovary with or without cyanuric acid in mice. Israel Journal of Veterinary Medicine; Vol. 69 (2); June 2014)

Cohorts 2A and 2B

The developmental neurotoxicity Cohorts 2A and 2B need to be conducted in case of a particular concern on (developmental) neurotoxicity as described in column 2 of 8.7.3., Annex X. When there are triggers for developmental neurotoxicity, both the Cohorts 2A and 2B are to be conducted as they provide complementary information.

You indicated in your dossier that "*There is no particular concern on (developmental) neurotoxicity. Neurotoxicity and developmental neurotoxicity were investigated already by An et al. 2011, 2013 and 2014, see Section 7.9.1. Specific neurotoxic effects were reported in rats in scientific investigations and not in a common neurotoxicity test.*
Effects are described only at doses that caused already urinary tract lesions and are therefore considered to be secondary effects, or at doses that already caused maternal toxicity and that are therefore also considered to be secondary effects. The authors themselves are not convinced on the relevance of their findings and they stated: "The result showed that prenatal melamine exposure probably impaired spatial learning and memory. " There is no need to further investigate these (secondary) effects. No further hints on possible neurotoxic effects were obtained from the many animal studies and also from the investigations of exposed infants, mainly in China."

However, existing information on the registered substance itself derived from available *in vivo* studies reported in the registration dossier (*An et al. 2011, 2013 and 2014)* show evidence of "*significant deficits of learning and memory induced by melamine*", impairment of learning and reversal learning abilities caused by the substance subject to this decision, and indicate that the function of cholinergic system was damaged associated with enhanced Acetylcholine esterase activity in melamine-treated rats. The authors from these studies concluded that "*melamine had a toxic influence on hippocampus, which induced the learning and memory deficits*". The outcome of investigations on whether prenatal melamine exposure induced cognitive deficits and impairment of synaptic plasticity in post-natal offspring suggested "*that prenatal melamine exposure probably impaired spatial learning and memory*" in male offspring rats. Recently published data associated impairement of spatial cognition after in utero and post-natal exposure of rats to melamine with histological evidence of toxicity in the hyppocampus (An *et al.*, *Neurotox Res (2016) 29:218-229*). Even though these studies have not been conducted according to internationally recognised test guidelines, ECHA considers that, taken together these findings constitute evidence of behavioural or functional adverse effects on the nervous system.

Validity of the studies by An et al for triggering of Cohorts 2A and 2B

Even though these studies have not been conducted according to internationally recognised test guidelines and despite limitations in the design of these studies, e.g. use of a single test dose and limited number of animals, ECHA considers that the reliability of the information obtained from these studies is adequate for establishing that there is a particular concern for (developmental) neurotoxicity and for establishing the design of the requested extended one-generation reproductive toxicity study. This is in line with the recommendations of the ECHA Guidance on information requirement and chemical safety assessment chapter R.7.5 – Appendix 5 addressing the quality and relevance of the triggers, their consistency, and their relationship with other signs of toxicity, the quality and reliability of the information which may be used as triggers in the context of setting the design of an EOGRTS.

According to this guidance, a trigger is "*an indication of concern which challenges the available data (..) and does not necessarily allow for conclusion on the hazardous properties to reproductive health – conclusion on classification or NOAEL values*" and results from scientifically evaluated (peer reviewed) publications may be used as triggers, where relevant. Therefore, ECHA considers that the studies by An et al constitute valid evidence for the purpose of establishing whether there is a particular concern for (developmental) neurotoxicity.

Relevance of the triggers in presence of kidney toxicity

According to the provisions of Annex X, section 8.7.3 column 2, the observation from existing information on the substance itself of abnormalities in the central nervous system or evidence of adverse effects on the nervous system in studies on adult animals or animals exposed pre-natally justify the identification of a particular concern. On the basis of the information provided, ECHA considers that it cannot be determined whether the observed learning and memory impairments are direct adverse effects or are caused by the urinary tract toxicity/ maternal toxicity of the substance subject to this decision. Specifically there is no indication of morbidity, death or other severe effects which would question the relevance of the neuro-behavioural findings. ECHA underlines that, for the purpose of identifying a particular concern for developmental neurotoxicity and a need to include the Cohorts 2A and 2B, ECHA Guidance on information requirement and chemical safety assessment Chapter R.7.6, Appendix 5 indicates that "*Generally triggers should be considered relevant even if observed at the same dose level than the (other) systemic toxicity findings if it cannot be justified why the triggers are secondary to (other) systemic toxicity*". Therefore, ECHA considers that the observations on learning and memory at doses which may or may not cause some kidney toxicity are relevant findings as triggers for inclusion of the Cohorts 2A and 2B in the design of the extended one-generation reproductive toxicity study.

Absence of existing information contradicting the concern

ECHA notes that histopathology was performed in brain and nervous tissue in the repeated-dose toxicity studies conducted by the NTP, Reno and Early. However, no neuro-behavioural investigations other than clinical observations have been conducted in any of the repeated-dose toxicity studies included in the dossier. The nature and results of these clinical observations cannot be independently evaluated from the robust study summaries in view of the poor reporting. These observations do not provide information on the learning and memory function, which is the specific neuro-behavioural aspect which is affected by exposure to melamine (An et al, 2011, 2013, 2014, 2016).
Further, the repeated dose toxicity studies involved exposure of adult rats only and, in contrast to the An, 2016 study, do not provide information on the potential of developmental neurotoxicity due to *in utero* and postnatal exposure to melamine. ECHA considers that time of exposure is a key parameter for developmental neurotoxicity and concludes that there is no information in the technical dossier which contradicts the concern for (developmental) neurotoxicity arising from *in utero* and postnatal exposure to melamine.

ECHA further highlights that the screening of exposed infants referred to by the registrant and reported in the dossier focused exclusively on the kidney toxicity of melamine and did not address or investigate the neurotoxicity of the substance.

ECHA concludes that the developmental neurotoxicity Cohorts 2A and 2B need to be conducted because there is a particular concern on (developmental) neurotoxicity based on the results from the above-identified *in vivo* studies on the registered substance itself.

Furthermore, ECHA considers that the observed effects on memory and learning in offspring are potentially adverse and give rise to concern both in terms of the nature of the effects and in terms of the dose-response relationship for these effects. The default standard investigations in the OECD TG 443 do not include such functional testing (cognitive). However, the OECD TG 443 states that "*if existing information indicates the need for other functional testing (e.g. sensory, social, cognitive), these should be integrated without compromising the integrity of the other evaluations conducted in the study.*" (see paragraph 50 of OECD TG 443). ECHA considers that this information on behavioural or functional adverse effects demonstrates the need for functional testing of memory and learning. ECHA therefore considers that investigations on learning and memory function conducted in accordance with the criteria described in paragraph 37 of the OECD TG 426 should be incorporated in the design of this study. ECHA considers that the Morris water maze (as used by An and co-workers, 2011, 2013, 2014, 2016) would be the preferred test of spatial learning and memory. However, if appropriately justified, and additionally considering the availability of historical control data and positive control data, an alternative test of spatial learning and memory could be used.

Since similar effects have been observed in the above-mentioned studies at weaning and in young adults, ECHA considers that the additional investigations conducted in the context of this extended-one generation study may be carried out only in young adults, i.e. subsequent to post-natal day 63. ECHA stresses that, in accordance with the recommendations presented in paragraph 50 of the OECD TG 443, "*If this testing is performed in the same animals as used for standard auditory startle, functional observational battery and motor activity testing, different tests should be scheduled to minimise the risk of compromising the integrity of these tests*".

Considering your further detailed reasons (your comments 4 to 13 on the draft decision) for disagreeing with the proposal to include Cohorts 2A and 2B in the design of the proposed extended one-generation reproductive toxicity study, ECHA moreover considered the following.

Modifications of the cholinergic system reported by An 2013 - comment 4: ECHA acknowledges the inconsistencies in the article by An et al, 2013 in the reporting of the variations of acetylcholine (ACh) levels between the control and the treated group. Figure 5 A illustrates a statistically significant increase in ACh level in the hippocampus whereas the results text and figure 5 legend refer to decreased ACh levels in the hippocampus. ECHA outlines that no figure was provided for this study in the robust study summary included in the technical dossier which constituted the basis for ECHA's scientific assessment. ECHA's statement in the draft decision indicating that "*the function of cholinergic system was damaged associated with decreased Ach level and enhanced Ach esterase activity in melamine treated group*" was a quote from the information provided by you in the executive summary of the study by An et al, 2013 in the technical dossier.

ECHA also stresses that ECHA does not refer to ACh esterase inhibition as a trigger for inclusion of Cohorts 2A and 2B in the design of the studies. Whilst this alteration of the ACh esterase activity does not correspond to the example presented in the ECHA guidance as a trigger for inclusion of the Cohorts 2A and 2B, ECHA considers that it constitutes evidence that this substance has an adverse effect on the functioning of the central nervous system together with the impaired learning and memory functions and histopathological findings reported in An et al 2016.

Impairment of learning and memory after in utero and post-natal exposure and associated histology by An, 2016 - comment 5: As you indicated in your comments, a dose of 400 mg/kg/d was used in both studies by An et al. conducted in 2014 and 2016. In both of these studies, dams were dosed either for the whole duration of the gestation period, or from post-natal day 21 until post-natal day 41. This corresponds to an exposure period of 20-21 days. According to the information reported in the article describing the 2014 study, the test dose of 400 mg/kg/d was established based on information from previous investigations in pregnant rats indicating that nephrotoxicity was observed in dams at a dose of 800 mg/kg/d. It is noted that no kidney effects were observed neither in dams dosed for 11 days at 400 mg/kg/d via the feed [redacted] 1996) nor in dams exposed for 14 days to 400 mg/kg/d by gavage (Kim 2011). It is acknowledged that haematuria was reported after gavage administration of 300 mg/kg/d of melamine to young male rats for 4 weeks, indicating that nephrotoxicity occurred in the conditions of that study.

Histopathology was performed in brain and nervous tissue in the repeated-dose toxicity studies conducted by the NTP, Reno and Early. No findings were reported in the robust study summaries (RSS) included in the technical dossier for these tissues. ECHA stresses that no detailed information on the central nervous system tissues subject to histopathology in these studies was provided in these RSS. ECHA cannot conclude on these studies because of the above-mentioned defects in reporting. The histopathological lesions identified by An et al. (2016) and referred to in the draft decision are specifically located in the hippocampus of adult male rats after *in utero* or post-natal exposure to melamine. The NTP, Reno and Early studies involved exposure of adult rats only and, in contrast to the An, 2016 study, do not provide information on the potential neurotoxicity of *in utero* exposure to melamine. ECHA considers that the life-stage of the exposure is a key parameter and that information after exposure to adults cannot be used to override the observed effects after *in utero* exposure.

Overall, the histopathological findings in the offspring of dams treated by oral gavage reported by An et al (2016) and impairment of the learning and memory function of the offspring in adulthood suggest that melamine may have the potential to cause persisting neurotoxic effects.

Reliability and relevance of the studies of An et al. (2011, 2013 and 2014) - comment 6: ECHA agrees with you on that the investigations conducted by An et al were not performed in accordance with GLP. The limitations identified in the design of each of these protocols, e.g. use of a single test dose and limited number of animals affect their suitability as definitive studies for risk assessment purposes. However, ECHA considers that the reliability of the information obtained from these studies is adequate for establishing that there is a particular concern for (developmental) neurotoxicity and for establishing the design of the requested extended one-generation reproductive toxicity study.This is in line with the recommendations of the ECHA Guidance on information requirement and chemical safety assessment chapter R.7.5 – Appendix 5 addressing the quality and relevance of the triggers, their consistency, and their relationship with other signs of toxicity, the quality and reliability of the information which may be used as triggers in the context of setting the design of an EOGRTS. According to this guidance, a trigger is "*an indication of concern which challenges the available data (..) and does not necessarily allow for conclusion on the hazardous properties to reproductive health – conclusion on classification or NOAEL values*" and results from scientifically evaluated (peer reviewed) publications may be used as triggers, were relevant.

In the light of this information, your arguments referring to the non-GLP status, limited number of animals and doses used in the An *et al.* investigations reported in the scientific literature are not a sufficient reason to dismiss the results of these studies as a basis for a particular concern.

You refer in your comments to conclusions from the authors of the study by An et al (2011) referring to the observation of haematuria as well as reduced spontaneous activity were observed in most of melamine rats and questions the interpretation of the findings reported in this study. You consider the reduced spontaneous activity and reported impaired performance in the water maze to be secondary to other sickness of the animals. ECHA considers that different views on the interpretation of a study results do not make the study unreliable *per se*.

You also referred in your comments to specific requirements on reporting of Cohort 2 parameters listed OECD test guideline 443 such as positive and historical control data in order to establish the sensitivity and reliability of the test method. ECHA points out that historical control data and positive control data are not required to raise a particular concern for the design of an extended one-generation reproductive toxicity study. ECHA takes the view that there are sufficient control data in these studies and that this is not a basis for considering that these studies are unreliable.

You indicated in your comment that no analysis for contamination of the feed used for conducting the An et al. studies was reported and you stressed possible toxic effects of melamine in the presence of cyanuric acid. No information on possible contamination of the anima feed is indeed reported in the publications. ECHA considers that it is not necessary to test for all possible dietary contaminants and notes that no indication of morbidity, death or other severe effects were noted in the studies by An et al.

Existing neuro-behavioural or neuro-pathological information - comment 7: ECHA accepts that histopathology was performed in brain and nervous tissue in the repeated-dose toxicity studies conducted by the NTP, Reno and Early.
ECHA notes that no neuro-behavioural investigations other than clinical observations were conducted as part of the NTP, Reno and Early studies. The nature and results of these clinical observations cannot be independently evaluated from the robust study summaries in view of the poor reporting. These observations do not provide information on the learning and memory function, which is the specific neuro-behavioural aspect which is affected by exposure to melamine according to An et al., (2011, 2013, 2014, 2016).

Interpretation of study results – comment 8: ECHA acknowledges that haematuria was observed in the 28-day study by An et al. (2011), showing that nephrotoxicity occurred at the dose of 300 mg/kg/d in the context of this study as indicated in your comments. ECHA emphasises that test guidelines, e.g. the OECD 414 for pre-natal developmental toxicity studies, require evidence of toxicity at the top dose but not death or severe suffering. However there is no evidence that the dose levels selected for the An et al. studies would be so high as to cause excessive toxicity or mortality. ECHA accepts that urinary tract lesions may have occurred in dams dosed during gestation with 400mg/kg/d . Based on the information provided by An et al. justifying the dose selection, the selection of 400 mg/kg/d was aimed at avoiding excessive maternal nephrotoxicity while ensuring fetal exposure. In that context, with regard to the animals exposed prenatally, nephrotoxicity in dams was reported in the study by Kim (2011) at 800 mg/kg/d leading to the identification of a maternal NOAEL of 400 mg/kg/d.

Secondary nature of the effects on learning and memory - comment 9: ECHA accepts that urinary tract lesions may have occurred in dams dosed with 400 mg/kg/d. However, ECHA considers that an essential part of the study design is to achieve toxicity at the top dose level. You have not demonstrated that this dose of 400 mg/kg/d is inappropriate for the study design. Since no overt toxicity was observed in dams exposed to 400 mg/kg/d, ECHA considers that this dose level is appropriate to investigate the pre-natal developmental toxicity of melamine. In this context, there is no evidence to support that the observations on learning and memory observed are secondary to other toxicity and, thus, these findings are considered relevant as triggers for inclusion of the Cohorts 2A and 2B in the design of the extended one-generation reproductive toxicity study.
You referred to recommendations for reporting results of parameters investigated in Cohort 2 animals, indicating that a "*Relationship of any other toxic effects to a conclusion about the neurotoxic potential of the test chemical, by sex and dose group*" should be documented. Whilst this reporting recommendation is important for an independent interpretation of the findings observed in Cohort 2 animals, ECHA is of the opinion that this is irrelevant in the assessment of triggers and design of an extended one-generation reproductive toxicity study which is based on available information.

Scope of the investigations conducted in the screening of exposed infants - comment 10: ECHA considers that you are making assumptions on epidemiological investigations being conducted and/or recorded as part of these screenings on a different organs/functions than the reported intended focus, i.e. kidney toxicity. No evidence of these investigations is provided to support these assumptions. In the absence of further details establishing the investigations of other functions than kidney toxicity conducted in these screenings, ECHA is of the opinion that the absence of reported neurotoxicity in the screening of infants exposed to melamine does not constitute reliable evidence that melamine has no neurotoxic properties.

Identification of a particular concern for neurotoxicity - comment 11: ECHA considers that an essential part of the study design is to achieve toxicity at the top dose level. You have not demonstrated that this dose of 400 mg/kg/d is inappropriate for the study design. Since no overt toxicity was observed in dams exposed to 400 mg/kg/d, ECHA considers that this dose level is appropriate to investigate the pre-natal developmental toxicity of melamine.

In this context, there is no evidence to support that the observations on learning and memory observed are secondary to other toxicity and, thus, these findings are considered relevant as triggers for inclusion of the Cohorts 2A and 2B in the design of the extended one-generation reproductive toxicity study.
ECHA is of the opinion that behavioural effects can be severe, particularly when observed in conjunction with pathological findings. In the light of the nature of effects reported in the data set that you provided or referred to in the draft decision, ECHA considers that these effects are severe and constitute evidence of adverse effects. Therefore there is a particular concern for (developmental) neurotoxicity.

Identification of a particular concern relating to learning and memory and following up on this concern - comments 12 and 13: ECHA is of the opinion that the observations on learning and memory observed are relevant irrespective if they may have occurred at that dose level with maternal kidney toxicity. Thus, these findings are triggers for inclusion of the Cohorts 2A and 2B in the design of the extended one-generation reproductive toxicity study irrespective of whether these findings are secondary to other toxicity.

ECHA considers that behavioural effects can be severe, particularly when observed in conjunction with pathological findings. In the light of the nature of effects reported in the data set provided by the registrant or referred to in the draft decision, ECHA considers that these effects are severe and constitute evidence of adverse effects. Therefore there is a particular concern for (developmental) neurotoxicity warranting extension of the investigations conducted on the Cohorts 2A and 2B in the design of the extended one-generation reproductive toxicity.

Therefore, ECHA concludes that the following investigations shall be performed in addition to the standard set of investigations required in the OECD TG 443 for the developmental neurotoxicity cohorts 2A and 2B:

- A test of associative learning and memory such as the Morris water maze test, as described in paragraph 37 of the OECD TG 426, in Cohort 2A, on the young adults (subsequent to PND 63) selected for the functional and motor activity testing required in the OECD TG 443.

Cohort 3

The developmental immunotoxicity Cohort 3 needs to be conducted in case of a particular concern on (developmental) immunotoxicity as described in column 2 of 8.7.3., Annex X.

Initially, you indicated in your testing proposal that "*there is no particular concern on (developmental) immunotoxicity. Sakazaki 2001, see Section 7.9.2, investigated immunotoxicity, without obtaining an indication on a possible immunotoxic action of melamine. No hints on possible immunotoxic effects were obtained also from the many animal studies and also from the investigations of exposed infants, mainly in China*".

A proposal for amendment requested the inclusion of Cohort 3 in the study design. ECHA considered the submitted scientific references (Yin *et al.* 2014, 2016 and 2017) and notes the information provided by these publications with regard to the immunological findings related to the substance.
ECHA further notes that existing information on the registered substance itself derived from an available *in vivo* study (Abd-Elhakim *et al.* 2016[5];), show evidence of immunotoxicity following exposure to melamine. In particular, the study showed statistically significant changes in both the innate and adaptive immune responses, such as decreases in phagocytic indices of the circulating white blood cells (-42.5%), a reduction in serum lysozyme activity (-38.1%), and a reduction in serum IgM and IgG levels (-24.1% and -66.1%, respectively). These changes are considered to be biologically relevant. In addition to the above mentioned findings, the study by Yin et al., 2014[6] showed increasing tendency in the expression rate of CD8+ spleen lymphocytes, *i.e.* cytotoxic T cells. Consequently, the CD4+/CD8+ ratio was decreased in melamine-treated study groups (significant with mid (32%) and high dose (30%). The lowered CD4+/CD8+ ratio could indicate an impairment of the immune system.
Therefore, ECHA considers that the criteria to include Cohort 3 are met and you agreed.

[5] Abd-Elhakim Y. M., Mohamed A. A-R., Mohamed W.A. Hemato-immunologic impact of subchronic exposure to melamine and/or formaldehyde in mice. *J Immunotoxicol* 2016;13(5).
[6] Yin, R. H., Liu, J., Li, H. S., Bai, W. L., Yin, R. L., Wang, X., ... & He, J. B. (2014). The toxic effects of melamine on spleen lymphocytes with or without cyanuric acid in mice. Research in veterinary science, 97(3), 505-513.

Outcome

Therefore, pursuant to Article 40(3)(b) of the REACH Regulation, you are requested to carry out the modified study with the registered substance subject to the present decision:
Extended one-generation reproductive toxicity study (test method EU B.56./ OECD TG 443), in rats, oral route, according to the following study-design specifications:

- At least two weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce some toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) with extension to mate the Cohort 1B animals to produce the F2 generation;
- Cohorts 2A and 2B (Developmental neurotoxicity) with the inclusion of the investigations on learning and memory function as described in paragraph 37 of the OECD TG 426 on animals of cohort 2A subsequent to PND 63; and
- Cohort 3 (Developmental immunotoxicity).

2. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.) in a second species

Pursuant to Article 40(3)(c) of the REACH Regulation, ECHA may require the Registrant to carry out one or more additional tests in case of non-compliance of the testing proposal with Annexes IX, X or XI of the REACH Regulation.

Pre-natal developmental toxicity studies on two species are part of the standard information requirements for substance registered for 1000 tonnes or more per year (cf Annex IX, Section 8.7.2., column 1, Annex X, Section 8.7.2., column 1, and sentence 2 of introductory paragraph 2 of Annex X of the REACH Regulation).

The technical dossier contains information on a pre-natal developmental toxicity study in rats by the oral route using the registered substance as test material.
However, there is no information provided for a pre-natal developmental toxicity study in a second species.

While you have not explicitly claimed an adaptation, you have sought to adapt the information requirement, possibly on the base of weight of evidence in the meaning of Annex XI, Section 1.2 with the following justification:

- *"Melamine has a low systemic toxicity, apart from the urinary lesions caused by the precipitating and stone forming test substance. No specific organ or tissue lesions - other than urinary system lesions - were detected in the 13-week toxicity studies with rats at doses 24 times higher than the NOAEL of 63 mg/kg bw.*
- *The maternal toxic NOAEL of ca. 400 mg/kg bw in both developmental toxicity studies with rats* [redacted] *1996 and Kim 2011) was ca. 6 times higher than the NOAEL in the 13-week toxicity study.*
- *The NOAEL developm. of ca. 1060 mg/kg bw (the highest dose applied in the key study of* [redacted] *1996) was even 17 times higher than the NOAEL in the 13-week toxicity study, render it unlikely that teratogenic effect could be observed at lower doses than the NOAELtoxic.*

- *Comparable toxic lesions were observed in the urinary tract in the systematically investigated species rat, mouse and monkey, and as far as is known from case and epidemiological studies also with intoxicated humans, dogs and cats. No other adverse effects at a comparable dose, or that are not sequels of the urinary tract lesions, were reported in these species.*
- *No teratogenic effect was detected in the rat in both studies ([redacted] 1996 and Kim 2011). No equivocal results were obtained but clear negative ones.*
- *There are no major toxicokinetic differences between species, as far as studied. Melamine is absorbed fast after oral exposure and it is excreted fast with no relevant metabolisation.*
  Overall it is unlikely that a developmental toxic effect could be detected in a second species"

However, ECHA notes that your adaptation does not meet the general rule for adaptation of Annex XI, Section 1.2. of the REACH Regulation.

ECHA acknowledges the observation of a consistent toxicity profile in repeated dose toxicity studies conducted with the substance subject to this decision in multiple species and revealing predominantly toxicity to the urinary tract.

However ECHA considers that this observation, and the absence of toxicokinetic differences between the species investigated, do not provide information on the developmental toxicity properties of the substance subject to this decision. ECHA points out that the lines of evidence provided in the registrant dossier for the endpoint developmental toxicity are all obtained in rats and therefore do not constitute a basis to assume that the substance has no developmental toxicity properties in a second species.

ECHA further outlines that you have not formally documented a weight of evidence approach to fulfil this information requirement, as required by the provisions of Annex XI, section 1.2 indicating that "*adequate and reliable documentation shall be provided*".

For all the reasons above, ECHA considers that the adaptation argument provided by the registrant is not acceptable and that there is a data gap for the information requirement of Annex X, 8.7.2.

Therefore, your adaptation of the information requirement is rejected.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

The test in the first species was carried out by using a rodent species (rat). According to the test method EU B.31./OECD 414, the rabbit is the preferred non-rodent species. On the basis of this default assumption, ECHA considers that the test should be performed with rabbit as a second species.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) R.7a, chapter R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Pre-natal developmental toxicity study (test method: EU B.31./OECD TG 414) in a second species (rabbit) by the oral route.

In your comments to the draft decision you agreed to conduct the requested study, by stating "*We agree to this proposal.*"

Deadline to submit the requested information in this decision

In the draft decision communicated to you, the time indicated to provide the requested information was 30 months. In your comments on the draft decision, you expressed your concerns on the possibility to comply with this timeline. Subsequently, in response to ECHA's invitation to provide a justification for these concerns, you indicated that on the basis of new information obtained from laboratories you inform ECHA that compliance with this 30-months deadline might still be achieved. Therefore, the deadline set in the draft decision was not amended.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposal for examination pursuant to Article 40(1) on 29 January 2016.

ECHA held a third party consultation for the testing proposal from 17 May 2016 until 1 July 2016. ECHA did not receive information from third parties.

This decision does not take into account any updates after **3 October 2016**, 30 calendar days after the end of the commenting period.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

ECHA notified you of the draft decision and invited you to provide comments.

ECHA took into account your comments, and amended the request(s).

The statement of reasons for the inclusion of Cohorts 2A and 2B has been amended based on the information provided in your comments.

In the initial draft decision issued to you, ECHA concluded on the basis of the information provided in your technical dossier that the consistent occurrence of effects in the thymus, spleen and thyroid in conjunction with kidney toxicity in multiple repeated dose toxicity studies of various duration conducted in multiple species constituted a particular concern on (developmental) immunotoxicity warranting the inclusion of Cohort 3 in the requested extended one-generation reproductive toxicity study.

You provided a summary of your comments disagreeing with the proposal to include Cohort 3 in the design of the proposed extended one-generation reproductive toxicity study. You provided further details explaining the basis for this disagreement in your comments numbered 15 to 24. ECHA has assessed the full extent of the detailed argument presented in your comments. The detailed information on the incidence and severity of the findings observed in the Early 2013a and 2013c studies conducted in rats indicates that the effects on the spleen, thymus and lymph nodes were detected at doses causing severe nephrotoxicity, lethality or moribundity in the test animals. Further, no dose dependence of the lymphoid depletion in the spleen and thymus in the Early, 2013b study.
In the light of these observations, ECHA concludes that the information included in the technical dossier, in your comments and in the scientific publications of the data referred to in your dossier does not constitute an appropriate basis to consider that there is a particular concern for (developmental) immunotoxicity. Therefore, ECHA has removed the request to include Cohort 3 in the design of the requested extended one-generation reproductive toxicity study from the draft decision.

ECHA notified the draft decision to the competent authorities of the Member States for proposal(s) for amendment.

ECHA received proposal(s) for amendment and did not modify the draft decision.

ECHA invited you to comment on the proposed amendments.

ECHA referred the draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-55 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided in your registration dossier is in compliance with the REACH requirements. The decision does not prevent ECHA from initiating a compliance check on the registration at a later stage.

2. Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, will result in a notification to the Enforcement Authorities of the Member States.

3. In relation to the information required by the present decision, the sample of the substance used for the new test(s) must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants. It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new test(s) is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant. If the registration of the substance by any registrant covers different grades, the sample used for the new test(s) must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the test(s) to be assessed.