Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo) tetraacetate

EC Number: 205-391-3
CAS Number: 140-01-2

CLH-O-0000001412-86-156/F

Adopted
9 June 2017

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON PENTASODIUM (CARBOXYLATOMETHYL)IMINOBIS(ETHYLENENITRILO)TETRAACETATE

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: pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo)tetraacetate
EC number: 205-391-3
CAS number: 140-01-2
Dossier submitter: Industry (Dow Chemical Company Ltd)

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
01.04.2016	Germany		MemberState	1
Comment received				
Substance ID: • In IUCLID section 1.2 a purity of 100% is given. This purity is also claimed in the CLH report. However, it is stated that a purity of 100% is not realistic and that typical impurities as well as water (substance is highly hydroscopic) are present. The corresponding impurities should be given in section 1.2 of the IUCLID file and should be flagged confidential, even though this information is only representative for the lead registrant and not for all SIEF members. Please correct the purity of the substance and add the missing information regarding impurities. • In the CLH report the correct purity should be stated. In a confidential Annex to the CLH report for Na·DTPA information on the impurities of the substance should be given. • In the registration dossier of the lead registrant additional information regarding physico-chemical properties are available. These information should be added in the CLH report and ideally in the IUCLID file. Classification: To serve as stand-alone document, it is recommended that the CLH report contains available data on all studies (both negative and positive results) to allow their independent assessment for classification purposes. From our point of view more detailed study descriptions are necessary (quantitative data e.g. incidences, degree of severity) to get a clear picture regarding the proposed classifications for pentasodium DTPA. It is difficult to assess the presented data. We think that this aspect is especially important, as it is noted that the French MS described in their RMO analysis (2014) that data concerning the reproductive toxicity seem to be sufficient to support a classification Repr. 1B (H360D).				
Dossier Submitter's Response				
Subtance identification: We appreciate and agree with your comment regarding the reporting of the impurities of our substance. The substance ID has been updated				

accordingly with a confidentiality claim in section 1.2 of the IUCLID and in the CLH dossier.
The classification proposal in the submitted CLH dossier and the comments made in this document aim to adress the classification of the pure substance Na5DTPA. Impurities were not taken into account.

Classification: We welcome your suggestion to include greater detail concerning the reproductive and developmental toxicity studies performed with DTPA. Please see below tables detailing all findings in the study conducted by BASF (1994) and the incidence/severity of developmental findings and where relevant, historical control data from the same species/strain from studies conducted at the same laboratory. All statistically significant values are shown in bold.

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 e.g. 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 (in number and g)

Findings	Control	DTPA-100	DTPA-400	DTPA-1000
Live foetuses	14.3±1.96	14.0±2.54	13.5±4.19	**11.9±3.78**
Foetal wt (all)	3.7±0.21	3.7±0.23	3.7±0.26	**3.4±0.29**
Foetal wt (♂)	3.8±0.21	3.8±0.25	3.8±0.24	**3.5±0.30**
Foetal wt (♀)	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.

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%)

Ranges indicated in brackets

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.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON PENTASODIUM (CARBOXYLATOMETHYL)IMINOBIS(ETHYLENENITRILO)TETRAACETATE

Date	Country	Organisation	Type of Organisation	Comment number
01.04.2016	France		MemberState	2
Comment received				
FR supports the assessment of DTPA-Na5 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 with its counter-ion. In this regard, FR agrees that comparison of DTPA-Na5 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-Na5 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 Na5-DTPA, like all chelating agents, exhibit effects via inducing a deficiency of essential minerals.				
RAC's response				
Thank you. Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
04.04.2016	Belgium		MemberState	3
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				
Please see tables included in response to comment 1. The increase in rate of skeletal retardations observed at 400 mg/kg bw/day is within historical control (table 4). The only effect observed at this dose level that is outside 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 would not be considered relevant for humans (gavage versus dietary). At 1000 mg/kg bw/day (the limit dose for OECD 414)				

malformations, variations and retardations were observed in conjunction with considerable maternal toxicity. Therefore, taking these factors into account it is considered that classification as a Category 2 developmental toxicant is most appropriate for DTPA.
RAC's response
Thank you. The new information supplied by the DS in comment number 1, will discuss the final classification (Category 1B versus 2) in RAC Plenay

Date	Country	Organisation	Type of Organisation	Comment number
01.04.2016	Germany		MemberState	4
Comment received				
P32ff: proposed C&L: Repr. 2; H361d: Suspected of damaging the unborn child if ingested Consent to the proposed classification as Repr. 2; H361d (oral). Adverse developmental effects of pentasodium DTPA (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 and body weight gain during and after cessation of treatment and were associated with lower litter sizes and foetal (male and female) weights. Quantitative data are missing in the CLH report. (2) Serious developmental defects were noted at 1000 mg/kg bw/d. There were significant increases in skeletal malformations (missing thoracic and lumbar vertebrae and bipartite sternebrae), in addition variations of the skeleton (shortened or absence 13th rib and rudimentary cervical ribs), and selected retardations (incomplete ossification of the skull and sternebrae). An increase in the rate of foetuses with skeletal retardations was also found in the mid-dose group at 400 mg/kg bw/d. It can be concluded that developmental effects are not induced secondary to the non-specific maternal toxicity. (3) Pentasodium DTPA 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				
We welcome your support for our proposal for classification of DTPA as Category 2. For clarification, the fact that DTPA exerts developmental effects secondary to zinc insufficiency and subsequent maternal zinc depletion is supported by several pieces of evidence presented in the CLH report and summarized below: 1) DTPA is minimally absorbed via the gut, as evidenced by stained faeces in the OECD 414 study at 1000 mg/kg bw/day, meaning the primary insufficiency is caused by chelation of dietary zinc still present in the GI tract. 2) Studies in which Zn-DTPA was administered or zinc supplementation employed indicate no potential for developmental toxicity or that previously developmentally toxic doses are no longer sufficient to cause developmental effects (Fisher et al 1975, Brummett and Mays 1976, Calder et al 1978). 3) In studies in which Ca-DTPA was administered and for which zinc levels were measured, an increase in zinc excretion relative to controls was observed (Cantilena				

and Klassen, 1982; Cohen and Guilmette, 1976; Domingo et al., 1988, Planas-Bohneet al., 1975 and 1976; Tandon et al., 1984)

4) Pregnant animals receiving zinc restricted diets report findings of increased malformations, increased resorptions and decreased fetal bodyweight relative to controls (Kechrid et al 2006).

Further support for zinc mediated developmental effects comes from studies involving Ca-EDTA and Zn-EDTA. Following administration of Ca-EDTA, developmental toxicity, increased zinc excretion and significantly reduced hepatic and plasma zinc concentrations were observed. In the same study Zn-EDTA did not cause developmental effects and measured maternal levels of zinc were increased following treatment (Brownie et al 1986).

It is accepted that the mechanism of action of DTPA and EDTA is identical, (i.e. chelation of essential metal ions) and that the Zn chelates of both EDTA and DTPA are without teratogenic potential. Further, in the EDTA Risk Assessment Report (2004), it was concluded;

'Since it has been demonstrated that zinc deficient diets per se lead to developmental and teratogenic effects in offspring (Hurley and Swenerton, 1966; Hurley et al., 1971), the depletion of zinc in the diet and/or the depletion of endogenous zinc tissue concentrations caused by EDTA treatment appear to be of specific significance for embryo/fetal impairment and the induction of malformations. With sufficient zinc supplementation fetotoxic and teratogenic effects could be prevented or minimised.'

If unknown mechanisms were responsible for the observed developmental toxicity then simple zinc supplementation should not be sufficient to counteract these effects as is demonstrated in the evidence provided. It is therefore concluded that zinc chelation is the primary reason for the developmental toxicity observed.

In addition, the risk assessment report for EDTA concluded no recommendation for classification for developmental toxicity based on the relatively high oral dose levels (i.e. 1000 mg/kg bw/day and above) at which developmental effects were observed. We are proposing Category 2, as classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and in the context of the information provided this would seem most appropriate.

References

Brownie CF, Brownie C, Noden D, Krook L, Haluska M and Aronson AL (1986). Teratogenic effect of calcium
edetate (CaEDTA) in rats and the protective effect of zinc. Toxicol. Appl. Pharmacol. 82, 426-443.
Brummett and Mays, Health Physics, 33, 624-626, 1977.
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Cohen and Guilmette, Bioinorg Chem 5(3), 203-210, 1976
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Domingo et al., Veterinary and Human Toxicology, 30(3), 224-228, 1988b
Fisher et al., Health Physics, 29, 780-782, 1975.
Hurley et al., Teratology 4, 199-204, 1971
Kechrid. Z. et al. (2006) The effect of zinc deficiency on zinc status, carbohydrate metabolism and progesterone level in pregnant rats. Turk. J. Med. Sci. 36 (6): pp 337-342
Planas-Bohne and Olinger, Health Physics 31, 165-166, 1976
Swenerton and Hurley, Science, 173, 62-64, 1971
Tandon et al., Environmental Research 35(1), 237-245, 1984

RAC's response
Thank you. The new information supplied by the DS in comment number 1, will discuss the final classification (Category 1B versus 2) in RAC Plenay

Date	Country	Organisation	Type of Organisation	Comment number
01.04.2016	Sweden		MemberState	5

Comment received

The Swedish CA do not agree with the rationale for classification of DTPA-Na5 (CAS No. 140-01-2) 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-Na5 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 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-Na5 is made available. Clear evidence of adverse effects on the development of the offspring was reported in the available prenatal developmental toxicity study in rat administered DTPA-Na5 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 middose (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).

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Dossier Submitter's Response
Please see the tables presented in response to comment 1 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 1). 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. The new information supplied by the DS in comment number 1, will discuss the final classification (Category 1B versus 2) in RAC Plenay

Date	Country	Organisation	Type of Organisation	Comment number
01.04.2016	France		MemberState	6
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-Na5. 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-Na5 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 therefore the key study for assessment of developmental toxicity of the substance. 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 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-Na 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 Na5-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. The new information supplied by the DS in comment number 1, will discuss the final classification (Category 1B versus 2) in RAC Plenay

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
04.04.2016	Belgium		MemberState	7
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 category 4 classification.				
RAC's response				
Thank you. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.04.2016	Germany		MemberState	8
Comment received				
P20ff: proposed C&L: Acute Tox.4; H332: Harmful if inhaled Consent to the proposed classification as Acute Tox.4; H332. No acute inhalation toxicity study with pentasodium DTPA is available. The classification proposal for pentasodium DTPA 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 11, p20) data from a sub-acute (5-day) inhalation toxicity study in Wistar rats according to OECD TG 412				

performed with Na2H2EDTA (purity: 91.7 %; particle size of the test substance not specified) 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. 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' (p 21) 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 pentasodium DTPA. The dossier submitter has not explained that the read across data from Na2H2EDTA relates to pentasodium DTPA for which the classification as Acute Tox.4, H332 is proposed. Justifications for the use of information from Na2H2EDTA and its support to the proposed classification were not provided. 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' (p21) the comprehensive comparison of the relevant available information in order to derive the classification proposed for pentasodium DTPA 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 pentasodium DTPA 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 Na5-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 – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
04.04.2016	Belgium		MemberState	9
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
01.04.2016	Germany		MemberState	10
Comment received				
P24ff, proposed C&L: STOT RE 2; H373: May cause damage to respiratory tract following prolonged or repeated inhalation exposure In general the proposed classification for pentasodium DTPA to the hazard class 'STOT RE 2; H373' cannot be supported. No repeated dose inhalation toxicity study with pentasodium DTPA is available. The classification proposal for pentasodium DTPA 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). 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 9. 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 Na5-DTPA.

RAC's response

Thank you. Noted.