Committee for Risk Assessment

(RAC)

Opinion

Pursuant to Article 77(3)(c) of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals

A reassessment at the request of the European Commission of the developmental toxicity of N-carboxymethyliminobis (ethylenenitrilo)tetra(acetic acid) (DTPA) and its pentasodium and pentapotassium salts

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OPINION OF THE COMMITTEE FOR RISK ASSESSMENT

A reassessment at the request of the European Commission of the developmental toxicity of N-carboxymethyliminobis (ethylenenitrilo)tetra(acetic acid) (DTPA) and its pentasodium and pentapotassium salts.

Pursuant to Article 77(3)(c) of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (the REACH Regulation), the Committee for Risk Assessment (RAC) has adopted an opinion on the classification for reproductive toxicity of DTPA-H5, DTPA-K5 and DTPA-Na5 and the setting of specific concentration limits.

I PROCESS FOR ADOPTION OF THE OPINION

Following a request from the European Commission on 4 December 2019, the Executive Director of ECHA in the mandate of 10 January 2020, requested RAC to prepare an opinion in relation to the classification for reproductive toxicity of DTPA-H5, DTPA-K5 and DTPA-Na5 and/or the setting of specific concentration limits within 9 months following receipt of the request.

Rapporteur, appointed by RAC: **Michal Martinek**

The draft opinion was made publicly available for targeted public consultation at https://echa.europa.eu/harmonised-classification-and-labelling-previous-targeted-consultations/-/substance-rev/25429/term on 7 April 2020. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by 21 April 2020.

The RAC opinion was adopted on **11 June 2020** by consensus of all members present and having the right to vote.

RAC General Comment

On 9 June 2017 RAC adopted opinions on the harmonised classification and labelling of N-carboxymethyliminobis (ethylenenitrilo)tetra(acetic acid) (DTPA) and its pentasodium and pentapotassium salts. While the industry consortium (BASF, Dow and Nouryon) submitting the CLH reports for these three closely related substances had proposed classification for each as Repr. 2; H361d (oral), RAC concluded that DTPA and its sodium and potassium salts should be classified as Repr. 1B; H360D based on malformations observed in rat offspring. RAC agreed with BASF, Dow and Nouryon that zinc depletion was a plausible mode of action (MoA), but considered this MoA to be relevant for humans.

Following this, BASF, Dow and Nouryon provided additional information to the European Commission, claiming that zinc kinetics are different in pregnant rats and humans and that the developmental findings in rats are not relevant for humans. As a result, the reproductive toxicity classification of DTPA and its salts was not included in the 14th Adaptation to Technical Progress (ATP) to the CLP Regulation (Reg. 2020/217). Instead, the Commission asked ECHA and RAC to review the information on interspecies differences provided by Industry and, if necessary, to amend the RAC opinion of 9 June 2017.

The information submitted by BASF, Dow and Nouryon to the European Commission was put to a targeted consultation and considered by RAC [Link].

Summary of the basis for the Art. 77(3)c request

BASF, Dow and Nouryon, suggested that the lower zinc requirement in pregnant women compared to pregnant rats and efficient homeostatic regulation would sustain sufficient internal body zinc levels in pregnant women for several months even if most of dietary zinc was bound in the gut by the chelateor. Low oral absorption and rapid excretion limits systemic exposure to DTPA and thereby its ability to reach zinc located outside the gastrointestinal tract. When additionally taking into account that the period of major organogenesis lasts only about 8 weeks in humans, BASF, Dow and Nouryon concluded that developmental effects are unlikely to occur in women exposed to DTPA.

Consequently, BASF, Dow and Nouryon proposed no classification for developmental toxicity. However, should the substance be classified in Category 2, they proposed that a specific concentration limit corresponding to low potency be assigned i.e. 3 to 10%.

BASF, Dow and Nouryon also noted that 2-ethylhexanoic acid (2-EHA), which had a harmonised classification as Repr. 2; H361d based on malformations and variations in rat offspring. A published study (Bui *et al.*, 1998) was considered to indicate that the developmental toxicity of 2-EHA might be partially related to effects on zinc metabolism and distribution in the parental animals. BASF, Dow and Nouryon argued that this is a reason to classify 2-E

Comments received during the targeted consultation

One MSCA was of the view that the information presented by BASF, Dow and Nouryon should not lead to revision of the RAC conclusion of Repr. 1B; H360D for DTPA and its

sodium and potassium salts. They did however support setting a higher specific concentration limit.

No other comments were received during the consultation.

Assessment and comparison with the classification criteria

RAC opinion from 2017

The key study used as the basis for classification in Category 1B was an oral prenatal developmental toxicity (PNDT) study with DTPA-Na_5 in the rat (BASF, 1994). This study showed an increased incidence of skeletal malformations (such as absent vertebrae) at the top dose of 1000 mg/kg bw/d and retarded ossification at the mid-dose of 400 mg/kg bw/d. The findings from this study are presented in detail in the initial opinion (RAC, 2017).

RAC also concluded in 2017 that "*there are adequate data to support the hypothesis that the teratogenicity resulting from dosing of rats with DTPA salts is as a result of an induced deficiency of zinc and presumably also other divalent cations in the mother which subsequently impacts the foetus.*" RAC considered this mode of action potentially relevant for humans.

Hazard versus risk

In the CLH report from 2016 as well as in the paper by Arts *et al.* (2018), low workplace exposure to DTPA is given as one of the key arguments for classification as Repr. 2 as opposed to Repr. 1B. The Committee notes that Classification according to the CLP regulation is based on the intrinsic hazards of substances and exposure levels resulting from use and any associated risks are not taken into account.

Susceptibility of pregnant women to DTPA-induced zinc deficiency

In the documents submitted by BASF, Dow and Nouryon a comparison of human and rat dietary zinc demand during pregnancy is presented. Their line of argumentation can be summarised as follows:

1. The whole-body zinc concentrations in humans and rats are comparable.
2. The additional dietary zinc requirement (per kg bw per day) during pregnancy is higher in rats than in humans. This is related to the fact that the percent body weight gain during pregnancy is greater and occurs within a shorter period of time in rats when compared to humans.
3. Due to a lower dietary zinc requirement (per kg bw per day) during pregnancy and a more efficient homeostatic control of zinc, pregnant women are less susceptible to zinc deficiency than pregnant rats when zinc intake is decreased.
4. Lower susceptibility to zinc deficiency of pregnant women compared to pregnant rats results in a lower sensitivity to zinc deficiency-induced developmental effects, such as those seen in the rat PNDT study with DTPA.

The total amount of zinc in the body of an adult woman is about 1500 mg. Zinc does not have an identified major storage site. Plasma zinc (ca. 2 mg) represents readily available zinc with a very rapid turnover. Furthermore, ca. 150 mg of zinc (or 10% of the whole-body zinc pool) represents a so-called rapidly exchanging pool, with a turnover rate in the

order of days. The majority of body zinc is part of a slowly exchanging pool that may not release zinc readily even in times of zinc deficiency (King *et al.*, 2000).

The daily urinary zinc loss is typically 0.3-0.6 mg/day. Faecal loss may be as high as tens of milligrams but may also be less than 1 mg/day depending on zinc intake; regulation of intestinal (re-)absorption seems to be the most important mechanism of maintaining zinc homeostasis in humans (Brown *et al.*, 2004; Miller *et al.*, 2000; Hess *et al.*, 1977).

The physiological zinc requirement of adult women has been estimated at 2-3 mg/day (Brown *et al.*, 2004; EFSA, 2014). After correction for oral absorption this leads to reference values of about 10 mg/day. A slightly higher amount (11-12 mg/day) is recommended for pregnant women but the available data suggest that the increased zinc demand during pregnancy is at least partly compensated by upregulated intestinal absorption (King, 2000).

The typical average zinc intake of European adult females is 10 mg/day, but approximately 5% of European women have a dietary zinc intake of 5 mg/day or less (EFSA, 2014).

The association between zinc deficiency and pregnancy outcome has been investigated in a number of epidemiology studies and controlled trials of supplemental zinc. Most of these studies investigated the relationship between maternal zinc status and birth weight. Although a few studies did indicate a relationship between zinc status and pregnancy outcome, overall the research appears inconclusive so far due to several factors, e.g. small sample size, lack of a reliable indicator of zinc status and insufficient control for confounding factors (King, 2000). Severe impact of low maternal plasma zinc on foetal development has been observed in females with *acrodermatitis enteropathica*, a rare hereditary disorder affecting zinc absorption. The human and animal data (rat, monkey) collectively suggest that zinc intake/absorption has to be reduced almost to zero in order to induce malformations while moderate reductions in zinc intake affect foetal growth negatively (King, 2000).

The zinc intake of the rats in the BASF (1994) study was quite high. The animals received a diet containing 60 mg zinc /kg feed while the recommended dietary concentration for pregnant rats is 25 mg/kg feed. Under these conditions, ten consecutive gavage doses of 1000 mg/kg bw/d DTPA-Na_5 were able to induce malformations, which indicates a profound effect on maternal zinc balance.

Only part of the diet in study BASF (1994) came into contact with DTPA in the gastrointestinal tract because rats feed mainly during the night and gavage administration of DTPA took place in the morning. The daily gavage dose of 1000 mg/kg bw was theoretically sufficient to bind the whole daily zinc intake of the animals in the rat PNDT study (molar ratio of DTPA to Zn was ca. 20). However, almost all of the dietary zinc would have to be chelated by DTPA in the gut to explain the observed malformations; this is unlikely to have occurred under the dosing regimen used. This indicates that the systemically absorbed DTPA also played an important role in mediating the adverse developmental outcome. Systemically absorbed DTPA reaches zinc in the plasma and other extracellular fluids and the resulting chelate is then excreted via urine.

Oral absorption of DTPA is relatively low but still significant. The CLH report from 2016 mentions two studies providing quantitative data on oral absorption of DTPA in humans. Resnick *et al.* (1990) reported that (2.8±1.6%) of ingested [^{99m}Tc]DTPA appeared in urine

of 11 healthy volunteers (males and females). Stevens *et al.* (1962) administered orally ^{14}C-DTPA (3 mg or 50 mg) to 7 patients with normal renal and intestinal function. Urinary excretion of ^{14}C was less than 3% of the administered dose in 4 patients but between 3 and 8% in 3 other patients. Based on this information, oral absorption of 4% will be assumed.

Kalkwarf *et al.* (1983) reported that an 18-mg urinary loss of body zinc (over 12 to 24 hours) was associated with each 1-g intravenous injection of $Na_3CaDTPA$ in an individual treated for americium poisoning. The equivalent oral dose of DTPA in humans (assuming oral absorption of 4% and body weight of 60 kg) is approximately 400 mg/kg bw. Taking into account that the average zinc intake is 10 mg/day, but some women ingest less than 5 mg zinc per day, a daily oral dose of 400 mg/kg bw DTPA is likely to deplete the rapidly exchangeable zinc pool within a few weeks and subsequently lead to adverse developmental outcomes including malformations. It should be noted that zinc deficiency may start before pregnancy and in this case the early periods of development would already be affected.

The lowest dose associated with malformations in the rat study with DTPA-Na_5 (BASF, 1994) was 1000 mg/kg bw/d and growth retardation was observed from 400 mg/kg bw/d. RAC notes two factors that reduced the sensitivity of the study for detection of developmental effects related to zinc deficiency: the use of zinc-supplemented diet (60 mg/kg diet vs the requirement of 25 mg/kg diet) and the fact that dosing ended on GD 15 whereas the foetal zinc demand is highest towards the end of gestation. If the study had been conducted at an adequate but not supplemental level of dietary zinc intake and DTPA administration had continued until the end of gestation, the LOAELs for malformations and growth retardation would most likely have been lower.

Pronounced developmental toxicity including malformations is expected in women orally exposed to 400 mg/kg bw/d DTPA. Less severe developmental effects (e.g. growth retardation) cannot be excluded at doses one order of magnitude lower especially in women with marginal zinc intake due to the combined effect of absorbed und unabsorbed DTPA (20-30 mg/kg bw/d DTPA would still be able to chelate all dietary zinc in humans assuming a chelation ratio DTPA to Zn of 20:1).

The above analysis was focused on the mode of action involving zinc deficiency. However, as mentioned in the initial RAC opinions from 2017, chelation of other essential metals (e.g. manganese) may further contribute to the developmental toxicity of DTPA. The relevance of these additional modes of action to humans has not been explored in the original BASF, Dow and Nouryon CLH reports, or in any subsequently submitted documentation.

Classification of 2-ethylhexanoic acid (2-EHA)

2-EHA currently has a harmonised classification as Repr. 2; H361d, transferred from the Dangerous Substance Directive. The available records from meetings of the Commission Working Group on the Classification and Labelling of Dangerous Substances in 1994, where classification of 2-EHA was discussed, do not mention any information related to mode of action. The study investigating the mode of action via altered zinc distribution (Bui *et al.*, 1998) was published only after the classification had been discussed by the Working Group. In addition, RAC finds the available evidence for involvement of this MoA in developmental

toxicity of 2-EHA very limited (for a more detailed discussion see the RAC opinion on 2-ethylhexanoic acid and its salts, 2020). Thus, the basis of the classifications of 2-EHA and DTPA are not related.

Overall RAC finds that the additional information received does not rule out human relevance of the developmental hazard identified in the rat PNDT study by BASF (1994).

Therefore, RAC reconfirms its proposal from 2017 to classify DTPA-H_5, DTPA-K_5 and DTPA-Na_5 with Repr. 1B; H360D.

Specific concentration limit (SCL)

SCLs are derived according to the procedure described in the Guidance on the application of the CLP criteria (version 5.0, section 3.7.2.6). In the first step, a preliminary potency group is assigned based on ED_{10} values or, if not available, LOAELs for the effects triggering classification. In the next step, the final potency group is selected after consideration of modifying factors.

The effect triggering classification of DTPA in Category 1B for development was skeletal malformations at 1000 mg/kg bw/d in the rat PNDT study BASF (1994) with DTPA-Na_5 (the equivalent doses of DTPA-K_5 and DTPA-H_5 are 1160 and 780 mg/kg bw/d respectively). Only delayed ossification, but no increase in malformations, was observed at the next lower dose of 400 mg/kg bw/d. ED_{10} values calculated by linear interpolation from litter-based incidence (i.e. mean percentage of affected foetuses per litter) and litter incidence for various malformations are provided in the table below. The source data can be found in the CLH proposals from 2016.

Effect	**ED_{10} (mg/kg bw/d)[a]**		**LOAEL (mg/kg bw/d)[a]**
	mean % of affected foetuses per litter	**% of affected litters**	
Total malformations	710	460	1000
Thoracic vertebrae absent	870	620	1000
Lumbar vertebrae absent	n.a.[b]	660	1000
Sternebra(e) bipartite, ossification centres dislocated[c]	n.a.[b]	570	1000

[a] values for DTPA-Na_5; conversion DTPA-H_5: multiply by 0.78; conversion to DTPA-K_5: multiply by 1.16
[b] rate of 10% not reached
[c] grey-zone anomaly

RAC notes that the LOAEL for malformations of 1000 mg/kg bw/d and the ED_{10} values calculated therefrom have probably been influenced by the use of zinc-supplemented diet. Had the animals been fed a diet with an adequate but not supplemental zinc content, the LOAEL and the ED_{10} values would most likely have been lower, possibly below 400 mg/kg bw/d (the boundary between the 'low' and 'medium' potency group). On the other hand, rats may be somewhat more sensitive to the MoA via zinc deficiency than humans due to a higher zinc requirement during pregnancy, and the potency, even when taking into account the use of zinc-supplemented diet in the key study, can still be considered relatively low. Thus, RAC agreed to accept BASF, Dow and Nouryon's proposal to set an

SCL corresponding to a 'low' potency, which is 3% for a substance classified as 1B according to the CLP guidance.

Conclusion on classification following the current assessment

RAC has evaluated the additional information provided by BASF, Dow and Nouryon in 2019. RAC confirms its previous conclusion from 2017 that the skeletal malformations observed in the BASF (1994) rat PNDT study with DTPA-Na_5 are considered relevant for humans and that classification with **Repr. 1B; H360D** is warranted.

RAC proposes to add an **SCL** of **3%** based on low potency in the key study (BASF, 1994) for DTPA-H_5, DTPA-K_5 and DTPA-Na_5.

References

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RAC (2017) Opinion proposing harmonised classification and labelling at EU level of N-carboxymethyliminobis (ethylenenitrilo)tetra(acetic acid). CLH-O-0000001412-86-155/F. Adopted 9 June 2017

RAC (2017) Opinion proposing harmonised classification and labelling at EU level of Pentapotassium 2,2',2'',2''',2''''-(ethane-1,2-diylnitrilo)pentaacetate, CLH-O-0000001412-86-157/F. Adopted 9 June 2017

RAC (2017) Opinion proposing harmonised classification and labelling at EU level of pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo)tetraacetate, CLH-O-0000001412-86-156/F. Adopted 9 June 2017

RAC (2020) Opinion proposing harmonised classification and labelling at EU level of 2-ethylhexanoic acid and its salts, CLH-O-0000006817-63-01/F. Adopted 11 June 2020

Annex: Records of the targeted public consultation on the developmental toxicity of N-carboxymethyliminobis (ethylenenitrilo)tetra(acetic acid) (DTPA) and its pentasodium and pentapotassium salts