# Committee for Risk Assessment

# RAC

## 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

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9 June 2017

CLH-O-0000001412-86-156/F

# OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

**Chemical name:** **pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo)tetraacetate**

**EC Number:** **205-391-3**

**CAS Number:** **140-01-2**

The proposal was submitted by **Dow Chemical Company Ltd** and received by RAC on **5 February 2016.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

# PROCESS FOR ADOPTION OF THE OPINION

**Dow Chemical Company Ltd** has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **17 February 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **4 April 2016**.

## ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **9 June 2017** by **consensus**.

**Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)**

| | Index No | International Chemical Identification | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hazard Class and Category Code(s) | Hazard statement Code(s) | Pictogram, Signal Word Code(s) | Hazard statement Code(s) | Suppl. Hazard statement Code(s) | | |
| Current Annex VI entry | No current Annex VI entry | | | | | | | | | | |
| Dossier submitters proposal | xxx-xxx-xx-x | pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo)tetraacetate | 205-391-3 | 140-01-2 | Acute Tox. 4<br>Repr. 2<br>STOT RE 2 | H332<br>H361d<br>H373 (Inhalation) | GHS08<br>GHS07<br>Wng | H332<br>H361d<br>H373 | | | |
| RAC opinion | xxx-xxx-xx-x | pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo)tetraacetate | 205-391-3 | 140-01-2 | Repr. 1B<br>Acute Tox. 4<br>STOT RE 2 | H360D<br>H332<br>H373 (Inhalation) | GHS08<br>GHS07<br><br>Dgr | H360D<br>H332<br>H373 | | | |
| Resulting Annex VI entry if agreed by COM | xxx-xxx-xx-x | pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo)tetraacetate | 205-391-3 | 140-01-2 | Repr. 1B<br>Acute Tox. 4<br>STOT RE 2 | H360D<br>H332<br>H373 (Inhalation) | GHS08<br>GHS07<br><br>Dgr | H360D<br>H332<br>H373 | | | |

# GROUNDS FOR ADOPTION OF THE OPINION

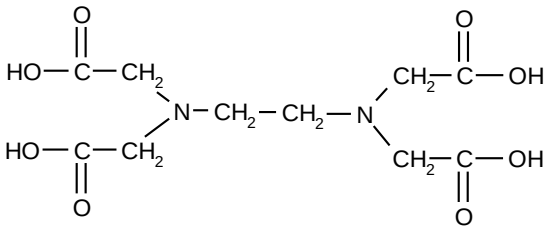
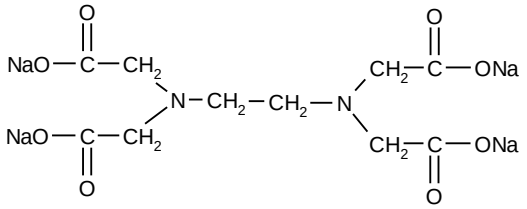
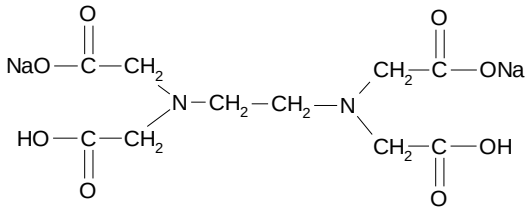
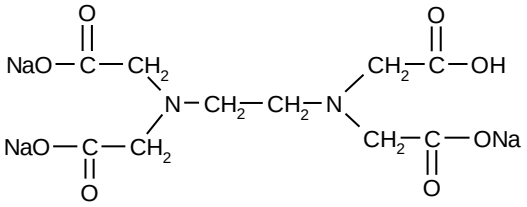
## RAC general comment

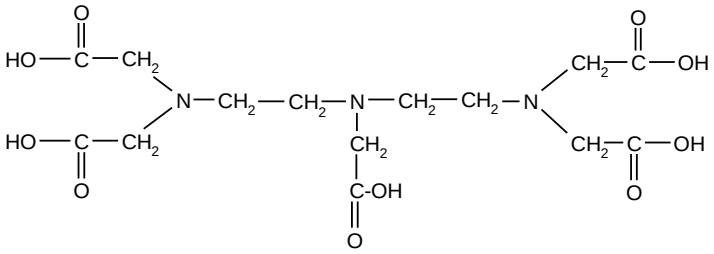
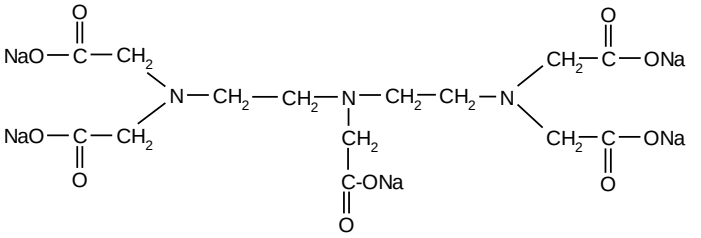
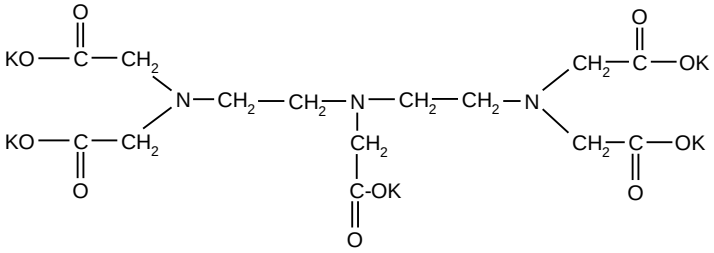
There is no current classification in Annex VI of Regulation (EC) No 1272/2008 for pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo)tetraacetate (DTPA-Na5). DTPA-Na5 is used in a wide number of industries including pulp and paper industries, laundry detergents, cleaners, soaps, and textiles. Read across from other un-complexed or "empty" DTPA chelates is considered acceptable for setting the classification of DTPA-Na5.

### *Justification for applying read-across within the aminocarboxylic acid-based chelants*

The acid ($H^+$) or salt ($Na^+$, $K^+$, $NH_4^+$) chelates are considered un-complexed chelates and are different from metal chelates, which are bound to a metal ion. The un-complexed chelates are called 'empty' chelates. The 'empty' chelates considered for this read-across analysis are shown in the Table below.

**Un-complexed chelates considered for the read-across in support of the classification of DTPA-Na5.**

| CAS number | EC number | Abbreviation | Molecular structure |
|---|---|---|---|
| 60-00-4 | 200-44-9 | EDTA-H4 |  |
| 64-02-8 | 200-573-9 | EDTA-Na4 |  |
| 139-33-3 | 205-358-3 | EDTA-Na2H2 |  |
| 150-38-9 | 205-758-8 | EDTA-Na3H |  |

| | | | |
|---|---|---|---|
| 67-43-6 | 200-652-8 | DTPA-H5 |  |
| 140-01-2 | 205-391-3 | DTPA-Na5 |  |
| 7216-95-7 | 404-290-3 | DTPA-K5 |  |

Members of this aminocarboxylic acid-based chelant category possess similar molecular structures that contain common functional groups (Table above). All members have a molecular structure with an ethylenediamine or a diethylenetriamine backbone, which has 4 or 5 acetic acid groups attached to the nitrogens. Specifically, the diethylenetriamine (DTPA) structures contain five acetic acid groups, while the ethylenediamine (EDTA) structures have four acetic acid groups (Table above). Therefore all category members have identical functional groups. It is the presence of multiple carboxylic acid groups on the amine that provide these chelants with their metal ion chelating or sequestering properties.

Because of this metal ion chelating property, metal EDTA and DTPA chelates have also been used for comparison with DTPA-Na5. Specifically the following metal ions $Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Cu^{2+}$, and/or $Fe^{3+}$, and $H^{+}$, $Na^{+}$, $K^{+}$, or $NH_4^{+}$ have been also considered as counter ions (Table below).

| **Metal chelates considered in the read-across in support of the classification of DTPA-Na5.** | | |
|---|---|---|
| **CAS number** | **EC number** | **Abbreviation** |
| 62-33-9 | 200-529-9 | EDTA-CaNa2 |
| 14025-15-1 | 237-864-5 | EDTA-CuNa2 |
| 74181-84-3 | 277-749-7 | EDTA-CuK2 |
| 67989-88-2 | 268-018-3 | EDTA-Cu(NH4)2 |
| 15708-41-5 | 239-802-2 | EDTA-FeNa |
| 54959-35-2 | 259-411-0 | EDTA-FeK |
| 14402-88-1 | 238-372-3 | EDTA-MgNa2 |
| 15375-84-5 | 239-407-5 | EDTA-MnNa2 |
| 68015-77-0 | 268-144-9 | EDTA-MnK2 |
| 94233-07-5 | 304-037-6 | EDTA-Mn(NH4)2 |
| 14025-21-9 | 237-865-0 | EDTA-ZnNa2 |
| 14689-29-3 | 238-729-3 | EDTA-ZnK2 |
| 67859-51-2 | 267-400-7 | EDTA-Zn(NH4)2 |
| 12389-75-2 | 235-627-0 | DTPA-FeHNa |
| 19529-38-5 | 243-136-8 | DTPA-FeNa2 |
| 85959-68-8 | 289-064-0 | DTPA-Fe(NH4)2 |

The table below summarises the available information for the hazards considered for classification of DTPA-Na5.

| **Toxicity studies considered for setting DTPA-Na5 classification.** | | |
|---|---|---|
| **Hazard** | **Source substance** | **DS proposal** |
| Acute inhalation toxicity | EDTA-Na2H2<br>DTPA-Na5 | Acute Tox. 4 (H332) |
| STOT RE | EDTA-Na2H2<br>DTPA-Na5 | STOT RE 2 (H373) |
| Reproductive toxicity | DTPA-Na5<br>Zinc and calcium salts of DTPA<br>Zn salt of DTPA | Repr. 2 (H361d oral) |

Common mechanism of action for un-complexed and metal chelates

The capability of chelants to remove or add ions to solution is the mechanism whereby these chemicals produce toxicity. Subtle differences in the toxicity of these chemicals are due to differences in affinity towards different metals. This determines whether the chelant alters the ion balance of the organism either by releasing ions through dissociation from the EDTA or DTPA salts or removing such ions through chelation. The table below summarises the affinity of EDTA and DTPA backbones to different ions.

| **Constants of affinity of EDTA and DTPA chelants for different ions** (Log K values) | | |
|---|---|---|
| **Ion** | **EDTA** | **DTPA** |
| $Mg^{2+}$ | 8.8 | 9.3 |
| $Ca^{2+}$ | 10.7 | 10.8 |
| $Mn^{2+}$ | 13.9 | 15.2 |
| $Fe^{2+}$ | 14.3 | 16.2 |
| $Zn^{2+}$ | 16.5 | 18.2 |
| $Cu^{2+}$ | 18.8 | 21.2 |
| $Fe^{3+}$ | 25.1 | 28.0 |

The highest affinity is for $Fe^{3+}$, while the lowest is for $Mg^{2+}$. It is also noted that although the order of affinities is the same between EDTA and DTPA, the affinity of DTPA is always higher than the affinity of EDTA for a specific ion. This can be explained by considering that the affinities increase with the number of functional groups (four for EDTA and five for DTPA; see Table " Un-complexed chelates considered for the read-across in support of the classification of DTPA-Na5", above).

Thus, it is expected that the main mechanism of toxicity of DTPA-Na5 would arise from chelation of metals present in the tissues with a specificity based on the data displayed in the table " Constants of affinity of EDTA and DTPA chelants for different ions", above.

Physico-chemical properties of un-complexed and metal chelates

The chemicals considered in Table 1 are produced as solid granular materials with molecular weights that range between 292 and 584 and possess similar physical/chemical properties. All compounds considered in this read-across assessment are very soluble in water and insoluble in organic solvents. The relative densities are around 1.3-1.8, and particle size distributions show that they comprise relatively large particles, *viz.* generally 10-50% are smaller than 100 microns (inhalable).

Toxicokinetics

Aerosolised DTPA and its salts are absorbed from the respiratory tract into the systemic circulation but the degree of absorption is dependent on the site of deposition. Dermal application

of radiolabelled EDTA-CaNa2 to human skin showed that 0.001% was found in the urine and none was found in the blood. Studies with EDTA-CaNa2, EDTA-Na2H2 and DTPA and its salts indicate that these complexes are poorly absorbed in mammals after oral administration. Most of the EDTA and its salts are eliminated from the body, 95% via the kidneys and 5% by the bile, along with the chelated metal.

Toxicological properties

In support of the read across proposal applied here, the acute toxicity, skin and eye irritation and skin sensitisation potential of un-complexed and metal chelates are compared and summarised in the BD, generally supporting similar toxicities for a range of relevant chelating compounds.

For genotoxicity, overall the findings indicate that EDTA-FeNa, DTPA-FeHNa and EDTA-CuNa2 lack significant genotoxic potential under conditions that do not deplete essential trace elements required for normal cell function. An oral two-year study with the 'empty' chelate EDTA-Na3H in mice and rats and an oral 2-year study with the metal chelate EDTA-CaNa2 in rats indicated no evidence of carcinogenicity (See BD).

The main target organ in repeated-dose toxicity studies was kidney. At high concentrations, the toxicity observed has been mainly attributed to nutrient metal deficiencies, resulting from chelation of critical metal species, most notably calcium and zinc, especially by the 'empty' chelates (See BD). It is concluded that at high testing levels, reproductive toxicity (both fertility and developmental) is due to an induced deficiency in zinc. NOAEL/LOAEL intervals for empty and metal chelates in medium- and long-term studies and in reproductive and developmental studies are comparable and overlapping, which suggests similar potencies towards reproductive and repeated toxicity.

***Conclusion***

RAC notes:

- The similar molecular structure of the un-complexed chelates considered in this read-across analysis and shown in the first Table in this section "Un-complexed chelates considered for the read-across in support of the classification of DTPA-H5".
- The common mechanism of action for un-complexed chelates based on altering the homeostasis of metal ions in the tissue milieu through chelation. Nevertheless, RAC also notes that EDTA salts are expected to be less toxic than DTPA salts due to their lower affinity to metal ions.
- The lack of significant differences in the physico-chemical properties among un-complexed chelates.
- The similar toxicokinetics among un-complexed chelates.
- The low acute toxicity by oral and dermal routes of the un-complexed and metal chelates.
- The lack of skin irritation and sensitisation capability of un-complexed and metal chelates.
- The lack of genotoxic potential of un-complexed and metal chelates.
- That the potential local effects should be carefully considered in the read-across analysis as it is not possible to determine, based on the available information, which chelates may be of similar, higher or lower local toxicity than DTPA acid and DTPA salts. This is because, for example, the acidifying properties in aqueous media may be different due to different capabilities to release $H^+$ to the media.

Thus, **RAC agrees with the DS, in line with Annex XI.1.5 of REACH that data from un-complexed chelates displayed in the Table " Un-complexed chelates can be considered**

**for the read-across in support of the classification of DTPA-Na5", partially supported by data from metal chelates, for the assessment of human health hazards of DTPA-Na5.**

# HUMAN HEALTH HAZARD EVALUATION

## RAC evaluation of acute toxicity

### Summary of the Dossier Submitter's proposal

Only the acute inhalation toxicity was considered for classification by the DS. The DS proposed classification as acute toxicity category 4; H332 (harmful if inhaled) on the basis of a well conducted study performed in accordance with GLP showing that 6 hours of exposure to 1000 mg EDTA-Na2H2/$m^3$ induced lethality in 6 of the 20 exposed animals.

### Comments received during public consultation

Two MSCA supported the DS's proposal of classification, although one of them highlighted some lack if justification for supporting such proposal.

### Assessment and comparison with the classification criteria

The table below summarises the acute inhalation toxicity studies.

| **Summary of acute inhalation toxicity studies for classification of DTPA-Na5.** | | | |
|---|---|---|---|
| **Method** | **Results** | **Remarks** | **Reference** |
| Rat (male only, Wistar)<br><br>Nose only<br><br>Dust aerosol<br><br>30, 300 and 1,000 mg/$m^3$<br><br>10/group: 30 or 300 mg/$m^3$<br><br>20/group: control or 1000mg/$m^3$<br>(exposed for 1 day only)<br><br>Exposure: 6 hours per day during 5 days<br><br>OECD TG 412 (Repeated dose inhalation toxicity 28/14 day) | Animals receiving 1000 mg/$m^3$ were exposed for 1 day only.<br><br>Exposure resulted in lethality of 6/20 male animals exposed to 1000 mg/$m^3$<br><br>Histological examination of the lung revealed congestion, oedema, multifocal haemorrhages and inflammatory infiltration | Study in accordance with GLP<br><br>Test material: EDTA-Na2H2<br><br>Purity 91.7% | BASF SE, 2010 |
| Rat (strain not given)<br><br>Vapour<br><br>12/sex/dose<br><br>Exposure: 8 hours per day for 5 days<br><br>Concentration not given | No mortalities observed | Test material: DTPA-Na5<br><br>Non-guideline<br><br>Non-GLP | BASF SE, 1968 |

***Comparison with the criteria***

In a study performed using DTPA-Na5, rats exposed for 8 h to the vapour generated at room temperature did not suffer any adverse effects. However, RAC notes that the concentration details were not provided and therefore this study cannot be considered for classification purposes.

A structurally-related compound, EDTA-Na2H2, caused 30% lethality after 6 h exposure to 1000 mg/m$^3$. The Guidance on Information Requirements and Chemical Safety Assessment (section R.7.4.4.1) establishes that a modification of the Haber's rule should be used for extrapolating the acute inhalation toxicity results obtained for exposure times different from the 4 h used in the standard acute toxicity studies. Specifically, the extrapolation should be performed using the following formula:

$$C^n.t = k$$

where a default value of n=3 should be considered for extrapolation to shorter periods of exposure (in this case from 6 to 4 hours). Therefore:

$$(1\ mg/L)^3 \times 6 = C^3 \times 4$$

and thus the extrapolation to 4 h of exposure of the data displayed in the table above yield an $LD_{30}$ of 1.14 mg EDTA-Na2H2/L. **RAC** notes that it is unlikely that the $LC_{50}$ for this substance would be higher than 5 mg/L, the threshold value for classification of dusts and mists and therefore RAC supports the DS's proposal for classification of DTPA-Na5 for **acute inhalation toxicity category 4 (H332: Harmful if inhaled).**

## RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

### Summary of the Dossier Submitter's proposal

The DS proposed classification of DTPA-Na5 as STOT RE category 2 (H373: May cause damage to organs) on the basis of slight histopathological effects in the larynx of rats exposed to 15 mg/m$^3$ via the inhalation route during 13 weeks (6 hours/day, 5 days/week).

### Comments received during public consultation

Two MSCAs disagreed with the DS's proposal and supported no classification of DTPA-Na5 for STOT RE on the basis of lack of severity and the reversibility of the effects and that these effects appeared always above the cut-off points established in the Regulation for warranting classification. The DS responded that the effects were consistent with chelation of calcium and possibly zinc ions in the cell junctions and membranes that would cause cellular detachment and death as a consequence of inflammation, necrosis and infiltration of inflammatory cells. Despite the effects having been reported as minor and reversible, it is highly likely that higher concentrations would cause more severe effects.

### Assessment and comparison with the classification criteria

The main relevant findings in the oral and inhalation repeated toxicity studies with un-complexed chelates are summarised in Tables 10 and 11.

| **Summary table of the animal repeated toxicity studies by oral route with un-complexed chelates.** | | | |
|---|---|---|---|
| **Method** | **Results** | **Remarks** | **Reference** |
| Rat (Sprague-Dawley)<br><br>5/sex/group<br><br>Oral: gavage<br><br>0, 83, 333, 1330 mg/kg bw/day<br><br>Exposure: daily, 28 days<br><br>OECD TG 407 | 1330 mg/kg bw/day: 4/5 males found dead at the end of the study; 1/5 females euthanized due to poor condition. Clinical signs: piloerection, hunched posture, abnormal gait, ptosis, decreased respiratory rate and diarrhoea. Significant bodyweight losses and reduced food consumption, changes in clinical chemistry parameters in both sexes, reduced absolute liver weight, watery contents in caecum of 3/4 surviving females, contracted spleen in 4 males and 1 female of rats that did not survive until end of study.<br><br>333 mg/kg bw/day: Clinical signs: hunched posture and abnormal gait in 1/5 males. Reduced bw gain and food intake in males. Slight changes in clinical chemistry and reduced liver weight in males only.<br><br>83 mg/kg bw/day: no substance related changes observed<br><br>Pallor seen in all dose groups but was considered related to blood sampling.<br><br>NOAEL: 83 mg/kg bw/day<br>LOAEL: 333 mg/kg bw/day | Reliability: 2<br><br>Test material: DTPA-K5. | Elliot *et al*., 1987 |
| Rat (Wistar)<br><br>5/sex/group<br><br>Oral: drinking water<br><br>600, 3000 or 12000 ppm<br><br>Exposure: Daily, 28 days<br><br>OECD TG 407 | 12000 ppm: (1775 mg/kg bw/day): discoloration of faeces, decreased food consumption and bodyweight, increased ALT in males only, increase in specific gravity, renal epithelial cells and casts and dark yellow coloured urine in males only, decrease in urine volume in both sexes, decrease ALP in females and transitional cell hyperplasia in the urinary bladder of 4 males and 2 females.<br><br>3000 ppm (420 mg/kg bw/day): significantly decreased bodyweight change in males in last test week (approx. 10% lower | Reliability: 1<br><br>Test material: DTPA-Na5 | BASF, 2002 |

| | | | |
|---|---|---|---|
| | than controls), increase in ALT in males and decrease in ALP in females<br><br>600 ppm (75 mg/kg bw/day): no substance related changes observed<br><br>NOAEL = 75/mg/kg bw/day<br>LOAEL = 420 mg/kg bw/day | | |

| **Summary table of the animal repeated toxicity studies by inhalation route with un-complexed chelates.** | | | |
|---|---|---|---|
| **Method** | **Results** | **Remarks** | **Reference** |
| Rat (male only, Wistar)<br><br>Inhalation: nose only, dust aerosol<br><br>30; 300 and 1,000 mg/m$^3$<br><br>10/group: 30 or 300 mg/m$^3$<br><br>20/group: control or 1000 mg/m$^3$<br><br>Exposure: 6 hours per day for 5 days<br><br>OECD TG 412<br><br>Purity 91.7% | 1000 mg/m$^3$: Mortality of 6/20 animals following single exposure. Multifocal haemorrhage and inflammatory cell infiltrates in lungs.<br><br>300 mg/m$^3$: Clinical signs: accelerated and noisy respiration, piloerection and reduced fur care. Decreased bodyweight gain, reduced food consumption on day 1. Increased lung weight. In the larynx, laryngeal, epithelial necrosis, multifocal in various levels. Inflammatory cell infiltrates and laryngeal squamous metaplasia, multifocal in various levels. In the lungs regenerative hyperplasia of bronchiolar epithelium and mucous cell hyperplasia of large bronchi. Interstitial infiltration of eosinophilic granulocytic cells.<br><br>30 mg/m$^3$: Increased lung weight. In the larynx laryngeal, epithelial necrosis, multifocal at the base of the epiglottis. Inflammatory cell infiltrates at the base of the epiglottis. In the lungs, regenerative hyperplasia of the bronchiolar epithelium and mucous cell hyperplasia of large bronchi. Interstitial infiltration of eosinophilic granulocytic cells.<br><br>No histopathological findings in recovery animals therefore all lesions in low/mid dose groups considered reversible.<br><br>LOAEC = 30 mg/m$^3$ | Reliability 1<br><br>Test material: EDTA-Na2H2 | BASF SE, 2010 |

| | | | |
|---|---|---|---|
| Rat (Wistar)<br><br>10/sex/group<br><br>Inhalation: nose only, dust<br><br>0.5, 3.0 and 15 mg/m$^3$<br><br>Exposure: 6 hours/day, 5 days/week for 13 weeks, total 65 exposures<br><br>OECD TG 413<br><br>Purity: 88.1% | <u>15 mg/m$^3$:</u> Increased absolute and relative lung weights. Focal hyperplasia of the laryngeal epithelium at the base of the epiglottis in one female. Slight granulocytic infiltrates at the base of the epiglottis of the larynx in two females.<br><br><u>3.0 mg/m$^3$:</u> Increased absolute lung weights in females only. No difference in relative lung weight.<br>No other compound related effects observed.<br><br><u>0.5 mg/m$^3$:</u> no substance related changes observed.<br><br>NOAEC = 3 mg/m$^3$<br>LOAEC = 15 mg/m$^3$ | Reliability: 1<br><br>Test material: EDTA-Na2H2 | BASF, 2014 |

***Comparison with the criteria***

The two available oral repeated toxicity studies with pentapotassium and pentasodium DTPA salts yielded LOAELs of 333 and 420 mg/kg bw/day, respectively after 28 daily exposures (Table "Summary table of the animal repeated toxicity studies by oral route with un-complexed chelates" above). The reported effects at these two LOAELs were slight reductions of bodyweight (around 10%), un-quantified reductions in liver weight, un-quantified changes in clinical chemistry and clinical signs in 1 of 20 exposed animals. According to the CLP Regulation, a substance should be classified as STOT RE 2 if can be presumed to have the potential to be harmful to human health following repeated exposure at concentrations below 100 mg/kg bw/day in the case of 90 days of exposure (300 mg/kg bw/day after correction with Haber's rule for 28 days of exposure). RAC notes that none of the effects reported after oral exposure at the reported LOAELs have the potential to cause serious damage to human health and in addition all appeared at concentrations above the guidance value in the CLP Regulation. RAC also considers that the effects, reported after oral exposure in studies and employed for justifying the read-across, do not support the classification for STOT RE (Table "Repeated dose toxicity, reproduction and developmental toxicity, and neurotoxicity of un-complexed and metal chelates" in the Section "RAC general comment", above).

The 13-week repeated dose toxicity study by the inhalation route reported increases in the absolute and relative lung weights. The incidence of this effect was not quantified and therefore RAC cannot consider it as significant for supporting STOT RE classification. However, this same study also reported focal hyperplasia of the laryngeal epithelium and slight granulocytic infiltrates at the base of the epiglottis in one and two females, respectively. These effects are considered by RAC potentially significant for supporting a STOT RE classification.

The 5-day repeated dose toxicity study by the inhalation route reported transient clinical signs and reductions in food consumption that were reversible and therefore not supportive of classification but also other adverse effects in lung (increases in weight, regenerative hyperplasia and interstitial infiltration of eosinophilic granulocytic cells) and larynx (epithelial multifocal necrosis). These effects in the lung and larynx were described in the CLH dossier as reversible. However, RAC considers that reversibility of at least necrosis is questionable and therefore considers these effects as relevant for classification as STOT RE.

RAC notes the following points:

- The local effects in the respiratory tract (impairments) after inhalation exposure appeared at concentrations relevant for classification.

- The classification proposal is based on weight of evidence rather than on considerations strictly relying on guidance values established in the CLP Regulation.

- The read-across of data from EDTA salts is justified because damage to the respiratory tract arises from a common mechanism of action based on the chelation of divalent ions in the cell junctions and membranes of respiratory tract that would cause cellular detachment and death as a consequence of inflammation, infiltration and necrosis.

- The local effects in the larynx and intestinal tract were not reported in the repeated dose oral toxicity studies.

- The severity and incidences of the effects reported in both repeated toxicity studies by inhalation were relatively low (Table " Summary table of the animal repeated toxicity studies by inhalation route with un-complexed chelates", above). However, both studies were performed with EDTA salts and according to the proposed mechanism of toxicity, a higher toxic potency is expected for DTPA salts.

- The percentage of inhalable material in both repeated toxicity studies displayed in the table "Summary table of the animal repeated toxicity studies by inhalation route with un-complexed chelates" (above) might be different; which adds uncertainty..

Taking into consideration all the factors stated above, RAC supports the DS's proposal for classification of DTPA-Na5 as STOT RE category 2, inhalation.

The DS proposed classification specifically by the inhalation route. Taking into consideration that the basis of the classification is the adverse effects on the respiratory tract, which could not be reproduced in the repeated dose toxicity studies conducted by the oral route and the extremely low bioavailability of these substance by the dermal route (dermal absorption of EDTA was reported to be 0.001%), RAC supports the DS's proposal for classifying DTPA-Na5 exclusively by the inhalation route.

Overall, RAC agrees to classify DTPA-Na5 as **STOT RE 2 (H373: May cause damage to organs through prolonged or repeated exposure via inhalation)**.

## RAC evaluation of reproductive toxicity

### Summary of the Dossier Submitter's proposal

The DS proposed classification of DTPA-Na5 as toxic to reproduction category 2 (H361d: Suspected of damaging fertility or the unborn child) by the oral route. This proposal was based on the hypothesis that the teratogenicity caused by DTPA-Na5 in a prenatal developmental toxicity study in rats (statistically significant increases in foetuses with malformations, retardations and skeletal variations, together with statistically significant reductions in the mean number of live foetuses/litter) appeared concurrently with maternal toxicity and as a result of an induced deficiency of zinc in the dams which subsequently impacted the foetus.

The DS proposed no classification of DTPA-Na5 for sexual function and fertility. No multi-generation reproductive toxicity studies were available. However, the DS postulated that specific degeneration of the testicular tissue and reduced fertility would have been expected due to parental zinc deficiency induced by chelation of this metal after DTPA-Na5 exposure.

## Comments received during public consultation

Two MSCAs supported the DS proposal for classification of DTPA-Na5 as Repr. Cat. 2; H361d by oral route and one MSCA also supported no classification for fertility.

Three MSCAs disagreed with the DS's proposal and supported a classification of toxicity to reproduction in category 1B, arguing that the skeletal malformations are of high concern and that the maternal toxicity effects do not seem to be sufficient to explain the foetal toxicity. The DS added more information for: i) a better clarification of the maternal toxicity; and, ii) a further assessment of teratogenicity (number of foetuses affected and historical control data for each of the impairments).

One MSCA also questioned restricting the classification to the oral route since there are no data to conclusively exclude a reproductive toxicity potential of DTPA-Na5 via the inhalation route. The DS replied that DTPA-Na5 has a large particle size (far in excess of 10 microns in diameter) when in powder form, and is not volatile when in solution and therefore significant exposure and subsequent absorption via inhalation is not foreseen.

## Assessment and comparison with the classification criteria

### *Sexual function and fertility*

No multi-generation reproductive toxicity studies were available.

### *Development*

Study 1 (key study): Developmental toxicity study in the rat (BASF SE, 1994)

The prenatal toxicity study was conducted under GLP principles. The test substance, DTPA-Na5 as administered to Wistar rats daily in water (by gavage) from day 6 to 15 post *coitum* at doses of 100, 400 and 1000 mg/kg bw/day. The number of pregnant females per dose group was 22. The study was considered to be of reliability 1 (Klimisch score).

The doses of 100 and 400 mg DTPA-Na5/kg bw/day caused no significant alterations in body weight, body weight gain or food consumption (Table "Maternal findings in the developmental toxicity study with DTPA-Na5", below). However, 1000 mg/kg bw/day caused statistically significant decreases in bodyweight gain (21% in the period between gestation days 6 and 15 and 16% over the 20 days of gestation) and overall bodyweights (approximately 7% by day 20 of gestation) as a result of reduced food consumption (approximately 10% between gestation days 6 and 10). Another effect was the dark yellow discolouration of the faeces found in all females. There were no substance-induced abnormal clinical findings in any dam exposed to 100 or 400 mg/kg bw/day.

Only at the limit dose (1000 mg DTPA-Na5/kg bw/day) a reduction of 21% the uterus weight was observed, although without any statistically significant effect on the corrected bodyweight gain (terminal body weight on day 20 before section minus weight of the uterus minus body weight on day 6) (Table " Maternal necropsy findings in the developmental toxicity study with DTPA-Na5", below). The uterus weights of the animals treated with the lowest and medium dose

were not altered (Table " Maternal necropsy findings in the developmental toxicity study with DTPA-Na5", below).

**Table 12: Maternal findings in the developmental toxicity study with DTPA-Na5 (BASF SE, 1994).** Fd-Food consumption (g), BW-Body weight (g), BWG-Body weight gain (g), GD-Gestation days. Bold indicates statistically significant differences regarding the control group.

| Findings | Control | DTPA-Na5 100 mg/kg bw/day | DTPA-Na5 400 mg/kg bw/day | DTPA-Na5 1000 mg/kg bw/day |
|---|---|---|---|---|
| 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** |

**Maternal necropsy findings in the developmental toxicity study with DTPA-Na5 (BASF SE, 1994).** Bold indicates statistically significant differences regarding the control group.

| Findings | Control | DTPA-Na5 100 mg/kg bw/day | DTPA-Na5 400 mg/kg bw/day | DTPA-Na5 1000 mg/kg bw/day |
|---|---|---|---|---|
| Uterus weight (g) | 80.8±10.75 | 80.1±13.95 | 76.9±22.87 | **64.2±20.01** |
| Carcass weight (g) | 324.8±19.20 | 324.6±24.55 | 325.9±23.11 | 314.5±13.83 |
| Adjusted weight gain (g) | 38.3±6.49 | 41.4±9.95 | 39.6±10.00 | 33.9±9.67 |

No treatment-related alterations were found during/ after the necropsies of any animal.

There were no substance-related differences between the groups in the conception rate (it varied between 92% in control group and 88% in all substance treated groups), in the mean number of corpora lutea and in implantation sites or post-implantation losses.

The highest exposure (1000 mg DTPA-Na5/kg bw/day) caused a statistically significant reduction in the number of live foetuses/dam (11.9±3.78 in exposed animals versus 14.3±1.96 in controls) and in the foetal weight (3.4±0.29 in exposed animals versus 3.7±0.21 in control animals) (Table "Litter findings in the developmental toxicity study with DTPA-Na5", below). The number of live foetuses/dam in the 1000 mg/kg bw/day group was still within the historical control range (11.1-14.9 live foetuses/dam).
The reduction of foetal body weight was similar in males and females. None of these parameters were significantly altered in the groups exposed to 100 and 400 mg/kg bw/day (Table "Litter findings in the developmental toxicity study with DTPA-Na5", below).

**Table 14: Litter findings in the developmental toxicity study with DTPA-Na5 (BASF SE, (1994)).** Bold indicates statistically significant differences relative to the control group.

| Findings | Control | DTPA-Na5 100 mg/kg bw/day | DTPA-Na5 400 mg/kg bw/day | DTPA-Na5 1000 mg/kg bw/day |
|---|---|---|---|---|
| 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** |

*External examinations of the foetuses*

The external examination of the foetuses revealed three malformations in total. One cleft palate and one unilateral microphthalmia were recorded in different foetus of the group exposed to the

low dose, while a unilateral anophthalmia was reported in one foetus exposed to 1000 mg/kg bw/day. One, three and four cases of placentae fused with ribs were reported in groups of foetuses exposed to 0, 400 and 1000 mg/kg bw/day, respectively. The external examination of the foetuses revealed no variations in any group.

*Soft tissue examinations of the foetuses*

The examination of the organs of the foetuses revealed two different malformations (dilatation of both ventricles and hyperplasia of the spleen) in one foetus exposed to 100 mg/kg bw/day and four different malformations (agenesis of kidney and ureters, hypoplasia of uterine horn and abnormal position of ovaries) in one foetus exposed to 1000 mg/kg bw/day. At the same time, variations (dilated renal pelvis and/or hydroureter) were detected in all groups without statistically significant differences among groups.

*Skeletal examinations*

The dose of 1000 mg/kg bw/day caused statistically significant increases in the total malformations relative to the controls (Table "Skeletal examinations in the developmental toxicity study with DTPA-Na5", below). The main reported malformations were thoracic vertebra absent, lumbar vertebra absent, sternebra bipartite and ossification centres dislocated. In all cases, the incidences were also higher than those recorded in the historical control data (both in terms of foetus and number of litters affected). The incidences of malformations reported in the groups exposed to both 100 and 400 mg/kg bw/day were not higher than the equivalent findings reported for the control and were always within the historical control data (Table "Skeletal examinations in the developmental toxicity study with DTPA-Na5", below).

A number of variations (mainly related to ribs) appeared statistically significantly more frequently at the highest dose level than in the concurrent controls or in the historical control data (Table "Skeletal examinations in the developmental toxicity study with DTPA-Na5", below). However, the incidence of variations observed in foetuses exposed to 100 and 400 mg/kg bw/day were not significantly different from the incidences found in the historical and concurrent controls (Table "Skeletal examinations in the developmental toxicity study with DTPA-Na5", below).

In all groups, signs of skeletal retardations such as incomplete or missing ossification of skull, vertebral column and sternebra were observed. These retardations followed a dose-response relationship with statistically significant differences with the concurrent control for foetuses exposed to 400 and 1000 mg/kg bw/day (Table "Skeletal examinations in the developmental toxicity study with DTPA-Na5", below). For these two groups of foetuses, the reported incidences for these malformations were found to also be above the historical control data.

**Table 15: Skeletal examinations in the developmental toxicity study with DTPA-Na5 (BASF SE, 1994).** In all cases it is shown in brackets the percentage with respect to the total. Bold indicates statistically significant differences relative to the control group.

| **Findings** | | **Control** | **DTPA-Na5 100 mg/kg bw/day** | **400 mg/kg bw/day** | **1000 mg/kg bw/day** | **Historical control data** |
|---|---|---|---|---|---|---|
| Total | Foetuses | 171 | 160 | 152 | 135 | 5776 |
| | Litters | 23 | 22 | 22 | 22 | 819 |
| **Malformations** | | | | | | |
| Total affected | Foetuses | 12 (7.0) | 3 (1.8) | 8 (5.3) | **38 (28)** | 243 (4.2) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Litters | 7 (30) | 3 (14) | 8(36) | **16(73)** | 191 (23.3) |
| Thoracic vertebra absent | Foetuses | 0 (0) | 0 (0) | 0 (0) | **15 (11)** | 5 (0.09) Range = 0-1.2% |
| | Litters | 0 (0) | 0 (0) | 0 (0) | **6 (27)** | 5 (0.6) Range = 0-9.1% |
| Lumbar vertebra absent | Foetuses | 0 (0) | 0 (0) | 0 (0) | **9 (6.7)** | 2 (0.03) Range = 0-0.6% |
| | Litters | 0 (0) | 0 (0) | 0 (0) | **5 (23)** | 2 (0.2) Range = 0-4% |
| Sternebra bipartite, ossification centres dislocated | Foetuses | 1 (0.5) | 0 (0) | 2 (1.3) | **7 (5.2)** | 37 (0.6) Range = 0-1.8% |
| | Litters | 1 (4) | 0 (0) | 2 (8) | **6 (27)** | 37 (4.5) Range = 0-13.6% |
| **Variations** | | | | | | |
| Total affected | Foetuses | 85 (50) | 79 (49) | 71 (47) | **105 (78)** | 2434 (42) |
| | Litters | 22 (95) | 21 (95) | 21 (95) | **21 (95)** | 763 (93) |
| Shortened 13th rib | Foetuses | 23 (14) | 19 (12) | 20 (13) | **65 (48)** | 470 (8) Range = 3-14% |
| | Litters | 11 (50) | 10 (48) | 10 (48) | **18 (86)** | 286 (35) Range = 14-57% |
| Rudimentary cervical rib(s) | Foetuses | 5 (3) | 5 (3) | 3 (2) | **28 (21)** | 152 (2.6) Range = 0-7% |
| | Litters | 2 (9) | 5 (24) | 3 (14) | **11 (52)** | 119 (15) Range = 0-33% |
| Absent 13th rib | Foetuses | 0 (0) | 0 (0) | 0 (0) | **30 (22)** | 4 (0.1) Range = 0-0.7% |
| | Litters | 0 (0) | 0 (0) | 0 (0) | **12 (57)** | 4 (0.5) Range = 0-5% |
| **Retardations** | | | | | | |
| Total affected | Foetuses | 80 (47) | 77 (48) | **97 (64)** | **105 (78)** | 2608 (45) |
| | Litters | 22 (96) | 22 (100) | **20 (91)** | **21 (95)** | 732 (89) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Skull incompletely ossified | Foetuses | 2 (1) | 2 (1) | **8 (5)** | **12 (9)** | 15 (0.3) Range = 0-1.3% |
| | Litters | 1 (4) | 2 (10) | **6 (30)** | **7 (32)** | 14 (2) Range = 0-8% |
| Sternebra not ossified | Foetuses | 9 (5) | 11 (7) | **26 (17)** | **69 (51)** | 443 (8) Range = 2-16% |
| | Litters | 8 (35) | 7 (32) | **11 (50)** | **18 (82)** | 295 (36) Range = 11-58% |

In conclusion, the maternal NOAEL for the developmental toxicity study in rat with DTPA-Na5 was set at 400 mg/kg bw/day; while the developmental NOAEL in the same study was 100 mg/kg bw/day.

Study 2 (supportive study): Prenatal developmental toxicity study with zinc and calcium DTPA salts in rats (Fukuda and Iida, 1983)

In this study, Fukuda and Iida (1983) used Wistar rats to clarify the safety of DTPA-Na3Ca and DTPA-Na3Zn as drugs for removing radionuclides. They injected (subcutaneously) daily on days 9-13 of gestation up to 536 and 600 mg/kg bw/day (equimolar doses) of DTPA-Na3Ca or DTPZ-Na3Zn, respectively. The number of injected pregnant females is unknown.

There were no clinical or pathological alterations in any dam injected with DTPA. However, DTPA-Na3Ca reduced the foetus survival rate from 90.3% found in the controls to 68%, while DTPA-Na3Zn caused no significant effects on this parameter. No cases of exencephaly, microphthalmia or anophthalmia were reported in controls or DTPA-Na3Zn exposed foetuses, but the incidences of these malformations in DTPA-Na3Ca-treated foetuses were 18 and 4%, respectively. Three percent of cases of exencephaly were also found in foetuses exposed to 357 mg DTP-Na3Ca/kg bw/day. The incidence of malformed foetuses and foetuses with first and second fused rib were 24% and 19%, respectively in foetuses exposed to DTPA-Na3Ca, while the incidences (for both malformations) in control and DTPA-Na3Zn-exposed foetuses were 12% and 13%, respectively.

In conclusion, the results of this paper suggest that calcium DTPA salts might be more potent teratogens than zinc DTPA salts.

Study 3 (supportive study): Prenatal developmental toxicity study with zinc and calcium DTPA salts in mice (Brummett and Mays, 1977)

In a study by Brummett and Mays, 1977, pregnant C57BL/Do mice were subcutaneously dosed with DTPA-Na3Zn daily either from gestation days 2-6 or 7-11. The doses of DTPA-Na3Zn used were either 0, 5720 or 11520 µmol/kg bw; these doses are equivalent to 0, 3163 and 6371 mg/kg bw/day, respectively. Due to the hypertonic nature of the test material an additional group of mice were treated with a solution of sodium chloride (1380 µmol NaCl/mL) at the same ion concentration, osmolality, pH and volume as the high dose DTPA-Na3Zn treatment. A DTPA-Na3Ca group (1440 µmol/kg bw/day = 715 mg/kg bw/day) dosed daily on days 7-11 was also included in this study. The pregnant mice were euthanized on day 18 of gestation and the foetuses removed and examined for gross malformations, visceral malformations and skeletal malformations.

Animals injected on days 2-6 with 5760 µmol DTPA-ZnNa3/kg bw/day were similar to controls in terms of embryo and foetal losses. However, those animals receiving 11,520 µmol DTPA-Na3Zn/kg bw/day had a 6-fold greater percentage of abortions (67% versus 11%) and more than twice the percentage of resorbed foetuses (25% versus 9%) than did the controls. The hypersaline injected animals also had a 6-fold greater percentage of abortions than controls, although no resorptions were found.

Animals injected on days 7-11 with 5760 µmol DTPA-ZnNa3/kg bw/day were not greatly different form the controls. However, with the 11.520 µmol DTPA-ZnNa3/kg bw/day dosage, the percentage of aborted litters was almost three times that of the controls (29% versus 11%) additionally to resorptions

The group receiving 1440 µmol DTPA-CaNa3/kg bw/day had nearly 4 times the percentage of aborted litters and 3 times the percentage of resorption sites than did the control animals.

In conclusion, this paper suggests that DTPA-Na3Zn is less teratogenic than DTPA-Na3Ca.

Other supportive teratogenicity studies with DTPA salts

Fisher *et al.* (1975) administered DTPA-Na3Zn (6 mice at 360 µmol/kg bw/day and 6 mice at 2900 µmol/kg bw/day), DTPA-Na3Ca (6 mice at 360 µmol/kg bw/day and 12 mice at 2900 µmol/kg bw/day) or saline solution (12 mice) to female mice (strain C57BL/Do) via daily subcutaneous injections. These doses are equivalent to 199 or 1600 mg DTPA-Na3Zn/kg bw/day and 179 or 1441 mg DTPA-Na3Ca/kg bw/day. The dosing period started 4 days after the mating period began and continued throughout pregnancy until the pups reached an age of 13 days. In the group of mice dosed with 2900 µmol DTPA-Na3Ca/kg bw/day there were no viable offspring observed. Only one stillborn pup was observed but it appeared based on macroscopic inspection normal. In the 360 µmol DTPA-Na3Ca/kg bw/day group there were no adverse effects on reproduction or developmental parameters. Both dose levels of DTPA-Na3Zn were reported to be 'completely harmless' to the mothers and the pups.

Fisher *et al*. (1976) administered a range of doses of DTPA-Na3Ca to pregnant mice (strain - C57BL/Do) via daily subcutaneous injections for different 4 day periods during pregnancy. The mice were separated into 3 groups and were dosed either on days 2-6, 7-11 or 12-16 during pregnancy. The mice received injections of 0, 720, or 1440 µmol/kg bw/day (days 2-6 or 7-11) or doses of 0, 720, 1440, or 2880 µmol/kg bw/day (days 12-16). These doses are equivalent to 0, 357, 715 and 1430 mg DTPA-Na3Ca/kg bw/day. The dams were sacrificed on day 18 of gestation and the foetuses examined for morphologic alterations. DTPA-Na3Ca (357 or 715 mg/kg bw/day) dosed either from day 2 to 6 or from day 7 to 11 of gestation resulted in an increase in resorptions compared to control. Neither of these doses caused an increase in resorptions (relative to controls) when dosed from day 12 to 16 whereas 1430 mg/kg bw/day did. Dosing with 715 mg/kg bw/day produced malformations in foetuses in all dosing period groups. The types of malformation and number of foetuses affected varied, however, with the dosing schedule. The malformations observed were exencephaly with ablepharia, exencephaly, spina bifida aperta and cleft palate. Dosing with 357 mg/kg bw/day only produced malformations when dosed from days 2 to 6 and these malformations were consistent with those observed with 715 mg/kg bw/day dosed for the same period.

***Mechanism of action for DTPA compounds-induced teratogenicity***

DTPA-Na5 is a chelating compound that predominantly binds divalent metals including zinc, iron, manganese, calcium, and monovalent ions metals such as sodium and potassium. Of these, DTPA binds most strongly to zinc (Log binding affinity = 18.3) compared to calcium (Log binding affinity

= 10.7) or potassium and sodium (Log binding affinity = 0.9 for both) (Table 4). Therefore in biological matrices where zinc is present, DTPA-Na5 will have a strong tendency to release Na ions in favour of binding zinc, calcium and other cations, although the differences of affinity might be of several orders of magnitude according to binding constants displayed in Table "Constants of affinity of EDTA and DTPA chelants for different ions" under "RAC general comments", above.

According to the DS there has been considerable work on the teratogenic effects of Zn deficiency. One study (Kechrid et al, 2006) reports that when pregnant Wistar rats were fed with diets containing adequate (54 mg/kg bw/day) or low (1 mg/kg bw/day) zinc quantities for 18 days the normal foetuses born from 10 pregnant females was reduced from 73 to 58, while the resorbed and malformed foetuses increased from 2 and 0 to 15 and 38, respectively.

The types of effects produced by Zn deficiency are many, e.g. brain defects, eye defects, cleft palates and skeletal defects, as well as gross malformations of the cardiovascular, respiratory and urogenital systems. It is also an important consideration that the extent, duration and timing of zinc deficiency during pregnancy will affect the severity and location of the effects on the developing foetus.

It seems that during periods of zinc deficiency the maternal liver sequesters zinc via induction of zinc-binding proteins as metallothionines, which restrict the supply of zinc to the embryo/foetus. It is noted by King (2000) that in humans and animals, transfer of sufficient zinc to the foetus is dependent on maintenance of normal maternal serum zinc concentrations and therefore the interference with maternal zinc status (as was presumably caused by DTPA-Na5 exposure) is the first step in producing zinc deficiency in the developing foetus.

Also supporting the hypothesis of teratogenicity induced by zinc deficiency is the fact that the ip administration of 1 g/kg bw of urethane to pregnant Sprague-Dawley rats on gestation day 11 caused a 14-fold induction of metallothionein concentrations, an increment in the hepatic zinc concentration, a decrease of approximately 30% of plasma zinc concentration, a reduction of at least 50% in the distribution of zinc to the embryonic tissues, a 18% decrease in foetal weight and a significant delay in skeletal ossification (Daston *et al.*, 1991). Thus, it supports the hypothesis that a decrease in plasma zinc concentration in pregnant female might be responsible for developmental toxicity.

Additional support for the mechanism of maternal zinc deficiency comes from studies where the zinc depleting agent was dosed in conjunction with a zinc supplemented diet. Indeed, doses that previously caused teratogenicity were found to be non-teratogenic in the presence of sufficient zinc (Swenerton and Hurley, 1971).

The above studies appear to demonstrate that DTPA-Ca salts are capable of causing foetotoxicity and malformations consistent with zinc deficiency; while DTPA-Zn dosed at significantly higher dose levels for equivalent dosing periods does not appear to cause malformations. A possible explanation for this phenomenon is that the toxicity is due to chelation of essential metals such as zinc and that the zinc salt of DTPA cannot chelate any additional zinc; while DTPA-Ca on the other hand will release calcium and bind the zinc in the body thus producing a zinc deficient state.

In summary, there are adequate data to support the hypothesis that the teratogenicity resulting from dosing of rats with DTPA salts is a result of an induced deficiency of zinc and presumably also other divalent cations in the mother which subsequently impacts the foetus.

## Comparison with the criteria

### *Sexual function and fertility*

RAC notes that, according to the DS, 2 available 28-days studies where DTPA salts were dosed showed clinical signs of a perturbation in nutrition (diarrhoea, decreased food consumption, decrease in bodyweight) without evidence of testicular toxicity. Therefore, **in the absence of any standard multi-generation reproductive toxicity studies, RAC considers that classification of DTPA-Na5 for sexual function and fertility is not possible due to lack of data.**

### *Development*

Human data are not present in the CLH report and therefore the classification as toxic to reproduction category 1A is not appropriate.

RAC notes that the available information clearly shows that DTPA salts are able to induce developmental toxicity:

- The malformation rate was notably increased (4 times) due to a high frequency of malformations of the vertebral column and sternebra together with statistically significant higher rates of retardations (1.7 times) and variations (1.5 times) in animals at a dose level of 1000 mg DTPA-Na5/kg bw/day (Table "Skeletal examinations in the developmental toxicity study with DTPA-Na5", above).

- Pregnant females exposed to 1000 mg DTPA-Na5/kg bw/day experienced a significant reduction (17%) of the mean number of live foetuses/litter and in foetal weight (8%) (Table 14).

RAC does not consider this developmental toxicity as secondary to maternal toxicity since the effects seen in pregnant females exposed to 1000 mg DTPA-Na5 were a reduction of 16% in body weight gain during the 20 days of gestation and an overall bodyweight reduction of 7%. In addition, statistically significant increases in number of retardations in ossification at 400 mg/kg bw/day without maternal effects were also reported. These statistically significant alterations appeared at a dose less than half of that causing severe effects such as malformations, which indicates a potential dose-response relationship supporting the developmental toxicity of this substance might be masked by the significant difference in the dose levels from 400 to 1000 mg/kg bw/day.

RAC also notes that the developmental toxicity of DTPA-Na5 salt is also supported by the following:

- The incidences of exencephaly, microphthalmia, anophthalmia, total malformed foetuses and foetuses with first and second fused ribs were increased in female pregnant rats subcutaneously exposed to 536 mg DTPA-Na3Ca/kg bw/day.

- A higher number of abortions and resorptions found in pregnant female mice subcutaneously exposed to 715 mg DTPA-Na3Ca/kg bw/day.

- A plausible mechanism of action potentially relevant for humans based on the chelation of zinc by DTPA compounds that would notably reduce the concentrations of zinc available to embryo and/or foetus. Also the chelation of other ions with affinities by DTPA comparable to Zn, as $Cu^{2+}$ or $Fe^{2+}$, might be causing the reported teratogenicity.

RAC therefore proposes to classify DTPA-Na5 as **toxic to reproduction category 1B (H360D: May damage the unborn child)**, in contrast with the DS proposal to classify the substance in Cat. 2.

The DS also proposed to state the route of exposure (oral) in the classification. This was because the relatively large particle size of the DTPA powder results in very limited absorption via the dermal route, and in its deposition in upper respiratory tract with subsequent oral ingestion.

RAC notes that according to the information contained in the CLH dossier, there have been a number of studies showing different degrees of bioavailability to humans of aerosolized DTPA complexes. Indeed, it is estimated as a worst case that a 20% of a dose may be dermally absorbed after administration of nebulized sprays. Other considerations also included in the CLH dossier reported that 10% of particles of less than 10 μm in diameter might be absorbed via the lungs. Thus, it is not conclusively proven that no other routes of exposure can cause the hazard. Therefore, RAC proposes not to state route of exposure together with the classification of DTPA-Na5 for reproductive toxicity.

## Additional references

Kechrid Z, Amamra S, Bouzerna N. The effect of zinc deficiency on zinc status, carbohydrate metabolism and progesterone level in pregnant rats. 2006; 36(6): 337-342.

## ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).