Committee for Risk Assessment

RAC

Annex 2
Response to comments document (RCOM)
to the Opinion proposing harmonised classification and labelling at EU level of

N-carboxymethyliminobis (ethylenenitrilo)tetra(acetic acid)

EC Number: 200-652-8
CAS Number: 67-43-6

CLH-O-0000001412-86-155/F

Adopted

9 June 2017

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON N-CARBOXYMETHYL IMINOBIS(ETHYLENENITRILO)TETRA(ACETIC ACID)

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: N-carboxymethyl iminobis(ethylenenitrilo)tetra(acetic acid)
EC number: 200-652-8
CAS number: 67-43-6
Dossier submitter: Akzo Nobel Functional Chemicals BV

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
15.07.2016	Germany		MemberState	1
Comment received				
The classification proposal for DTPA-H5 as 'Repr. 2; H361d' is based on read across data from DTPA-Na5 (CAS 140-01-2; EC 205-391-3). We noted that the FR CA has described in their RMO analysis for DTPA-Na5 and DTPA-H5 (2014) that data concerning the reproductive toxicity seem to be sufficient to support a classification 'Repr. 1B (H360D: May damage the unborn child)' according to CLP Regulation. In 2015 industry has submitted a CLH dossier for DTPA-Na5. For the endpoint reproductive toxicity the dossier submitter has proposed 'Repr.2; H361d: Suspected of damaging the unborn child if ingested'. The examination of the data provided showed that the proposed classification as Repr.2; H361d (oral) is warranted. Concerning substance identity we would like to note that in Part B, section 1.3, table 8 of the CLH report the physical-chemical properties of the substance are given but corresponding references (respectively comments) are missing in the table which would make the data more comprehensible.				
Dossier Submitter's Response				
Classification: We are happy to see that you agree with the proposal for classification as Repr. 2, H361d (oral) based on the available data. Substance identity: We do not really understand this remark as phys/chem data are not part of substance identity. References and comments with regard to phys/chem properties of this substance can be found in the REACH dossier and on the ECHA website.				

RAC's response
Thank you.

Date	Country	Organisation	Type of Organisation	Comment number
15.07.2016	Netherlands		Company-Downstream user	2

Comment received
Further data is available showing that the effect of DTPA in lung is lower than that of Na2H2EDTA reported in the submission (page 28). This additional data would be useful to consider in relation to the relevant route of exposure for the classification of DTPA and its salts. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment No. 02
Dossier Submitter's Response
Data from disodium EDTA is considered appropriate for read across to the DTPA category since the mode of action for these effects is considered to be related to the chelation of calcium and both these agents have a similar affinity for calcium. The sites of irritation observed following inhalation exposure are consistent with the areas where – in view of the generated particle size distribution - a high degree of test material impaction would occur. Upon impaction, the disodium EDTA complexes with calcium and perhaps zinc, removing it from cell junctions and membranes. The removal of these metals from intercellular junctions causes cells to detach from one another resulting in cell shedding. This leads to tissue regeneration and metaplasia in the affected areas. As cells which have become detached die, inflammation occurs leading to signs of necrosis and infiltration of inflammatory cells. This effect is similar to that observed in the intestines of rats given high oral bolus doses of chelating agents such as EDTA and DTPA. Given the effects of the substances are similar in nature, involve the same mode of action, are concentration dependent, threshold mediated and do not involve metabolic processes, it is therefore considered relevant to read across from disodium EDTA to the DTPA category of substances for the inhalation endpoint. Whilst we agree the irritation effects observed in the repeat dose inhalation study are minor, adaptive and reversible it is highly likely that at higher concentrations more severe effects would be observed in a greater number of animals. We, however, interpreted the kind of effects as relevant for STOT RE 2 classification and as a consortium have implemented self-classification of DTPA. We however welcome clarity from RAC concerning whether assignment of STOT RE2 is relevant for H5-DTPA.
RAC's response
Thank you. Noted.

Date	Country	Organisation	Type of Organisation	Comment number
13.07.2016	France		MemberState	3

Comment received
The same comments made by FR for DTAP-Na5 are considered appropriate for DTPA-H. FR supports the assessment of DTPA-H in the context of the category of amino carboxylic acid-based chelants on the basis of close structural similarity and especially of a similar toxicological mode of action through chelation of essential minerals. The ability of each compound within the category to capture essential minerals such as zinc or calcium from the organism and to create deficiencies is however highly dependent of the stability of each compound. In this regard, FR agrees that comparison of DTPA-H with what is mentioned as "empty" chelates (acid forms and sodium and potassium salts, which are weakly bound) is much more relevant compared to metal chelates. However, FR emphasizes that within the subcategory of "empty" chelates differences in the chelation

power are expected. In particular, as noted in the OECD SIAR (2012): "The chelant DTPA, possessing 8 potential electron-pair donor groups, forms the strongest chelant complexes. EDTA and PDTA, each with 6 potential electron-pair donor groups, and HEDTA, with 5 potential electron donor-groups, form comparably less strong complexes." Therefore, DTPA-H is expected to be one of the "empty" chelates that have the stronger potential to induce deficiencies in essential minerals in particular compared to EDTA empty compounds and more severe effects linked to essential minerals deficiencies are expected compared to EDTA empty compounds.

Dossier Submitter's Response

We welcome your support for use of read-across within the 'empty chelates' category and note your acceptance that H5-DTPA, like all chelating agents, exhibit effects via inducing a deficiency of essential minerals. In addition, it is indeed true that DTPA as higher stability constants than EDTA or HEDTA as also indicated in the table below.

Table: Stability constants (Log K values)

Metal ion	**EDTA**	**DTPA**	**HEDTA**
Mg 2+	8.8	9.3	7.0
Ca 2+	10.7	10.8	8.1
Mn 2+	13.9	15.2	11.1
Fe 2+	14.3	16.2	12.2
Zn 2+	16.5	18.2	14.6
Cu 2+	18.8	21.2	17.4
Fe 3+	25.1	28.0	19.7

Values are based on Martell AE, Smith RM, NIST Critically selected stability constants of metal complexes (NIST standard reference database 46, Version 7.0, 2003)

RAC's response

Thank you. Noted.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2016	Belgium		MemberState	4

Comment received

Based on the results of the BASF SE 1994 study, BE CA does not agree with the proposal to classify the substance in category Repr. 2. In this study, significant increase in rate of skeletal retardations (63.8% at 400 mg/kg bw/d vs 47.4% in controls) were already observed in fetuses at the mid dose group without any maternal effects only observed in the highest dose group (1000 mg/kg bw/d). Furthermore, the maternal toxicity effects (reduced body weight and food consumption) observed at 1000 mg/kg bw/d do not seem to be enough to explain the foetal toxicity at this highest dose.
Therefore, BE CA does not agree with the justification given in the CLH report for the occurrence of developmental toxicity which was considered by the dossier submitter as secondary to primary maternal zinc deficiency. Thus BE CA support a classification in Repr. 1B H360D.

Dossier Submitter's Response

As dossier submitter for H5-DTPA we have valuable information regarding the data used to support the classification as Reprodevelopmental toxicant Category 2.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON N-CARBOXYMETHYL IMINOBIS(ETHYLENENITRILO)TETRA(ACETIC ACID)

With regard to the dossier Na5-DTPA the consortium received a number of comments concerning the OECD 414 Prenatal developmental toxicity study (BASF 1994) in which a number of malformations, retardations and variations were apparent in the offspring of animals dosed at 1000 mg/kg bw/day or 400 mg/kg bw/day. In response to those comments, we submitted historical control data and we would ask that the data be taken into consideration for both H5-DTPA and K5-DTPA.

From the data, it is clear that at 1000 mg/kg bw/day (the limit dose for this study), statistically significant decreases in bodyweight gain (21%), and overall bodyweights (Day 17 and 20) as a result of reduced food consumption (approx. 7% during treatment period GD 6-15) were apparent (Tables 1 and 2). At this dose level, a statistically significant reduction in live fetuses/litter, mean fetal bodyweights and increased malformations, variations and retardations were observed (Tables 3 and 4).

At 400 mg/kg bw/day no overall effect on maternal parameters was observed (Tables 1 and 2). However an increase in skeletal retardations versus concurrent controls was apparent. Whilst this increase in retardations was statistically significant, the overall number of retardations was still well within historical control. Actually, when comparing the variations and retardations observed to historical control data, the only finding of significance at this dose level would be a slight increase in fetuses showing incomplete ossification of the skull (Table 4).

We have concluded based on data available on EDTA (see further response to comment 4), these developmental findings are secondary to zinc insufficiency as a result of DTPA chelating zinc both in the diet and the zinc available systemically in the dam, and at high levels, also secondary to induced maternal toxicity.

At 1000 mg/kg bw/day significant malformations were observed in conjunction with considerable maternal toxicity manifested as significantly reduced bodyweights, a significant reduction in body weight gain and reduced food consumption. As already mentioned, at 400 mg/kg bw/day retardations in ossification were observed in the absence of overt maternal toxicity.

It should be considered however when conducting a standard OECD 414 study, the number of investigations performed is very limited compared to an OECD 407 study (or OECD 408) in which the investigations are more extensive and detailed in the parent animals e.g. full histopathology, haematology, clinical chemistry and others. Indeed for pentasodium DTPA, a 28-day oral (drinking water) repeat dose study in rats was conducted and dose levels of approximately 420 mg/kg bw/day resulted in changes in clinical chemistry parameters. It is therefore likely in the developmental toxicity study at 400 mg/kg bw/day that maternal toxicity was present but was not detected because of the limited number of investigations performed.

It is well known that skeletal ossification is a zinc-dependent process, and is severely impacted in cases of zinc deficiency. It is also worth noting that as a finding, incomplete ossification of the skull is considered a retardation of low to moderate concern (Moore et al 2013; ECETOC 2002) i.e. minor variations to the norm that would not normally justify classification.

The most plausible explanation for the developmental findings is that at 400 mg/kg bw/day, the DTPA administered is chelating sufficient dietary zinc to induce a deficient state in the mother but no outward signs of maternal toxic effects as found at 1000 mg/kg bw. Under conditions of zinc deficiency, the dam maintains liver zinc levels via increased metallothionein expression at the expense of the circulating plasma concentration and a concomitant reduction in foetal zinc levels would occur. Such processes would help maintain sufficient internal zinc levels in the dam such that outward signs of toxicity would not be apparent.

Given the effects observed at 400 mg/kg bw/day were retardations of low concern, were secondary toxicities associated with primary zinc depletion, and were apparent only when following a dosing regimen that is non-representative of potential human exposure (bolus gavage versus continuous, dietary) we conclude that classification of Na5-DTPA as developmentally toxic, category 2 is considered most appropriate.

References

ECETOC. (2002). Guidance on Evaluation of Reproductive Toxicity Data. Monograph No. 31. European Centre for Toxicology and Ecotoxicology of Chemicals, Brussels

N.P. Moore et. al. (2013) Guidance on classification for reproductive toxicity under the globally harmonized system of classification and labelling of chemicals. *Crit. Rev. Toxicol.* 43(10): pp 850-891

Table 1: Maternal in-life findings

Findings	Control	DTPA-100	DTPA-400	DTPA-1000
Fd GD6-8	26.1±2.04	25.3±2.18	26.4±1.91	**22.7±2.06**
Fd GD8-10	26.0±1.94	25.7±2.46	26.1±1.85	**23.4±2.75**
BW GD17	352.6±21.32	349.5±25.55	350.5±27.63	**332.8±18.25**
BW GD20	405.6±26.64	404.6±28.35	402.8±37.95	**378.7±26.93**
BWG GD6-8	7.9±4.05	6.7±2.81	7.0±2.90	**3.6±5.33**
BWG GD15-17	22.4±4.11	22.0±5.06	20.5±6.54	**17.5±5.11**
BWG GD6-15	43.7±8.01	44.5±6.25	43.6±8.75	**34.6±10.23**
BWG GD15-20	75.4±9.88	77.1±12.04	72.8±17.61	**63.5±13.57**
BWG GD0-20	148.0±16.88	150.3±19.09	141.4±26.70	**125.2±19.41**

Fd – Food consumption (g), BW – Body weight (g), BWG – Body weight gain (g), GD – Gestation days

Table 2: Maternal necropsy findings

Findings	Control	DTPA-100	DTPA-400	DTPA-1000
Uterus wt (g)	80.8±10.75	80.1±13.95	76.9±22.87	**64.2±20.01**
Carcass wt (g)	324.8±19.20	324.6±24.55	325.9±23.11	314.5±13.83
Adjusted wt gain (g)	38.3±6.49	41.4±9.95	39.6±10.00	33.9±9.67

Table 3: Litter findings

Findings	Control	DTPA-100	DTPA-400	DTPA-1000
Live foetuses (no.)	14.3±1.96	14.0±2.54	13.5±4.19	**11.9±3.78**
Foetal wt (all) (g)	3.7±0.21	3.7±0.23	3.7±0.26	**3.4±0.29**
Foetal wt (♂) (g)	3.8±0.21	3.8±0.25	3.8±0.24	**3.5±0.30**
Foetal wt (♀) (g)	3.6±0.22	3.7±0.25	3.6±0.29	**3.4±0.28**

Table 4: Skeletal examination

Values for each endpoint are number of affected litters per group and percentage of affected foetuses per litter (ranges in brackets)

Findings	Control	DTPA-100	DTPA-400	DTPA-1000	Historical Control
No. Litters	23	22	22	22	819
Malformations					
Total	7	3	8	16	191
	6.7±14.03%	1.9±4.82%	4.7±6.58%	**27.7±31.15%**	23.3%
Thoracic vertebra absent	0	0	0	**6**	5
	0.0±0.00%	0.0±0.00%	0.0±0.00%	**12.8±29.39%**	0.6% (0.0-9.1%)
Lumbar vertebra absent	0	0	0	**5**	2
	0.0±0.00%	0.0±0.00%	0.0±0.00%	**5.9±15.71%**	0.2% (0.0-4.0%
Sternebra(e) bipartite, ossification centres dislocated	1	0	2	**6**	37
	0.5±2.61%	0.0±0.00%	1.2±4.06%	**5.4±10.11%**	4.5% (0.0-13.6%)
Variations					
Total	22	21	21	21	763
	49.6±26.19%	48.6±20.09%	46.7±23.60%	**78.4±26.74%**	93.2%
Shortened 13th rib	11	10	10	**18**	286
	13.6±18.37%	12.3±18.91%	13.0±18.25%	**47.5±32.46%**	34.9% (13.6-57.1%)
Rudimentary cervical rib(s)	2	5	3	**11**	119
	2.7±10.63%	2.9±5.42%	1.8±4.61%	**21.3±31.20%**	14.5% (0.0-33.3%)
Absent 13th rib	0	0	0	**12**	4
	0.0±0.00%	0.0±0.00%	0.0±0.00%	**21.8±30.57%**	0.5 (0.0-4.8%)
Retardations					
Total	22	22	20	21	732
	47.4±24.75%	48.4±26.66%	**63.8±33.50%**	**78.0±31.29%**	89.4%
Skull incompletely ossified	1	2	**6**	**7**	14

	1.0±4.63%	1.2±3.95%	**4.6±8.65%**	**8.5±16.07%**	1.7% (0.0-8.3%)
Sternebra(e) not ossified	8	7	11	**18**	295
	4.8%	6.8%	**17.1%**	**50.7%**	36% (11.1-58.3%)

RAC's response

Thank you. The new information supplied by the DS will be included in the assessment and the Category (1B versus 2) will be discussed in RAC Plenary session.

Date	Country	Organisation	Type of Organisation	Comment number
15.07.2016	Germany		MemberState	5

Comment received

The German CA agrees with the proposed classification as Repr. 2; H361d (oral).
No reproductive/developmental toxicity study with DTPA-H5 is available.
The classification proposal for DTPA-H5 as 'Repr. 2; H361d' is based on read across data from DTPA-Na5 (CAS 140-01-2). It is pointed out that currently DTPA-Na5 has no harmonized classification (no existing entry in Annex VI, Table 3.1 to CLP). In 2015 industry has submitted a CLH dossier for DTPA-Na5.
In Section '4.11.4 Summary and discussion of reproductive toxicity', part 'Developmental toxicity' the dossier submitter has not explained that the read across data from DTPA-Na5 relates to DTPA-H5 for which the classification as Repr. 2; H361d is proposed.
Adverse developmental effects of DTPA-Na5 (purity 43.7 %) have been observed in an oral prenatal developmental toxicity study according to OECD TG 414 in Wistar rats.
Justification: (1) Signs of slight maternal toxicity were noted in the high-dose (1000 mg/kg bw/d), but not in the other treatment groups. They were limited to reduced food consumption between GD6-10 and body weight at GD17 and GD20, and reduced body weight gain during and after cessation of treatment and were associated with lower live litter sizes (11.9 vs 14.3 in control) and foetal (male and female) weights.
(2) Serious developmental defects were noted at 1000 mg/kg bw/d. There were significant increases in malformation rate (15.4 % affected foetus/litter vs 3.5 % affected foetus/litter in controls), predominantly caused by increase in skeletal malformations (missing thoracic and lumbar vertebrae and bipartite sternebrae), and in addition variations of the skeleton (shortened or absence 13th rib and rudimentary cervical ribs) (78.4 % affected foetuses/litter vs 49.6 % affected foetuses/litter in controls), and selected retardations (incomplete ossification of the skull and sternebrae) (78 % affected foetuses/litter vs 47.4 % affected foetuses/litter in controls). An increase in the rate of foetuses with skeletal retardations was also found in the mid-dose group at 400 mg/kg bw/d (63.8 % affected foetuses/litter vs 47.4 % affected foetuses/litter in controls). It can be concluded that developmental effects are not induced secondary to the non-specific maternal toxicity.
(3) DTPA-Na5 is a chelating agent with a strong affinity for zinc and copper. Zinc deficiency in maternal and also foetal organism cannot be ruled out as a possible cause of developmental toxicity. There was no information on the zinc status in either maternal or foetal organisms. Moreover no control group with zinc supplementary feeding was available. Therefore there is no evidence of the hypothesis that the occurrence of developmental toxicity is primary induced by altered zinc status in the mothers. It cannot be excluded that hitherto unknown mechanisms might be the cause of the observed developmental toxicity.
Finally, as the skeletal malformations were observed only at 1000 mg/kg bw/d, which is

the limit dose, the proposal on Category 2 can be supported.
Dossier Submitter's Response
See also our response at comment no. 4; we are happy to see that our proposal for Repr. cat. 2 H361d (oral)is supported.
RAC's response
Thank you. In the plenay session, RAC will discuss the final classification (Cat. 1B versus 2) considering the new information supplied by the DS in comment number 4.

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2016	Switzerland	Dow Europe GmbH	Company-Manufacturer	6
Comment received				
We support the dossier submitters proposal for Repr. 2 H361d specifically via the oral route and attach comments providing relevant additional data to support such a position. ECHA note – An attachment was submitted with the comment above. Refer to non-confidential attachment No. 01				
Dossier Submitter's Response				
See also our response at comment no. 4.				
RAC's response				
Thank you. Noted. The possibility of statement of oral route in the classification will be discussed during RAC Plenary session.				

Date	Country	Organisation	Type of Organisation	Comment number
08.07.2016	Sweden		MemberState	7
Comment received				
The Swedish CA do not agree with the rationale for classification of N-carboxymethyliminobis(ethylenenitrilo)tetra(acetic acid) (DTPA acid) (CAS No. 67-43-6) in Repr. 2 H361d (oral) as specified in the proposal. We do not support to specify the oral route since there is no data to conclusively exclude a reproductive toxic potential of DTPA acid via inhalation. We do not agree with the DS conclusion stating that only unrealistic exposure scenarios would lead to zinc deprivation and subsequent developmental toxicity in humans and that consequently classification in category 2 is warranted. As CLP is hazard-based, it does not take exposure into consideration in arriving at either a classification or appropriate labelling. Moreover, there is no possibility to assess the exposure potential for the substance in different uses and there is no clear mechanistic information that raises doubt about the relevance for humans. DTPA acid induces zinc deficiency in the mothers and as a consequence also in the fetus leading to developmental toxicity. Zinc deficiency in fetus and the developmental toxicity that follows cannot be considered as an unspecific effect of maternal toxicity. We propose that classification in Repr. 1B may be considered provided that some clarification of the data from the prenatal developmental toxicity study of DTPA (BASF SE, 1994) is made available. The briefly reported data makes it difficult for the reader to evaluate the studies thoroughly and thus, a higher level of detail is indeed desirable. Nevertheless, clear evidence of adverse effects on the development of the offspring was reported in the available prenatal developmental toxicity study in rat administered DTPA				

via oral gavage at the highest dose (1000 mg/kg bw/day): statistically significant induction of malformations (15.4% affected fetus/litter vs 3.5% affected fetus/litter in controls), and statistically significant decreased fetal viability (11.9 vs 14.3 in control group). The reported skeletal malformations are considered to be of high concern (missing thoracic and lumbar vertebrae) but data is lacking that clarifies the number of fetuses that were affected by one or more of these malformations and no historical control data is available. Moreover, it is unclear if the whole segment or unit is missing of the thoracic and lumbar vertebraes, and if the rib also is missing. Skeletal variations (in total 78.4% affected fetuses/litter vs 49.6% affected fetuses/litter in controls) manifested as absent 13th rib is also considered to be of high concern, however, also in this case data is lacking that clarifies the number of fetuses that had a missing 13th rib and no historical control data is available.

At high dose, only moderate maternal toxicity manifested as decreased corrected body weight gain (11.5%, not stat. sign.) was reported and reduced body weight were noted at GD 17 and GD 20 (but it is unclear to what extent and if statistical significant). 7% less food intake during the treatment period was also reported and dark yellow discolouration of the faeces in all females was observed.

The mid-dose (400 mg/kg bw/day) also caused a statistically significant increase in rate of fetuses with skeletal retardations (63.8% affected fetuses/litter vs 47.4% affected fetuses/litter in controls) but information is lacking on what types of skeletal retardations that were observed and the number of fetuses that were affected by one or more of the retardations. At this dose, no maternal toxicity and no effect on fetal weight was observed. Thus, this confirms that the developmental effects are not secondary to non-specific maternal toxicity (i.e. reductions in maternal weight gain).

To clarify and allow a transparent interpretation of the results of the developmental toxicity data, please provide the number of fetuses that were affected by one or more of the malformations, retardations and variations respectively, as well as the types and incidences of each individual malformation, skeletal variation and skeletal retardation, respectively, for each dose group and the control group. Please specify if the whole segment or unit is missing of the thoracic and lumbar vertebraes, and if the rib also is missing.

Regarding fertility, we question the conclusion that effects on male fertility (testicular toxicity) is a secondary non-specific consequence due to zinc deficiency caused by the chelating ability of DTPA acid. Ideally, such a statement should be supported with relevant data demonstrating that.

Dossier Submitter's Response

Please see the tables presented in response to comment no. 4 for clarification of observations noted at each dose level.
We are fully aware that CLP is based on intrinsic hazard. Please note the exposure considerations were presented to provide information concerning the likelihood that such developmental effects could occur in humans following use of DTPA in the workplace (or in consumers) as part of a weight of evidence assessment of the relevance of the effects.
With regards to selection of the oral route specifically, DTPA has a large particle size (far in excess of 10 microns in diameter) when in powdered form, and is not volatile when in solution and therefore significant exposure and subsequent absorption via inhalation is not foreseen. If inhalation would occur, particles would deposit in the upper respiratory tract with subsequent oral uptake. Though no data are available concerning dermal absorption for DTPA, data on EDTA indicate that a very small proportion is absorbed (0.001%) (and EDTA is a smaller molecule than DTPA), thus the potential for developmental effects following dermal exposure are considered negligible.
When analyzing the findings from the BASF study it is quite clear that the developmental effects occurring following administration of Na5-DTPA were associated with considerable maternal toxicity of a type indicative of zinc deficiency at 1000 mg/kg bw (see response to comment no. 4). At 400 mg/kg bw/day the only effect outside of historical control range is

the rate of incomplete ossification of the skull. Given this effect is a retardation i.e. the pattern of development is normal but delayed, it is unlikely to affect overall survival or health of the animal and as such it is considered an effect of low concern. In addition, these effects occurred following an exposure regimen that should not be considered relevant for humans (gavage versus dietary). We therefore maintain that the most appropriate category for classification is Category 2.
RAC's response
Thank you. In the plenay session, RAC will discuss the final classification (Cat. 1B versus 2) considering the new information supplied by the DS in comment number 4.

Date	Country	Organisation	Type of Organisation	Comment number
13.07.2016	France		MemberState	8
Comment received				

Fertility
Effects on sperm are observed with high doses of some metal chelates within the category whereas metal chelatants are expected to have less sequestration ability of zinc and other essential minerals than DTPA-H. On "empty" chelates, data presented in the CLH report are restricted to repeated toxicity studies of 28 days by oral route and the short duration of exposure is clearly inappropriate to be able to detect effects on spermatogenesis or male reproductive organs. No relevant fertility data is presented for members of the category with a higher chelating power such as DTPA-H whereas the effects observed on metal chelates and the mode of action expected to be more effective with "empty" chelates clearly raises a concern for fertility effects. Moreover, additional data showing effects in particular on sperm parameters are reported in the OECD SIAR (2012) with "empty" chelates such as Na2EDTA (Yang 1952) and PDTAH4 (Carney 2000). All this information needs to be considered in relation to a potential fertility classification in category 2. In any case, absence of classification could only be justified by the absence of relevant data.

Development
The study BASF 1994 is performed on DTPA-Na5 by oral route and is also considered a key study for assessment of developmental toxicity of the substance DTPA-H. The following effects were observed at the dose of 1000 mg/kg: resorptions (post-implantation loss), reduction of 8% in the fetal body weight and significantly increased incidence of skeletal malformations and variations. At this dose, maternal toxicity was minimal with a non-significant decrease in adjusted maternal body weight gain of 11.5% and the net reduction of maternal adjusted body weight is most probably negligible. Therefore the developmental effects cannot be explained by the minimal maternal toxicity observed. Besides, the induction of resorptions is supported by results obtained with Zn- and Ca-DTPA salts by subcutaneous route as well as at high doses of EDTA-Na2H2 by oral route. This effect is observed at different levels of doses, which is consistent with the relative strength of chelation expected from the various compounds. Skeletal malformations are observed with DTPA-Na5 only whereas gross malformations are observed with other compounds, which is consistent with the understanding of the mode of action in particular by sequestration of Ca ions and reduced capacity of Zn- and Ca-DTPA to chelate Ca. For skeletal effects, it is although noted that skeletal retardations are observed at the mid-dose of 400 mg/kg without any maternal toxicity, which confirm that these effects are not secondary to maternal toxicity and that chelation of essential minerals and in particular Ca is observed at lower doses with DTPA-Na5 compared to other tested compounds. The clear developmental effects observed at the high dose in the study BASF 1994 are therefore considered by FR to justify a classification Repr 1B for developmental toxicity.

Regarding the mode of action (MoA), FR agrees that capture and elimination of essential

minerals from the maternal body is the likely mode of action and FR would like to emphasize that although this has been substantiated by a study on EDTA-Na2H2 with Zn supplementation, the mode of action of DTPA-Na5 developmental toxicity can involve deficiencies in other essential minerals such as Ca as well as Mn, Fe and other metals with high affinity for the DTPA. This understanding of the MoA supports the observation of developmental effects and the fact that they are not a secondary non-specific consequence of maternal toxicity but secondary of a very specific property of the substance to induce a maternal loss of essential minerals. It is very likely that the needs of developing embryos in essential minerals is much more critical that for the dams and it is not unexpected that critical effects for the embryos are observed at doses that do not induce critical deficiencies and toxicity in the dams.

Considering the relevance for humans of this MoA, FR notes that many arguments relates to assessment of human exposure and risks and are not relevant for classification considerations. It is acknowledged that mechanisms of compensation of Zn in case of decreases in nutritional intake exists. However, there is no information showing that specific mechanisms occur in humans that are not present in rodents and experimental results therefore support that compensation mechanisms can be insufficient in case of prolonged essential minerals depletion and increased needs of developing organisms. In addition, dietary deficiencies in Zn as well as in other essential minerals can be expected in humans depending on specific diet with low intake or conditions that can be associated with malabsorption (diabetes, celiac disease). In conclusion, FR considers that the likely mode of action is relevant for human.

Overall, FR considers that available data therefore support a classification Repr 1B for developmental toxicity.
Regarding route of exposure, it is expected that effects by oral route result from both retention of essential minerals from the diet in the gastro-intestinal tract (reduced systemic intake) as well as sequestration and elimination of essential minerals from the systemic circulation secondary to presence of the (low) fraction of absorbed DTPA (increased systemic elimination). The role of increased systemic elimination is supported by positive effects observed by subcutaneous route and the developmental effects of DTPA-H can therefore not be considered as specific to the oral route (also noting that absorption by inhalation is significant), although effects are expected to be more pronounced by oral route.

Dossier Submitter's Response

Fertility: We note the comments concerning fertility. Indeed as we summarized in the CLH report, it is highly likely that should studies be conducted with H5-DTPA, the DTPA would complex with enough of the zinc in the diet leading to an insufficient zinc intake in the animals. This would lead to evidence of male reproductive toxicity (specifically degeneration of the testicular tissue and reduced fertility), developmental toxicity such as terata of the skeleton and viscera and many of the symptoms of zinc deficiency such as alopecia, diarrhea, eye and skin lesions etc. Such a study would therefore not provide evidence of the reproductive or developmental toxicity of DTPA but rather the toxicity associated with a deficiency in zinc. Conversely, if we conducted studies with zinc supplementation, it is unlikely that effects on fertility or development would be observed.
Developmental toxicity: As mentioned in response to previous comments the most plausible explanation for the developmental findings is that at 400 mg/kg bw/day, the DTPA administered is chelating sufficient dietary zinc to induce a deficient state in the mother. Under conditions of zinc deficiency, the dam maintains liver zinc levels via increased metallothionein expression at the expense of the circulating plasma concentration and a concomitant reduction in foetal zinc levels would occur. Such processes would help maintain sufficient internal zinc levels in the dam such that outward signs of toxicity would not be apparent. As noted it is highly likely that the critical need of zinc is greater in the developing foetus than in dams which already have zinc stores sufficient to maintain normal physiological function for a limited period of time.
Considering relevance for humans, the method of dosing employed in the study is not reflective of human exposure where bolus administration would be unlikely. The exposure considerations presented indicate that significant DTPA intake would

have to occur on a daily basis to have any effect on overall zinc status. Further, we agree that the mode of action is relevant for humans and as such forms the reasoning behind the proposal for Category 2 as most relevant.
RAC's response
Thank you. In the plenary session, RAC will discuss the final classification (Cat. 1B versus 2) considering the new information supplied by the DS in comment number 4.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2016	Belgium		MemberState	9
Comment received				
Based on the limited available data, BE CA can support the proposal to classify the substance as Acute toxicity category 4.				
Dossier Submitter's Response				
We welcome your support for Acute tox cat. 4 classification.				
RAC's response				
Thank you. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
15.07.2016	Germany		MemberState	10
Comment received				
The German CA agrees with the proposed classification as Acute Tox.4; H332. No acute inhalation toxicity study with DTPA-H5 is available. The classification proposal for DTPA-H5 as 'Acute Tox.4, H332' is based on read across data from Na2H2EDTA (CAS 139-33-3). It is pointed out that currently Na2H2EDTA has no harmonized classification (no existing entry in Annex VI, Table 3.1 to CLP). Justification: (1) In the CLH report (Chapter '4.2 Acute toxicity', Table 9, p16) data from a sub-acute (5-day) inhalation toxicity study in male Wistar rats according to OECD TG 412 performed with Na2H2EDTA (purity: 91.7 %; particle size of the test substance not specified, Annex 2, p73: 'respirable dust aerosol') were given. There is no doubt that the observed results from the sub-acute inhalation toxicity study in rats with Na2H2EDTA have shown clear acute toxic effects to fulfil CLP criteria for classification as 'Acute Tox.4, H332'. Lethality of 6/20 male rats exposed to 1000 mg/m3 as dust aerosol for 6 hours was noted after exposure for one day. (2) In Section '4.2.3 Summary and discussion of acute toxicity' (p17) a short summary on the acute inhalation toxicity data and conclusion on the relevance of the provided data is not specified for this CLH dossier on DTPA-H5. The dossier submitter has not explained that the read across data from Na2H2EDTA relates to DTPA-H5 for which the classification as Acute Tox.4, H332 is proposed. Missing is also the hint on the justifications for the use of information from Na2H2EDTA in Annex 2, p66ff. Furthermore an adjustment of the 6-hour LC50 value to a 4-hour equivalent using Haber's law is not presented. (3) In Section '4.2.4 Comparison with criteria' (p17) the comprehensive comparison of the relevant available information in order to derive the classification proposed for DTPA-H5 is missing. Data from the sub-acute inhalation toxicity with Na2H2EDTA showed the LC30 value of 1000 mg/m3/6h. The adjusted 4-hour equivalent LC30 value (LC30=1000 mg/m3/6h→1.144 mg/L/4h) should be compared with the criteria for classification as specified for acute inhalation toxicity in Annex I to CLP, for each hazard class. Then a conclusion could be drawn that the substance meets the criteria for classification in acute toxicity hazard categories (e.g. dusts and mists of category 4: 1.0<ATE≤5.0 mg/L/4h). Although the exact LC50 value for DTPA-H5 was not estimated, it is likely that the LC50				

value would be lower than 5 mg/L/4h and warrants classification.
Dossier Submitter's Response
We welcome your support for Acute tox category 4 classification and agree the data would indicate the LC50 would be lower than 5mg/L/4h for H5-DTPA. Data from disodium EDTA is considered appropriate for read across to the DTPA category since the mode of action for these effects is considered to be related to the chelation of calcium and both these agents have a similar affinity for calcium. The sites of irritation observed following inhalation exposure are consistent with the areas where a high degree of test material impaction would occur (however, please note that for the inhalation studies the particle size of test material (disodium EDTA) was reduced to comply to the general requirement of a particle size distribution with an MMAD between 1-3 microns and a gsd between 1.5 and 3). Upon impaction, the disodium EDTA complexes with calcium and perhaps zinc, removing it from cell junctions and membranes. The removal of these metals from intercellular junctions causes cells to detach from one another resulting in cell shedding. This leads to tissue regeneration and metaplasia in the affected areas. As cells which have become detached die, inflammation occurs leading to signs of necrosis and infiltration of inflammatory cells. This effect is similar to that observed in the intestines of rats given high oral bolus doses of chelating agents such as EDTA and DTPA. Given the effects of the substances are similar in nature, involve the same mode of action, are concentration dependent, threshold mediated and do not involve metabolic processes, it is therefore considered relevant to read across from Na2H2EDTA to the DTPA category of substances for the inhalation endpoint.
RAC's response
Thank you. Noted.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2016	Belgium		MemberState	11
Comment received				
BE CA agrees to classify the substance as Eye Irrit. 2 despite the divergent conclusions in the studies and the relatively low reliabilities.				
Dossier Submitter's Response				
We welcome your support for Eye Irrit. 2 classification.				
RAC's response				
Thank you. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
15.07.2016	Germany		MemberState	12
Comment received				
The German CA agrees with the proposed classification as Eye Irrit.2; H319. The classification proposal for DTPA-H5 as 'Eye Irrit.2; H319' is based on read across data from DTPA-K5 (CAS 7216-95-7). It is pointed out that currently DTPA-K5 has no harmonized classification (no existing entry in Annex VI, Table 3.1 to CLP). Justification: (1) In the CLH report, Chapter '4.4.2.1 Non-human information', Table 10 (p18ff) for DTPA-H5 only data from an incompletely reported study (not assignable, 4) is available in which an eye irritating potential was reported in rabbits. Furthermore read across data from structural analogue are presented: Two experimental studies on eye irritation with DTPA-K5 according to EU B.5/OECD TG 405, and 8 studies with DTPA-Na5 (140-01-2). As shown in Table 10, DTPA-K5, when applied to the eye of three rabbits, produces cornea score of 1 of max. 4 calculated as the mean scores following grading at 24, 48 and 72 hours after instillation. The finding in the cornea was fully reversible within 7 days in 2 animals and within 14 days in the third. A second study using a 40 % solution of DTPA-K5 did not induce eye irritation. From studies in rabbits with the structural analogue DTPA-Na5 a lack of eye irritation potential was noted in 4 studies. In two further studies in				

rabbits performed as declared by the submitter 'in accordance of internal procedure' 'moderately' and 'highly' irritating results were observed. In two further incompletely reported studies two opposing results were noted in treated rabbits - 'not irritating' and 'highly irritating'. (2) In Section '4.4.2.4 Comparison with criteria' (p23) the comprehensive comparison of the relevant available information in order to derive the classification proposed for DTPA-H5 is missing. The available eye irritation study results in rabbits have shown that DTPA-K5 has the potential to induce reversible eye irritation. Corneal findings were noted 24, 48 and 72 hours post exposure (scores 1 of max. 4) which were fully reversible within 14 days. No effects were noted in iris and conjunctiva (erythema and swelling). Based on the available data (cornea effects, scores ≥1, reversible in the post exposure period), DTPA-K5 meets the criteria for classification and labelling as 'Irritating to eyes (Category 2)'.
Dossier Submitter's Response
We welcome your support for Eye Irrit. 2 classification.
RAC's response
Thank you. Noted.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2016	Belgium		MemberState	13
Comment received				
Based on the limited available data in the CLH report, it is difficult to conclude. The information provided in the registration dossier revealed that only 1/10 females showed a focal hyperplasia of the laryngeal epithelium at the base of the epiglottis and only 2/10 females developed slight granulocytic infiltrates at the base of the epiglottis of the larynx. The incidence is not severe nor significant and the study is a read across, however, the tested dose was low 15 mg/m³ thus a greater incidence can be expected at higher dose.				
Dossier Submitter's Response				
We agree that the changes observed in the repeat dose inhalation study with disodium EDTA were not severe, likely adaptive responses and most probably reversible upon cessation of exposure. The concentration at which such effects occurred was 15 mg/m3, this is slightly below the cut-off limits for STOT RE2 (0.02 <C<0.2 mg/L). At higher concentrations, more significant lesions would probably occur and in a greater proportion of animals. In view of the effects observed in the 5-day inhalation study at 300 mg/m3 (above the cut off value but exposure was only for 5 days, not 90 days) in our opinion the prudent approach would be to assign STOT RE2 in the absence of specific inhalation data at higher concentration levels. We however welcome clarity from RAC concerning assignment of STOT RE2. We again would like to note that we take hazard classification into account, as with regard to human exposure, no such small particles will be present, neither for EDTA nor for DTPA.				
RAC's response				
Thank you. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
15.07.2016	Germany		MemberState	14
Comment received				
In general the proposed classification for DTPA-H5 to the hazard class 'STOT RE 2; H373' cannot be supported. No repeated dose inhalation toxicity study with DTPA-H5 is available. The classification				

proposal for DTPA-H5 as STOT RE 2; H373 is based on read across data from Na2H2EDTA (CAS 139-33-3). It is pointed out that currently Na2H2EDTA has no harmonized classification (no existing entry in Annex VI, Table 3.1 to CLP).
In Section '4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation' the dossier submitter has not explained that the read across data from Na2H2EDTA relates to DTPA-H5 for which the classification as STOT RE 2; H373 is proposed.
The observations of local effects on the respiratory tract caused by Na2H2EDTA may be considered as a borderline case regarding classification for target organ toxicity arising from repeated inhalation exposure.
Reasons: Two studies are available to evaluate the potential toxicity of the structurally related compound Na2H2EDTA following repeated inhalation exposure: a sub-acute (5-day) inhalation toxicity study (according to OECD TG 412) and a sub-chronic (90-day) inhalation toxicity study (according to OECD TG 413) both in rats. (1) In the short-term study treatment-related significant toxic effects, of relevance to human health were seen at a concentration of 0.3 mg/L/6h/d approximately equal to the STOT RE 2 guidance values according to CLP (for very short study durations: 10 times of the default guidance values, inhalation (rat) dust/mist/fume: 0.2<C≤2.0 mg/L/6h/d). After a recovery period of 14 days, all findings had disappeared and may question the severity of the observed inflammatory/metaplastic/necrotic effects in the larynx and bronchiolar airways. (2) More weight is given to the sub-chronic (90-day) inhalation toxicity study, where no relevant toxic effects were noted at the highest tested concentration of 0.015 mg/L/6h/d. However, it should be noted that the highest tested concentration was below the guidance value for STOT RE 2 according to CLP (inhalation (rat) dust/mist/fume: 0.02<C≤0.2 mg/L/6h/d).

Dossier Submitter's Response

Please see the response to comment 13. Data from disodium EDTA is considered appropriate for read across to the DTPA category since the mode of action for these effects is considered to be related to the chelation of calcium and both these agents have a similar affinity for calcium. The sites of irritation observed following inhalation exposure are consistent with the areas where – in view of the generated particle size distribution - a high degree of test material impaction would occur. Upon impaction, the disodium EDTA complexes with calcium and perhaps zinc, removing it from cell junctions and membranes. The removal of these metals from intercellular junctions causes cells to detach from one another resulting in cell shedding. This leads to tissue regeneration and metaplasia in the affected areas. As cells which have become detached die, inflammation occurs leading to signs of necrosis and infiltration of inflammatory cells. This effect is similar to that observed in the intestines of rats given high oral bolus doses of chelating agents such as EDTA and DTPA. Given the effects of the substances are similar in nature, involve the same mode of action, are concentration dependent, threshold mediated and do not involve metabolic processes, it is therefore considered relevant to read across from disodium EDTA to the DTPA category of substances for the inhalation endpoint. Whilst we agree the irritation effects observed in the repeat dose inhalation study are minor, adaptive and reversible it is highly likely that at higher concentrations more severe effects would be observed in a greater number of animals. We, however, interpreted the kind of effects as relevant for STOT RE 2 classification and as a consortium have implemented self-classification of DTPA. We however welcome clarity from RAC concerning whether assignment of STOT RE2 is relevant for H5-DTPA.

RAC's response

Thank you. Noted.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON N-CARBOXYMETHYL IMINOBIS(ETHYLENENITRILO)TETRA(ACETIC ACID)

NON-CONFIDENTIAL ATTACHMENTS

1. *Dow comments DTPA acid and pentapotassium DTPA CLH dossiers.docx*. Submitted on 14/07/2016 by Dow Europe GmbH. [Please refer to comment No. 6]

CONFIDENTIAL ATTACHMENTS

1. *Confidential attachment to the public consultation of DTPA.pdf*. Submitted on 15/17/2016 by <confidential>. [Please refer to comment No. 2]