Helsinki, 28 May 2018

Substance name: Methacrylamide
EC number: 201-202-3
CAS number: 79-39-0
Date of latest submission(s) considered[1]: 22 March 2017
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)
Addressee(s): Registrant(s)[2] of methacrylamide (Registrant(s))

DECISION ON SUBSTANCE EVALUATION

Based on Article 46(1) of the REACH Regulation (Regulation (EC) No 1907/2006), you are requested to submit the following information on the registered substance:

1. Developmental Neurotoxicity Study, oral route; test method: EU B.53/OECD 426 in rats.

2. Adequate substance-specific justification for deviating from the default assessment factors for derivation of the DNEL for dermal long-term systemic effects.

You have to provide an update of the registration dossier(s) containing the requested information, including robust study summaries and, where relevant, an update of the chemical safety report by **6 March 2020**. The deadline takes into account the time that you may need to agree on which of the registrant(s) will perform the required tests.

The reasons of this decision and any further test specifications are set out in Appendix 1. The procedural history is described in Appendix 2. Further information, observations and technical guidance as appropriate are provided in Appendix 3. Appendix 4 contains a list of registration numbers for the addressees of this decision. This appendix is confidential and not included in the public version of this decision.

Who performs the testing?

Based on Article 53 of the REACH Regulation, you are requested to inform ECHA who will carry out the study on behalf of all registrant(s) within 90 days. Instructions on how to

[1] This decision is based on the registration dossier(s) at the end of the 12-month evaluation period.

[2] The terms registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of registrants addressed by the decision.

do this 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, has to be submitted to ECHA in writing. An appeal has a suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals

Authorised[3] by Leena Ylä-Mononen, Director of Evaluation

[3] 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

Based on the evaluation of all relevant information submitted on methacrylamide and other relevant available information, ECHA concludes that further information is required to enable the evaluating Member State competent authority (MSCA) to complete the evaluation of whether the substance constitutes a risk to human health.

The evaluating MSCA will subsequently review the information submitted by you and evaluate if further information should be requested to clarify the concerns for developmental neurotoxicity and derivation of the DNELs.

1. Developmental Neurotoxicity Study, oral route; test method: EU B.53/OECD 426 in rats

The concern(s) identified

You have self-classified methacrylamide as STOT SE 2 and STOT RE 2 for effects on the nervous system. In a two-generation reproductive toxicity study combined with a dominant lethal assay according to the US NTP modified Reproductive Assessment by Continuous Breeding (RACB) protocol (hereinafter referred to as the RACB study, further described below), methacrylamide caused neurotoxic effects in young F1 mice at low doses raising concern that it may be also a developmental neurotoxicant. The NOAEL for developmental toxicity was considered to be less than the lowest dose (6.8 mg/kg bw/day) in this study (OECD SIDS, 2002). You have derived the DNEL for dermal long-term systemic effects from a NOAEL of ca. 9.1 mg/kg bw/day based on neurotoxic effects at higher dose levels observed in adult rats in a 12-month oral study with methacrylamide. For several of the exposure scenarios for industrial and professional workers, the RCRs for dermal long-term systemic effects obtained by using the DNEL derived from the NOAEL 9.1 mg/kg bw/day are very close to 1 (when applying your choice of assessment factors (AFs)) or more than 1 (when applying the default AFs recommended in the ECHA Guidance[4]). This shows a potential risk for industrial and professional workers during the manufacture and the identified uses of methacrylamide.

Why new information is needed

The information available:

In the key and supporting acute oral toxicity studies in the rats according to OECD 401 reported in the registration dossier(s), methacrylamide caused neurotoxic effects above 1000 mg/kg bw including clinical signs (such as sedation and ataxia) and histopathological changes (such as necrosis of Purkinje cells, necrosis of neurocytes in cerebellar and amigdala nuclei, necrosis of neurocytes and gliosis in hippocampus, and degeneration of sciatic nerve fibres).

[4] ECHA Guidance on information requirements and chemical safety assessment, Chapter R.8: Characterisation of dose [concentration]-response, Version 2.1, November 2012.

Methacrylamide caused structural and functional neurotoxic effects also in the key and supporting oral repeated dose toxicity studies reported in the registration dossier(s) with the lowest NOAEL being 9.1 mg/kg bw/day in the male rats in a 12-month study.

In the 12-month repeated dose toxicity studies, methacrylamide was administered by drinking water for 4, 8 or 12 months to male Wistar rats (at 0, ca. 4.6, 9.1, 19.5 and 31.6 mg/kg bw/day) and male ddY mice (at 0, ca. 24.3, 49.6, 120 and 220 mg/kg bw/day). In the rats at 19.5 and 31.6 mg/kg dose groups, reduced rotarod performance, shrinkage and loss of myelinated fibres of the sciatic nerve, and atrophy of the gastrocnemius muscle were observed. At 31.6 mg/kg, abnormal gait, decreased grip strength and paralysis of hindlimb were also observed. In the mice at 49.6, 120 and 220 mg/kg dose groups, paralysis of hindlimb, and shrinkage and loss of myelinated fibres of the sciatic nerve were observed. At 120 and 220 mg/kg dose groups, reduced rotarod performance, reduced grip strength, abnormal gait, and atrophy of the gastrocnemius muscle were observed.

In the 28-day repeated dose toxicity study according to OECD 407, methacrylamide was administered by oral gavage to Sprague-Dawley (Crj: CD) rats at 0, 30, 100 and 300 mg/kg bw/day. "Males and females at 300 mg/kg/day showed staggering gait starting at day 20 or 21 of administration. Regarding functional observation, males and females at 300 mg/kg/day showed a decrease in muscle tone and ataxia. In males at 300 mg/kg/day, a decrease in grip strength was noted. Males at 100 mg/kg/day and higher and females at 30 mg/kg/day and higher showed a decrease in locomotor activity. These functional changes were observed continuously throughout the recovery period. Histopathological examination revealed degeneration of sciatic nerve fibers and axonal swelling in the cerebellar peduncle in males and females at 300 mg/kg/day" (OECD SIDS, 2002). Also absolute brain weights were decreased in males and females at 300 mg/kg/day.

An OECD 413 study in Wistar rats is reported in the registration dossier(s) as the key repeated dose toxicity study via the inhalation route. Methacrylamide was administered via nose-only exposure for 6 hours – once daily, 5 days per week, for 13 weeks – at 0, 10, 25 and 62.5 mg/m^3. Additional neurobehaviour examinations in this study included assessment of locomotor activity (compared with controls over a period of 60 minutes with 10-minute intervals during week 12 in males and week 13 in females), forelimb and hindlimb grip strength, landing foot splay and Preyer's reflex. Statistically significant increases in motor activity were observed at 50 minutes in mid- and high-dose group females, at 60 minutes and for the data pooled over the total 60 minutes in mid-dose group females, and at 60 minutes in only mid-dose males. You have concluded that "*Motor activity values on some occasions statistically significantly higher in Groups 3 (Mid Dose) and/or 4 (High Dose) than in concurrent controls were not attributed to treatment with the test item, because they lacked dose relationship and were evident only towards the end of the 60 minute recording period*". Absolute brain weights were decreased in only mid-dose group males (4.3% less compared to controls) and without any histopathological effects in the brain.

A 14-day range-finding study performed according to GLP in Sprague-Dawley male rats is reported as a supporting repeated dose toxicity study via the inhalation route in the registration dossier(s). Methacrylamide was administered via nose-only exposure for 6 hours – once daily, 7 days per week, for 2 weeks – at 0, 12.8, 62.6 and 286 mg/m^3. In this study there were no adverse effects on the brian weights, histopathology of the sciatic and tibial nerves or on the grip strength after 14 days treatment period.

A study via dermal route in New Zealand white rabbits is reported as a supporting repeated dose toxicity study in the registration dossier(s). Methacrylamide was administered for 12 weeks at 0, 5 and 50 mg/kg bw/day or for 5 weeks at 0, 5 and 500 mg/kg bw/day. Starting at day 23, the incidence and severity of clinical signs of neurotoxicity were steadily increased by 5 weeks and reversed within 20 days after treatment in the 500 mg/kg bw/day group.

You have reported an OECD 421 study as one of the two key studies under the 'Toxicity to reproduction' section. Methacrylamide was given via oral gavage to Sprague-Dawley (Crj: CD) rats at 0, 12.5, 50 and 200 mg/kg bw/day. The neurotoxic effects observed in this study were dragging of hindlimb in all of the high-dose F0 animals.

You have reported the RACB study as the other key study under the 'Toxicity to reproduction' section and also as one of the two key studies under the 'Developmental toxicity / teratogenicity' section. In the RACB study (NTP, 1992 and Chapin et al., 1995), methacrylamide was evaluated for reproductive toxicity, neurotoxicity and dominant lethal effects in Swiss CD-1 mice dosed via drinking water. Following seven days of premating exposure while singly housed, the F0 animals were given methacrylamide as breeding pairs for 98 days at 24, 80 and 240 ppm (corresponding to 4.5, 15.4 and 49 mg/kg bw/day). The F1 animals were given the same concentrations as the F0 animals (24, 80 and 240 ppm corresponding to 6.8, 23.8 and 71.3 mg/kg bw/day) since weaning (PND 21) until necropsy (week 16) and were mated at 74 (±10) days. The dose levels for F0 animals were set so that the highest dose was expected to cause decreased nerve function halfway through the treatment period and was lower than the maximum tolerated dose. The parameters assessed in F0 and F1 animlas were similar including clinical signs, body weights, fertility (ability to produce any live pups), number of litters/pair, number of live pups/litter, sex ratio of the pups, the mean pup weights taken at birth (both absolute and adjusted for pup number), study day of delivery, food and water consumption, and at necropsy the data collected included body and selected organ weights, epididymal sperm number, motility, morphology, and testicular spermatid head count (expressed both as heads/gram of testis and as heads/testis). To assess the dominant lethal effects, the F0 males treated with methacrylamide for 100 days were cohabited with untreated females for maximum four nights. As an indicator for neurotoxicity, grip strength was evaluated in F0 animals at weeks 0, 6, 9, 12, 15 and 26, and in F1 animals at weeks 3, 5, 7, 10 and 16.

In F0 animals, there were no treatment-related effects on body weights and also no consistent changes in food and water consumption. There were no treatment-related neural, reproductive or somatic organ histopathological effects in F0 animals except statistically significant decrease in epididymal sperm concentrations and spermatid

heads/gram of testes in only the mid-dose group, and statistically significant decrease of epididymal sperm motility in the high-dose group. There were no dominant lethal effects (increase in the number of early resorptions/female, the number of dead foetuses, or in total postimplantation loss).

In F0 males, on weeks 12, 15 and 26, hindlimb grip strength was statistically significantly increased inconsistently at one or more doses. There was also a dose-response related increase in hindlimb grip strength in F0 males in week 6 but these changes did not reach statistical significance. There were no effects on grip strength of forelimb in F0 males and neither of forelimb nor hindlimb in F0 females.

In F1 animals there were no treatment-related effects on neural, reproductive or somatic organ histopathology, and on fertility, reproductive performance or terminal body weights. However, the body weights of F1 males were statistically significantly reduced (7% lower compared to controls) in week 3 (at PND 21) in the high-dose group. Whereas in the low- and mid-dose groups the body weights of F1 males at the same age were 8 and 5% lower compared to controls, respectively (not statistically significant). The body weights of F1 females at PND 21 were statistically significantly reduced in low-, mid-, and high-dose groups (7, 6, and 7% lower compared to controls, respectively). At PND 74 (±10), there were no statistically significant changes in F1 female body weights but that of F1 males were reduced in low-, mid-, and high-dose groups (5, 5, and 6% lower compared to controls, respectively).

During week 3 in F1 males, there was a statistically significant reduction in forelimb grip strength in mid- and high-dose groups (26 and 29% lower compared to controls, respectively; 15% lower in low-dose group – not significant) and in hindlimb grip strength at all dose levels (19, 12, and 31% lower compared to controls in the low-, mid-, and high-dose groups, respectively). During week 3 in F1 females the forelimb grip strength was not affected but the hindlimb grip strength was statistically significantly reduced at all dose levels (28, 19, and 32% lower compared to controls in the low-, mid- and high-dose groups, respectively). However, as the F1 animals grew older the grip strength effects became insignificant and were gone by week 5 except during week 16 when the hindlimb grip strength in the high-dose F1 females was ca. 13% lower compared to controls (not statistically significant) and the hindlimb grip strength of F1 males during week 16 showed dose-response reduction but did not reach statistical significance (ca. 2, 7, and 8% lower compared to controls).

Because of the statistically significant reduction in the hindlimb grip strength during week 3 even in the low-dose F1 animals, it was concluded in the OECD SIDS (2002) evaluation for methacrylamide that the NOAEL for developmental toxicity is less than 6.8 mg/kg bw/day in the RACB study. You, however, report in the registration dossier(s) that the highest dose level (71.3 mg/kg bw/day) is the NOAEL for developmental toxicity in this study because "*the observation of a temporarily slightly diminished grip strength in juvenile mice […] is considered as irrelevant for the NOAEL determination*" and you reason that the slightly diminished grip strength cannot be confirmed due to the bias caused by diminished body weights of the treated versus the control animals. You refer to Maurissen et al. (2003) study which studied the correlation between feed restriction-

induced loss of body weight of the rats and the grip strength. In this study, following 13 days of diet restriction, the rats weighed ca. 10% less compared to controls but neither forelimb nor hindlimb grip strengths were affected. Following 24 days of diet restriction, the rats weighed 26% less compared to controls and the forelimb and hindlimb grip strengths were reduced by 18 and 17%, respectively. It is important to note that 11 week old rats were used in the beginning of the Maurissen et al. study. In the RACB study, body weights of F1 animals of all dose groups in week 3 (at PND 21) were <10% lower compared to controls yet the hindlimb grip strength reduction ranged between 12 and 31% lower compared to controls in F1 males and between 19 and 32% lower compared to controls in F1 females. Therefore, the reductions in the grip strength of the young F1 mice in the RACB study are not correlated to the degree of their body weight loss. Thus, the evaluating MSCA, in agreement with the OECD SIDS evaluation, considers 6.8 mg/kg bw/day as the LOAEL for developmental toxicity in the RACB study.

You have reported also other published *in vivo* and *in vitro* studies including the evaluation of the neurotoxicity of methacrylamide in the 'Specific investigations' and 'Additional toxicological data' sections of the registration dossier(s).

"Male Wistar rats were treated for 120 days with (23.5 mM corresponding to ca. 480 mg/kg) methacrylamide in drinking water. Signs of neurotoxicity were observed after 120 days of treatment (Ataxia and a tendency towards spreading and draging of hindlimbs). After terminal kill, the spinal cords were removed and pellets enriched in neurofilamental proteins were prepared. The NF-proteins were isolated and separated by SDS-immunoblotting technique. A reduction in the degradation of neurofilament proteins, in particular the NF68K-protein, was observed in treated rats compared to controls" (Tanii et al., 1988(a) described in OECD SIDS 2002).

"25 mM of methacrylamide had no effect on the resting potential of the isolated desheathed sciatic nerve of the isolated retina of a frog [Boehling et al., 1977]. Methacrylamide also had no effect on neurite-extending chick dorsal root ganglion (DRG) cells in terms of alterations in morphology and function up to 16.6 mM for 16 hours [Martenson et al., 1995]. On the other hand, methacrylamide inhibited the neurite growth from rat dorsal root ganglion in culture. The half-maximum inhibition concentration was 30 mM [Tanii et al., 1991]" (OECD SIDS, 2002).

Methacrylamide caused cytotoxicity in primary cell cultures of embryonic rat brain enriched in nerve cells which was demonstrated by a decreased cumulative glucose consumption with an ED_{50} of 15 mM (Hayashi et al., 1989). Methacrylamide caused dose-related cytotoxicity in mouse neuroblastoma N18TG-2 cells and rat Schwannoma RT4 cells with ED_{50} of 8 mM and >20 mM, respectively (Tanii et al., 1988(b)). "Different concentrations of methacrylamide were added to rat brain homogenates in vitro and the inhibition of enolases was determined (I_{50} varied between 6.2 and 6.7 mM for the different isoenzymes)" (Tanii & Hashimoto, 1984 described in the registration dossier(s)). Methacrylamide inhibited the activitiy of the mouse brain total enolase and bovine neuron specific enolase with I_{50} of 6.6 and 6.8 mM, respectively. In the same study, methacrylamide up to 3 mM did not affect mouse brain glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphofructokinase (PFK) activity (Sakamoto

and Hashimoto, 1985). However, methacrylamide inhibited the rat brain GAPDH with an I_{50} of 16.2 mM but did not affect PFK activity up to 30 mM (Tanii and Hashimoto, 1985).

The information lacking for a robust conclusion:

The available information clearly establishes the neurotoxicity of methacrylamide in adult experimental animals. However, there is a concern that methacrylamide may be a developmental neurotoxicant as well. As shown in the RACB study, the grip strength of F1 young mice was affected for which no NOAEL could be identified as described above. There were adverse effects on locomotor activity of the adult rats in the 28-day (via oral route) and 90-day (via inhalation route) repeated dose toxicity studies. However, apart from the grip strength measurements, the locomotor activity and several other neurobehavioural assessments are not investigated in young experimental animals. Therefore, information is lacking for a robust conclusion on the developmental neurotoxicity potential of methacrylamide.

Further, other effects on the nervous system/cells observed in the *in vivo* and *in vitro* studies mentioned above add to the concern for developmental neurotoxicity potential of methacrylamide.

What is the possible regulatory outcome

Information from a developmental neurotoxicity study can be used for classification for Reproductive toxicity Category 1B (H360D) or Category 2 (H361d), and is needed for risk assessment of methacrylamide.

Considerations on the test method and testing strategy

Developmental neurotoxicity study (OECD 426) is designed to provide information on the potential functional and morphological hazards to the nervous system arising in the offspring from exposure of the mother during pregnancy and lactation. These studies investigate changes in structure and function of the central nervous system and the peripheral nervous system using extensive neuropathology (structure) and behavioural (function) surveys (ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7a, version 6.0, July 2017).

According to the OECD 426 test guideline, the rat is the preferred species. Regarding the route of administration, although dermal and inhalation are relevant exposure pathways, oral route is the most appropriate for solids like methacrylamide to focus on the detection of hazardous properties as its dustiness is low (particle size distribution – d90: 1644 µm; d50: 967 µm; d10: 348 µm; d1.15: 47.94 µm).

Consideration of alternative approaches

The request for an OECD 426 study is suitable and necessary to obtain information that will allow to clarify whether there is a risk for developmental neurotoxicity. More explicitly, there is no equally suitable alternative way available of obtaining this information. ECHA notes that there is no experimental study available at this stage that

would generate the necessary information and would not involve testing on vertebrate animals.

Consideration of your comments on the draft decision

Your comments on the draft decision

You have acknowledged that methacrylamide is neurotoxic in adults and pups might be more sensitive than adults; however, you do not agree with the evaluating MSCA's conclusions of the RACB study. You consider that the reversible nature of the grip strength effects in F1 animals in the RACB study is apparent; and that a differentiation between adverse and non-adverse nature of these effects can be made. You also commented that reversibility of neurofunctional effects in adult animals was observed in some older studies (performed in the 1960s) which are not part of the registration dossier(s) and are of lower reliability (low number of animals and limited study parameters evaluated). Moreover, you pointed out that, in the existing studies, functional neurotoxic effects were observed at or below the dose levels causing neuropathological effects.

Finally, you have questioned the need for a developmental neurotoxicity study while mentioning the words "animal welfare", "resource efficiency" and that the exposure to methacrylamide is restricted to well-controlled industrial uses and a meagre professional use.

Response to your comments on the draft decision

According to ECHA Guidance the neurotoxicity occurring only during development should also be regarded as an adverse effect. "The nervous system possesses reserve capacity for repairing. We may for example, find the nervous system impaired during puberty, whereas the adult nervous system seems intact. In such a case, however, one should still realise that not only the trajectory from birth to puberty differed between control and substance-exposed individuals, but the trajectory from puberty to adulthood also differed. So even when a developmental neurotoxicant may not show adverse effects in the adult, the trajectories towards adulthood have been affected and the consequences of this are so far unknown. The nervous system may compensate for damage but the resulting reduction in reserve capacity is of concern and neurotoxicity occurring during development should be regarded as an adverse effect." (ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7a, version 6.0, July 2017).

It is also important to note that the high dose level was lower than the maximum tolerated dose in the RACB study and that several neurobehavioural parameters (which would be assessed in a developmental neurotoxicity study) were not investigated in the pups. Moreover, the adverse effects on locomotor activity were not reversible in adults in a study with high reliability (an OECD 407 study in rats).

Because of the reasons mentioned in this and other sub-headings above (The concern(s) identified, The information lacking for a robust conclusion and Consideration of alternaive

approaches), a developmental neurotoxicity study is needed to clarify the developmental neurotoxicity concern for methacrylamide.

Consideration of the PfA(s) and your comments on the PfA(s)

One MSCA made proposals for amendment (PfA) to modify the Appendix 1 (reasons). The Appendix 1 was modified according to the PfA to refer to the latest version of the ECHA Guidance R.7a, and further justification was included for the route of administration. The part of the PfA (and your comments on it) that did not lead to any modifications to the Appendix 1 are explained below.

You agreed with the PfA that the transient nature of neurotoxic effects in the RACB study does not seem to be addressed in the draft decision and that the impact of the quotation of the ECHA Guidance R.7a (under the sub-heading "*Response to your comments on the draft decision"*) is not clear. The quotation was made in response to your earlier comments on the draft decision that the reversible nature of the grip strength effects in F1 animals in the RACB study is apparent and that according to your understanding, the neurotoxicity concerns are a misinterpretation of the RACB study results. As explained above (see the paragraph starting at the end of page 6 above), ECHA considers these transient effects to be adverse and the lowest dose (6.8 mg/kg bw/day) to be the LOAEL for developmental toxicity in the RACB study. In the same section it is also explained why your justification for setting a higer NOAEL is not acceptable. The quotation from ECHA Guidance R.7a clarifies why these transient effects on grip strength in the lowest dose group in the RACB study are considered as adverse (and thereby setting the LOAEL). You have, however, misinterpreted the PfA referring to a generic quotation of the ECHA Guidance R.7a and consequently you compared the exposure periods of an OECD 426 study with that of the available RACB study. Indeed the exposure periods of the RACB study covered the life stages that would be exposed in a developmental neurotoxicity study. However, the developmental neurotoxicity study is needed because, apart from the grip strength measurements, the locomotor activity and several other neurobehavioural assessments were not investigated in the RACB study.

You have provided the Chapin et al. (1995) publication as an attachment to your comments on the PfA, and mentioned that the complete RACB study report would be provided on request. The evaluating MSCA had already evaluated the Chapin et al. (1995) publication (please see the reference under the RACB study summary) and it had access to the complete report of the US National Toxicology Program (NTP) funded RACB study via the NTP archives. You wanted to outline that apart from grip strength measurements, the microscopic examination of sural and gastrocnemius nerves was performed. In the summary of the RACB study (see 3rd paragraph on page 6 above) it is already acknowledged that there were no treatment-related effects on neural histopathology. You further maintained your position that you "see no additional benefit for hazard assessment in a new OECD 426 study" as the "most relevant neurotoxic effects expected in this substance group" are represented in the RACB study. However, the available information does not allow to conclude that the effects on grip strength are

the most relevant neurotoxic effects of methacrylamide as it also caused adverse effects on locomotor activity in the repeated dose toxicity studies.

You also agreed with the PfA that your comments on the draft decision regarding low exposure in industrial and professional use and on animal welfare did not seem to be addressed. The PfA further mentioned that in order to alleviate the concerns for animal welfare you had proposed combining the OECD 414 and OECD 426 studies which you later agreed was not feasible. This latter issue has been addressed under "Note for your consideration" below. Concerning your comment regarding low exposure, the reasons for considering that the exposure is significant and leading to potential risk is explained under "The concern(s) identified" above, stating the following "For several of the exposure scenarios for industrial and professional workers, the RCRs for dermal long-term systemic effects obtained by using the DNEL derived from the NOAEL 9.1 mg/kg bw/day are very close to 1 (when applying your choice of assessment factors (AFs)) or more than 1 (when applying the default AFs recommended in the ECHA Guidance[5]). This shows a potential risk for industrial and professional workers during the manufacture and the identified uses of methacrylamide." In your comments on the PfA you further mentioned that if the "sole professional use is critical for the study requirement", you "are open to discuss an advice again[st] this use in the dossier and, consequently, down the supply chain in Europe". However, as explained above, the concern has been identified not just for the professional use but also for industrial uses.

Conclusion

Therefore, based on the substance evaluation and in accordance with Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the registered substance subject to this decision: Developmental Neurotoxicity Study, oral route; test method: EU B.53/OECD 426 in rats.

Note for your consideration

Please note that the registered substance has also been investigated in a compliance check, ECHA's compliance check decision of 28 May 2018 (Decision/annotation number CCH-D-2114394755-33-01/F), where information requirements for Annex X, Section 8.7.2. (Pre-natal developmental toxicity study in a second species (rabbit), oral route) were addressed.

In your comments on the draft decision you proposed to combine in one study the Developmental neurotoxicity study in rats (B. 53/OECD 426) requested in this decision and the Pre-natal developmental toxicity study in rabbit (B. 31/OECD TG 414) requested in the compliance check decision (Decision/annotation number CCH-D-2114394755-33-01/F). Since the two studies are to be carried out in different species, this is not possible. However, the timelines set for the testing required have been aligned in such a

[5] ECHA Guidance on information requirements and chemical safety assessment, Chapter R.8: Characterisation of dose [concentration]-response, Version 2.1, November 2012.

way to allow you to consider the results of the Developmental neurotoxicity study in rats before initiating the testing required in the compliance check decision.

2. Adequate substance-specific justification for deviating from the default assessment factors for derivation of the DNEL for dermal long-term systemic effects.

The concern(s) identified

You have used a NOAEL of 9.1 mg/kg bw/day from a 12-month oral repeated dose toxicity study in rats as a starting point to derive the DNEL for dermal long-term systemic effects. This NOAEL was based on effects observed at next dose levels including reduction in the rotarod performance and shrinkage and loss of myelinated fibers of sciatic nerve. You have not applied the default AFs recommended in the ECHA Guidance (ECHA Guidance on information requirements and chemical safety assessment, Chapter R.8: Characterisation of dose [concentration]-response, Version 2.1, November 2012). There is an inadequate substance-specific justification (discussed below) for deviating from the default AFs in the registration dossier(s). There is a risk of dermal long-term systemic effects for industrial and professional workers as the RCRs would be above 1 when default AFs are applied.

Why further justification is needed

You have not applied the default AF of 2.5 for remaining interspecies differences with the justification that "*The substances are metabolised via general metabolic pathways that are common and very similar to rodents and humans and the absence of any specific target organs indicating a specific MOA at high concentrations there is no reason to believe that an additional AF of 2.5 for remaining differences is justified*". You have not applied the default AF of 5 for intraspecies differences either. Instead, you have applied an AF of 3 with the justification that the "*Known mode of action involving ubiquitous and non-specific enzyme systems (carboxylesterases, tricaboxylic acid cycle) makes a lower variability likely, hence the AF of 3 by ECETOC (2010) is sufficiently conservative for workers*".

You hypothesize that the major metabolic pathway for methacrylamide is its "*degradation via methacrylic acid and further via citric acid cycle to physiological metabolites, as already described for the metabolism of methacrylic acid (esters)*". However, there are no toxicokinetic studies in the registration dossier(s) in support of this hypothesis. One *in vitro* study on the metabolism of methacrylamide in the registration dossier(s) demonstrates 2-fold increase in the reaction rate after phenobarbital induction that suggests a cytochrome P-450 dependent metabolism. The neurotoxicity of methacrylamide in adult experimental animals is well established. However, there is no information in the registration dossier(s) describing the mode of action for neurotoxicity. Therefore, the justification for deviating from the default AFs provided by you is not backed by sound substance-specific information.

Consideration of your comments on the draft decision

You have agreed to review the currently applied assessment factors for all DNELs and modify them to default values or justify them adequately.

Conclusion

Therefore, based on the substance evaluation and in accordance with Article 46(1) of the REACH Regulation, ECHA concludes that you are required to provide adequate substance-specific justification for deviating from the default assessment factors for derivation of the DNEL for dermal long-term systemic effects.

References

Boehling, H.G., Borchard E., & Dronin H. 1977. Monomeric Methyl-methacrylate (MMA) Acts on the Desheathed Myelinated Nerve and on the Node of Ranvier. Arch. Toxicol. 38(3): 307-314.

Chapin, R.E., Fail, P.A., George, J.D., Grizzle, T.B., Heindel, J.J., Harry, G.J., Collins, B.J. & Teague J. 1995. The reproductive and neural toxicities of acrylamide and three analogues in swiss mice, evaluated using the continuous breeding protocol. Fundamental and Applied Toxicology, 27: 9-24.

Hayashi, M., Tanii, H., Horiguchi, M. & Hashimoto, K. 1989. Cytotoxic effects of acrylamide and its related compounds assessed by protein content, LDH activity and cumulative glucose consumption of neuron-rich cultures in a chemically defined medium. Arch Toxicol., 63: 308-313.

Martenson, C.H., Sheetz M.P. & Graham D.G. 1995. In vitro acrylamide exposure alters growth cone morphology. Toxicology and Applied Pharmacology 131: 119-129.

Maurissen, J.P.J., Marable, B.R., Andrus, A.K. & Stebbins, K.E. 2003. Factors affecting grip strength testing. Neurotoxicology and Teratology 25: 543–553.

NTP (US National Toxicology Program). 1992. Final Report of the Reproductive Toxicity of Methacrylamide (MACR) (CAS: 79-39-0) in CD-1 Swiss mice. PB93-149284.

OECD Screening Information DataSet (SIDS) for methacrylamide. Initial Assessment Report for SIAM 15, Boston, October 22-25, 2002.

Sakamoto, J. & Hashimoto, K. 1985. Effect of acrylamide and related compounds on glycolytic enzymes in mouse brain in vitro. Arch Toxicol., 57: 276-281.

Tanii H. & Hashimoto K. 1984. Inhibition of brain enolases by acrylamide and its related compounds in vitro, and the structure-activity relationship. Experimentia 40(9): 971-972.

Tanii, H. & Hashimoto, K. 1985. Effect of Acrylamide and related compounds on glycolytic enzymes of rat brain. Toxicology Letters, 26: 76-84.

Tanii, H. & Hashimoto, K. 1991. In vitro neurotoxicity study with dorsal root ganglia for acrylamide and its derivatives. Toxicology Letters, 58: 209-213.

Tanii, H., Hayashi, M. & Hashimoto, K. 1988(a). Neurofilament degradation in the nervous system of rats intoxicated with acrylamide, related compounds or 2,5-hexanedione. Arch Toxicol., 62: 70-75.

Tanii, H., Miki, N., Hayashi, M. & Hashimoto, K. 1988(b). Cytotoxicity of acrylamide and related compounds to mouse neuroblastoma and rat Schwannoma cells. Arch Toxicol., 61: 298-305.

Appendix 2: Procedural history

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to neurotoxic properties and exposure of workers, methacrylamide (CAS No 79-39-0, EC No 201-202-3) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2016. The updated CoRAP was published on the ECHA website on 22 March 2016. The competent authority of Sweden (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

In accordance with Article 45(4) of the REACH Regulation, the evaluating MSCA carried out the evaluation of the above substance based on the information in your registration(s) and other relevant and available information.

In the course of the evaluation, the evaluating MSCA identified additional concerns regarding derivation of the DNELs.

The evaluating MSCA considered that further information was required to clarify the abovementioned concerns. Therefore, it prepared a draft decision under Article 46(1) of the REACH Regulation to request further information. It subsequently submitted the draft decision to ECHA on 3 March 2017.

The decision making followed the procedure of Articles 50 and 52 of the REACH Regulation as described below.

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

Registrant(s)' commenting phase

ECHA received comments from you and forwarded them to the evaluating MSCA without delay.

The evaluating MSCA took the comments from you into account and they are reflected in the reasons (Appendix 1). The requests and the deadline were not amended.

Proposals for amendment by other MSCAs and ECHA and referral to the Member State Committee

The evaluating MSCA notified the draft decision to the competent authorities of the other Member States and ECHA for proposal(s) for amendment.

Subsequently, the evaluating MSCA received proposal(s) for amendment to the draft decision and modified the draft decision. They are reflected in the reasons (Appendix 1).

ECHA referred the draft decision, together with your comments, to the Member State Committee.

ECHA invited you to comment on the proposed amendment(s).

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

MSC agreement seeking stage

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-59 written procedure 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 by you in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on your dossier(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

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

3. In relation to the required experimental study, the sample of the substance to be used ('test material') has to have a composition that is within the specifications of the substance composition that are given by all registrant(s). It is the responsibility of all the registrant(s) to agree on the test material to be subjected to the test subject to this decision and to document the necessary information on the composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation.

4. In relation to the experimental study the legal text foresees the sharing of information and costs between registrant(s) (Article 53 of the REACH Regulation). You are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who will carry out the study on behalf of the other registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at:
https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx

   Further advice can be found at
http://echa.europa.eu/regulations/reach/registration/data-sharing. If ECHA is not informed of such agreement within 90 days, it will designate one of the registrants to perform the stud(y/ies) on behalf of all of them.