MCL Report for:

N-carboxymethyliminobis (ethylenenitrilo)tetra(acetic acid) (DTPA) and its pentasodium and pentapotassium salts

Proposal for mandatory classification and labelling (MCL) based on Annex VI, Part 2 of the retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain

EC Number: 200-652-8; 404-290-3; 205-391-3
CAS Number: 67-43-6; 7216-95-7; 140-01-2

July 2024

Contents

1. Identity of the substance

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance DTPA-H5 (CLH, 2016a)

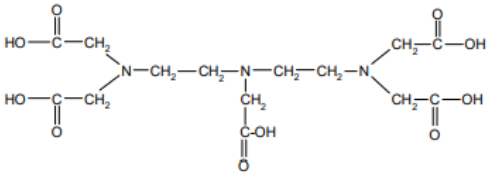
Name(s) in the IUPAC nomenclature or other international chemical name(s)	2-[bis[2-(bis(carboxymethyl)amino)ethyl]amino]acetic acid)
Other names (usual name, trade name, abbreviation)	DTPA-H5
ISO common name (if available and appropriate)	N/A
EC number (if available and appropriate)	200-652-8
EC name (if available and appropriate)	N-carboxymethyliminobis(ethylenenitrilo)tetra(acetic acid)
CAS number (if available)	67-43-6
Other identity code (if available)	N/A
Molecular formula	C14H23N3O10
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	393.3465
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	N/A
Description of the manufacturing process and identity of the source (for UVCB substances only)	N/A
Degree of purity (%) (if relevant for the entry in Annex VI)	100%
Reference	CLH, 2016a

Table 2: Substance identity and information related to molecular and structural formula of the substance DTPA-K5 (CLH, 2016b)

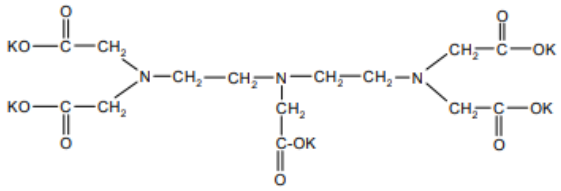
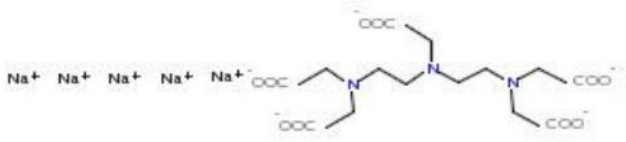
Name(s) in the IUPAC nomenclature or other international chemical name(s)	Pentapotassium 2,2',2'',2''',2''''-(ethane-1,2-diylnitrilo)pentaacetate
Other names (usual name, trade name, abbreviation)	DTPA-K5
ISO common name (if available and appropriate)	N/A
EC number (if available and appropriate)	404-290-3
EC name (if available and appropriate)	N/A
CAS number (if available)	7216-95-7
Other identity code (if available)	*[For example CIPAC number]*
Molecular formula	C14H23N3O10K5
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	583.8
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	N/A
Description of the manufacturing process and identity of the source (for UVCB substances only)	N/A
Degree of purity (%) (if relevant for the entry in Annex VI)	100%
Reference	CLH, 2016b

Table 3: Substance identity and information related to molecular and structural formula of the substance DTPA-Na5 (CLH, 2015)

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Pentasodium 2,2',2",2''', 2''''-(ethane-1,2-diylnitrilo) pentaacetate
Other names (usual name, trade name, abbreviation)	DTPA-Na5
ISO common name (if available and appropriate)	N/A
EC number (if available and appropriate)	205-391-3
EC name (if available and appropriate)	Pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo)tetraacetate
CAS number (if available)	140-01-2
Other identity code (if available)	*[For example CIPAC number]*
Molecular formula	C14H23N3O10Na5
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	503.2557
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	N/A
Description of the manufacturing process and identity of the source (for UVCB substances only)	N/A
Degree of purity (%) (if relevant for the entry in Annex VI)	100%
Reference	CLH, 2015

1.2 Composition of the substance

Table 4: Constituents (non-confidential information) of DTPA-H5 (CLH, 2016a)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current MCL on GB MCL list (if applicable)**	**Current self-classification and labelling (GB CLP)**
N-carboxymethyliminobis(ethylenenitrilo)tetra(acetic acid) EC – 200-652-8 CAS – 67-43-6	**100%**	**Acute Tox. 4 (H332)** **STOT RE 2 (H373) (inhalation)** **Eye Irrit. 2 (H319)**	**n/a**

Table 5: Constituents (non-confidential information) of DTPA-K5 (CLH, 2016b)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current MCL on GB MCL list (if applicable)**	**Current self-classification and labelling (GB CLP)**
Pentapotassium 2,2',2'',2''',2''''-(ethane-1,2-diylnitrilo)pentaacetate **EC – 404-290-3** **CAS – 7216-95-7**	**100%**	**Acute Tox. 4 (H332)** **STOT RE 2 (H373) (inhalation)** **Eye Irrit. 2 (H319)**	**n/a**

Table 6: Constituents (non-confidential information) of DTPA-Na5 (CLH, 2015)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current MCL on GB MCL list (if applicable)**	**Current self-classification and labelling (GB CLP)**
Pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo)tetraacetate EC – 205-391-3 CAS – 140-01-2	**100%**	**Acute Tox. 4 (H332)** **STOT RE 2 (H373) (inhalation)**	**n/a**

Impurities (non-confidential information) if relevant for the classification of the substance

N/a

Additives (non-confidential information) if relevant for the classification of the substance

N/a

Test substances (non-confidential information)

N/a

2. Proposed mandatory classification and labelling

Table 7: Proposed mandatory classification and labelling according to the GB CLP criteria (DTPA-H5)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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 GB MCL list entry	607-735-00-1	N-carboxymethyliminobis(ethylenenitrilo) tetra(acetic acid)	200-652-8	67-43-6	Acute Tox. 4 STOT RE 2 Eye Irrit. 2	H332 H373 (inhalation) H319	GHS08 GHS07 Dgr	H332 H373 (inhalation) H319		Inhalation: ATE = 1.5 mg/l (dusts or mists)	
Agency proposal	607-735-00-1	N-carboxymethyliminobis(ethylenenitrilo) tetra(acetic acid)	200-652-8	67-43-6	**Retain** Acute Tox. 4 STOT RE 2 Eye Irrit. 2 **Add** Repr. 1B	**Retain** H332 H373 (inhalation) H319 **Add** H360D	**Retain** GHS08 GHS07 Dgr	**Retain** H332 H373 (inhalation) H319 **Add** H360D		**Retain** Inhalation: ATE = 1.5 mg/l (dusts or mists)	
Resulting entry on GB MCL list	607-735-00-1	N-carboxymethyliminobis(ethylenenitrilo) tetra(acetic acid)	200-652-8	67-43-6	Acute Tox. 4 STOT RE 2 Eye Irrit. 2 Repr. 1B	H332 H373 (inhalation) H319 H360D	GHS08 GHS07 Dgr	H332 H373 (inhalation) H319 H360D		Inhalation: ATE = 1.5 mg/l (dusts or mists)	

Table 8: Proposed mandatory classification and labelling according to the GB CLP criteria (DTPA-K5)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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 GB MCL list entry	607-734-00-6	Pentapotassium 2,2',2'',2''',2''''-(ethane-1,2-diylnitrilo)pentaacetate	404-290-3	7216-95-7	Acute Tox. 4 STOT RE 2 Eye Irrit. 2	H332 H373 (inhalation) H319	GHS08 GHS07 Dgr	H332 H373 (inhalation) H319		Inhalation: ATE = 1.5 mg/l (dusts or mists)	
Agency proposal	607-734-00-6	Pentapotassium 2,2',2'',2''',2''''-(ethane-1,2-diylnitrilo)pentaacetate	404-290-3	7216-95-7	**Retain** Acute Tox. 4 STOT RE 2 Eye Irrit. 2 **Add** Repr. 1B	**Retain** H332 H373 (inhalation) H19 **Add** H360D	**Retain** GHS08 GHS07 Dgr	**Retain** H332 H373 (inhalation) H319 **Add** H360D		**Retain** Inhalation: ATE = 1.5 mg/l (dusts or mists)	
Resulting entry on GB MCL list	607-734-00-6	Pentapotassium 2,2',2'',2''',2''''-(ethane-1,2-diylnitrilo)pentaacetate	404-290-3	7216-95-7	Acute Tox. 4 STOT RE 2 Eye Irrit. 2 Repr. 1B	H332 H373 (inhalation) H319 H360D	GHS08 GHS07 Dgr	H332 H373 (inhalation) H319 H360D		Inhalation: ATE = 1.5 mg/l (dusts or mists)	

Table 9: Proposed mandatory classification and labelling according to the GB CLP criteria (DTPA-Na5)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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 GB MCL list entry	607-736-00-7	Pentasodium (carboxylatomethyl)iminobis (ethylenenitrilo)tetraacetate	205-391-3	140-01-2	Acute Tox. 4 STOT RE 2	H332 H373 (inhalation)	GHS08 GHS07 Dgr	H332 H373 (inhalation)		Inhalation: ATE = 1.5 mg/l (dusts or mists)	
Agency proposal	607-736-00-7	Pentasodium (carboxylatomethyl)iminobis (ethylenenitrilo)tetraacetate	205-391-3	140-01-2	**Retain** Acute Tox. 4 STOT RE 2 **Add** Repr. 1B	**Retain** H332 H373 (inhalation) **Add** H360D	**Retain** GHS08 GHS07 Dgr	**Retain** H332 H373 (inhalation) **Add** H360D		**Retain** Inhalation: ATE = 1.5 mg/l (dusts or mists)	
Resulting entry on GB MCL list	607-736-00-7	Pentasodium (carboxylatomethyl)iminobis (ethylenenitrilo)tetraacetate	205-391-3	140-01-2	Acute Tox. 4 STOT RE 2 Repr. 1B	H332 H373 (inhalation) H360D	GHS08 GHS07 Dgr	H332 H373 (inhalation) H360D		Inhalation: ATE = 1.5 mg/l (dusts or mists)	

Table 10: Reason for not proposing mandatory classification and status under public consultation DTPA-H5; DTPA-K5 and DTPA-Na5

Hazard class	Classification / reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	Repr. 1B; H360D	Yes
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3. History of the classification and labelling

The classification and labelling of N-carboxymethyliminobis (ethylenenitrilo)tetra(acetic acid) (DTPA) and its pentasodium and pentapotassium salts (referred to as DTPA-H5 DTPA-K5 and DTPA-Na5 respectively in this report) were considered by the EU Committee for Risk Assessment in 2017 (ECHA, 2017a, b, c). RAC concluded that all three substances should be classified as Acute Tox. 4 (H332), STOT RE 2 (H373, inhalation) and Repr. 1B (H360D), with DTPA-H5 and DTPA-K5 additionally classified as Eye Irrit. 2 (H319).

The Repr. 1B classification was based on the results of a prenatal developmental toxicity study in rats dosed with DTPA-Na5. In this study, an increased incidence of skeletal malformations (such as absent vertebrae) was reported at the top dose of 1000 mg/kg bw/d test material and retarded ossification at the mid-dose of 400 mg/kg bw/d. The results of this study were also used to classify DTPA-H5 and DTPA-K5; the read-across was based on the similar molecular structures, common functional groups, shared mechanism of action (MoA), similar physico-chemical properties, similar toxicokinetics and similar systemic toxicity profiles of the substances.

The harmonised classification and labelling (CLH) reports (CLH 2015; 2016a,b) on which RAC based their opinions had been prepared by an industry consortium (BAS, Dow and Nouryon), who suggested that the MoA was likely to be zinc deficiency due to chelation of zinc by DTPA, and proposed Repr. 2 rather than Repr. 1B based on low workplace exposure. Following the adoption of the RAC Opinion, the industry consortium provided additional information to the European Commission and argued that the developmental effects seen in rats are not relevant to humans, owing to differences in zinc kinetics. In addition, the consortium provided arguments to justify the setting of a specific concentration limit (SCL) higher than the generic concentration limit, in the event that the proposal for a classification for reproductive toxicity would be maintained. Consequently, the reproductive toxicity classification of DTPA and its salts was not included in the 14th Adaptation to Technical Progress (ATP) to the EU CLP Regulation (Reg. 2020/217), and the Commission asked RAC to review the information on interspecies differences provided by industry and, if necessary, amend the RAC opinions (ECHA, 2017a,b,c) in relation to the classification for reproductive toxicity and/or the setting of specific concentration limits.

The information submitted by industry was subject to a targeted public consultation. RAC considered the information submitted by industry and the comments received during the targeted public consultation and reconfirmed its conclusion from 2017 that DTPA-H5, DTPA-K5 and DTPA-Na5 should be classified with Repr. 1B; H360D. However, they did

support the application of a specific concentration limit (SCL) of 3%, based on the low potency indicated by the results of the key study.

The Annex VI entries for DTPA-H5, DTPA-K5 and DTPA-Na5 were updated to include Repr. 1B; H360D and the SCL by the 18th ATP to EU CLP (Reg. 2022/692).

4. Justification that action is needed

The EU Committee for Risk Assessment (RAC) has concluded that DTPA-H5, DTPA-K5 and DTPA Na-5 meet the classification criteria for Repr. 1B (H360D). Therefore, the Agency considers it appropriate to review the available data and decide whether it is necessary to update the GB MCL list entries for these substances.

5. Identified uses

DTPA is a chelating compound used industrially and pharmacologically to bind metals. The substance is widely used in the pulp and paper industry. It is also used in laundry detergents, cleaners, soaps, textiles, setting retarder in plaster production, scale remover for substances such as barium sulphate and as complexing agents of metals used as micronutrients for plants. Consumer exposure is expected to be low and significantly less than workers, since the substance is only used in trace amounts in final products (<2% cleaning products and <0.1% personal care products), is poorly absorbed dermally and does not volatilise (ECHA, 2015; 2016a,b).

6. Data sources

This report was compiled using publicly available information on the ECHA website. See references section for further details.

7. Physicochemical properties

Table 11: Summary of physiochemical properties

Property	Value (DTPA-h5)	Value (DTPA-k5)	Value (DTPA- Na5)
Physical state at 20°C and 101,3 kPa	Solid	Solid	Solid
Melting/freezing point	Decomposes without melting	Decomposes without melting	Decomposing without melting
Boiling point	Decomposes without melting	Decomposes without melting	Decomposing without melting
Relative density	No data	No data	600
Vapour pressure	Based on the presence of multiple carboxylic acid salt functions in the molecular structure, the neat material will exhibit negligible vapor pressure. Some commercial product mixtures contain water and will exhibit a vapor pressure corresponding to that of water	Based on the presence of multiple carboxylic acid salt functions in the molecular structure, the neat material will exhibit negligible vapor pressure. Some commercial product mixtures contain water and will exhibit a vapor pressure corresponding to that of water	Based on the presence of multiple carboxylic acid salt functions in the molecular structure, the neat material will exhibit negligible vapor pressure. Some commercial product mixtures contain water and will exhibit a vapor pressure corresponding to that of water
Surface tension	N/A	N/A	N/A
Water solubility	0.5 g/100 mL	No data	No data
Partition coefficient n-octanol/water	-4.09 (measured)	16.35 (calculated)	-3.05 (measured)
Flash point	N/A	N/A	N/A
Flammability	N/A	N/A	N/A
Explosive properties	N/A	N/A	N/A
Self-ignition temperature	N/A	N/A	N/A
Oxidising properties	N/A	N/A	N/A
Granulometry	27 -50% considered inhalable, ca. 5% smaller than 10 micron (considered at 20C	Mainly produced as a 40 wt% aqueous solution	No data
Stability in organic solvents and identity of relevant degradation products	No data	No data	No data

Property	Value (DTPA-h5)	Value (DTPA-k5)	Value (DTPA- Na5)
Dissociation constant	pK1: 1.79; pK2: 2.56; pK3: 4.42; pK4: 8.76 and pK5: 10.42. All conducted at 20C	pK1: 1.79; pK2: 2.56; pK3: 4.42; pK4: 8.76 and pK5: 10.42. All conducted at 20C	Key study is available for DTPA Acid (CAS# 67-43-6) however the dissociation constants (pKa) are the same for DTPA, regardless of acid or salt form (Pentasodium DTPA 88% purity). 5 pKa values are available: 16 pK1: 1.79; pK2: 2.56; pK3: 4.42; pK4: 8.76 and pK5: 10.42. All conducted at 20 C
Viscosity	N/A	N/A	N/A

8. Evaluation of physical hazards

Not considered in this report.

9. Toxicokinetics (absorption, metabolism, distribution and elimination)

9.1 Short summary and overall relevance of the provided toxicokinetic information

The following summary is based on information in the CLH reports for DTPA-H5, DTPA-K5 and DTPA Na5 (CLH 2015; 2016a,b) and Arts *et al.* (2018).

Absorption

The average intestinal absorption of DTPA and its salts has been determined to be 3-5% across rats, dogs and humans (Dudley *et al.,* 1980a, b; Stevens *et al.,* 1962; Resnick *et al.,* 1990).

Dermal absorption is expected to be very low; 0.001% or less. This is based on data from a structurally related chelating agent, EDTA, as data on DTPA were unavailable.

Absorption via the inhalation route depends on whether DTPA is aerosolised or powdered and the size of those particles. The degree of absorption is dependent upon the site of deposition within the respiratory tract; studies performed in dogs (Dudley *et al.,* 1980b) demonstrate that the percentage of the applied dose absorbed through the respiratory tract increases the further into the respiratory tract the substance is deposited (i.e., 23% absorbed in the nasopharyngeal region vs 90% absorbed in the pulmonary region). A study in humans (Jolly *et al.,* 1972) noted an average of 20% absorption with droplet sizes of between 0.3 and 2 µm. This represents a worst case scenario as the nebuliser used in the study is designed to produce small particles. The absorption of powdered particles also depends on how far down the respiratory tract the particles reach, since mucociliary transport deals with particles in the upper respiratory tract. The ICRP (1994) estimated that only 10% of an administered dose of powdered particles (<10µm) are available for absorption by the lungs. The remaining powder is transported back to the mouth where it is swallowed.

Distribution & Metabolism

Following oral exposure in humans and rats, the absorbed fraction is not metabolised and is rapidly excreted with a very short half-life (2 h) via the urine; any systemically absorbed fraction is completely excreted unmetabolized within 24 h (ECB, 2004). Therefore,

excretion of these chelates is almost exclusively via the faeces. The passage of the DTPA through the gut varies between individuals; however, there is almost complete excretion of the substance within 5 days of administration (Stevens *et al*., 1962). DTPA is not taken up or concentrated in any particular tissue, and in pregnant rats did not pass into fetal circulation (Zylicz *et al.,* 1975).

Excretion

Absorbed DTPA is excreted rapidly via the urinary system with a half-life of 2-4 hours (Stevens *et al.,* 1962). Unabsorbed oral doses are excreted via the faeces. There is no evidence to suggest that DTPA bioaccumulates or is sequestered by any organs/tissues.

DTPA and metal excretion

According to the original CLH reports for DTPA-H5, K5 and Na5, the effects of administering DTPA to humans and animals on the excretion of essential nutrients have been studied. Systemic administration of DTPA (i.e., intravenous, intraperitoneal, subcutaneous) causes an increase in urinary excretion of zinc, calcium and to a lesser extent iron and manganese. The increase in urinary excretion of these metals occurs because DTPA forms complexes with ‘free’ metals in the blood and lymph; it is these complexes which are then excreted in the urine. DTPA has a high affinity for zinc, therefore zinc is one of the metals most affected by administration of DTPA. In humans, the increased excretion of zinc following prolonged administration of DTPA results in a zinc deficiency, treatable with supplementation of zinc sulphate, or administration of the zinc complex of DTPA (CLH 2015; 2016a,b).

10. Evaluation of health hazards

10.1 Acute toxicity – oral route

Not considered in this report.

10.2 Acute toxicity – dermal route

Not considered in this report.

10.3 Acute toxicity – inhalation route

Not considered in this report

10.4 Specific target organ toxicity – single exposure (STOT SE)

Not considered in this report

10.5 Skin corrosion/irritation

Not considered in this report

10.6 Serious eye damage/eye irritation

Not considered in this report

10.7 Respiratory sensitisation

Not considered in this report.

10.8 Skin sensitisation

Not considered In this report

10.9 Specific target organ toxicity – repeated exposure (STOT RE)

Not considered in this report

10.10 Germ cell mutagenicity

Not considered in this report

10.11 Carcinogenicity

Not considered in this report

10.12 Reproductive toxicity

10.12.1 Adverse effects on sexual function and fertility

Not considered in this report.

10.12.2 Adverse effects on development

Table 12: Summary of oral animal studies on adverse effects on development (adapted from CLH, 2015)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD Guideline 414 (Prenatal Developmental Toxicity Study) rat (Wistar) 22 pregnant females/group 1 (reliable without restriction) key study	Test material Pentasodium DTPA, CAS number: 140-01-2 oral: gavage 0, 100, 400 and 1,000 mg/kg bw/d Vehicle: water Exposure: day 6 through day 15 post coitum (daily) Purity 43.7%	**Maternal findings** **1000 mg/kg bw/d:-** - Reduced bodyweight and food consumption - Dark yellow discolouration of the faeces in all females. - Statistically significant lower mean gravid uterus weights **Foetal findings** **1000 mg/kg bw/d:-** - Statistically significant reduction in live fetuses/litter (11.9 vs 14.3 in control group) - Slight increase in number of resorptions and non-significant increase in post-implantation loss value/ approx. - 8% lower mean fetal bodyweights. - Statistically significant increase in malformation rate (15.4% affected fetus/litter vs 3.5% affected fetus/litter in controls), predominantly caused by increase in skeletal malformations and variations (78.4% affected fetuses/litter vs 49.6% affected fetuses/litter in controls), and retardations (78% affected fetuses/litter vs	BASF SE (1994)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		47.4% affected fetuses/litter in controls). **400 mg/kg bw/day:-** - Statistically significant increase in rate of fetuses with skeletal retardations (63.8% affected fetuses/litter vs 47.4% affected fetuses/litter in controls).	

Table 13: Summary of Subcutaneous animal studies on adverse effects on development (adapted from CLH, 2015)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
rat (Wistar) Equivalent or similar to EPA OPP 83-3 No. of animals not specified Subcutaneous 2 (reliable with restrictions) supporting study (Prenatal Developmental Toxicity Study)	Test material (Common name): Zinc and Calcium salts of DTPA (CAS number. not given) 0 µmol/kg body weight (nominal conc. (negative control and another group administered with 0.60 ml of isotonic saline)) 30 µmol/kg body weight (nominal conc. (equivalent to 1 human dose (30 µmol/kg body weight))) 180 µmol/kg body weight (nominal conc. (equivalent to 6 human dose)) 360 µmol/kg body weight (nominal conc. (equivalent	**<u>DTPA-Ca</u>** **1080 µmol/kg bw** Decrease in the survival rates **360, 720 and 1080 µmol/kg bw** Abnormalities such as exencephaly, microphthalmia, anophthalmia and fused ribs were observed **<u>DTPA- Zn</u>** Nothing significant noted	Fukuda *et al* (1982)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	to 12 human dose)) 720 and 1080 µmol/kg body weight (nominal conc. (equivalent to 24 and 36 human dose)) Vehicle: isotonic saline solution, pH adjusted to 7.2 Exposure: days 9-13 of gestation (daily from days 9-13 of gestation) Purity: not specified		
mouse (C57BL/Do) Equivalent or similar to EPA OPP 83-3 No. of animals not specified subcutaneous 2 (reliable with restrictions) supporting study (Prenatal Developmental Toxicity Study)	Test material (Common name): Zinc DTPA (CAS Number not given) 11520 µmole Zn-DTPA/kg/day (nominal conc. (vehicle - saline)) 5760 µmole Zn-DTPA/kg/day (nominal conc. (vehicle - saline)) 1440 µmole Ca-DTPA/kg/day (nominal conc. (vehicle - distilled water)) Vehicle: saline solution, pH 7.0- 7.2 Exposure: days 2-6 or 7-11 of gestation (daily either from days 2- 6 or 7-11 of gestation) Purity: not specified	**Day 2-6** **11520 µmole Zn-DTPA/kg/day**: 6-fold greater percentage of abortions and 2-fold increase in percentage of resorbed fetuses vs controls. **5760 µmole Zn-DTPA/kg/day**: similar to controls in terms of embryo and fetal loss. Hypersaline injected animals also had 6 fold increase in percentage of abortions but neither of the 2 animals carrying to term had uterine resorption sites. **Day 7-11** **11520 µmole Zn-DTPA/kg/day**: 3-fold greater percentage of abortions compared to controls and all 25 fetuses (5 litters) were resorbed. **5760 µmole Zn-DTPA/kg/day**: similar to controls. Hypersaline injected animals had twice the percentage of abortions and twice the percentage of resorbed	Brummett *et al* (1977)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		fetuses compared to controls. **1440 µmole Ca-DTPA/kg/day**: 4 times the percentage of aborted litters and 3 times the percentage of resorption sites vs controls.	

Developmental toxicity study in the rat (BASF SE, 1994) – GLP compliant

Twenty-two pregnant female Wistar rats were administered, via drinking water, the test material 'Trilon C Flussig' (containing DTPA pentasodium salt in water; degree of purity: >43.7% pentasodium DTPA[1]) at 100, 400 and 1000 mg/kg bw/d of DTPA-Na5 from days 6-15 (post coitum). Within the EU, this was considered to be the key study.

Maternal toxicity

At **100 and 400 mg/kg bw/d** test material, no significant alterations were reported in body weight (bw), body weight gain (bwg) or food consumption of the dams, and no treatment-related clinical findings were reported.

At **1000 mg/kg bw/d** test material, the dams showed statistically significant decreases in bwg (↓16% over the 20d gestation period) and bw (↓7% by gestation day 20) because of reduced food consumption between days 6-10 gestation (↓10%). A dark yellow discolouration of the faeces was also observed. A 21% reduction in uterus weight was observed but this had no statistically significant effect on corrected bwg.

Fetal toxicity

At **100 and 400 mg/kg bw/d** test material, there were no significant reductions in the number of live fetuses nor were the fetal body weights affected. Skeletal examination revealed no significant alterations/retardations at 100 mg/kg bw/d. At 400 mg/kg bw/d a statistically significant increase, compared to controls, in the number of skeletal retardations (incomplete/missing ossification of skull, vertebral column and sternebra) was observed. The incidences of these findings were outside the range of the historical control data (HCD). These skeletal retardations followed a dose response (total fetal incidence (%) was 47, 48, 64 and 78 at 0, 100, 400 and 1000 mg/kg bw/d, respectively).

At **1000 mg/kg bw/d,** a statistically significant reduction in the number of live fetuses/dam (11.9 vs 14.3 in control) was observed. However this was within the range of the HCD (11.1-14.9). The mean bw of the fetuses was 8% lower than controls. Skeletal malformations consisted of: absent thoracic vertebra; absent lumbar vertebra; sternebra bipartite/dislocated ossification centres. All of these findings were statistically significant, and the incidences (fetuses/litter) were above the range of the HCD. Furthermore, statistically significant increases in the incidence of certain rib variations (shortened 13th rib, rudimentary cervical rib(s) and absent 13th rib) were reported, and these were all outside the HCD. Lastly, there were increases in skeletal retardations (described in the previous paragraph). The figure and table below outline the key observations.

[1] Information on the test material was taken from the EU REACH registration dossier for pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo)tetraacetate

Figure 1: Two tables copied directly from the RAC opinion (ECHA 2017 a,b,c) showing maternal body weight and organ weights, number of live fetuses and fetal weight.

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

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**

Figure 2: Table copied from the RAC Opinion (ECHA 2017a,b,c) summarising the skeletal findings in the developmental toxicity study with DTPA-Na5 (BASF SE, 1994) (continued on the next page).

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)

Additional studies

In addition to the key prenatal developmental toxicity study (BASF SE, 1994), there were further studies performed via the subcutaneous route of exposure, two of which are outlined in the study summary table above. The other performed by Fisher *et al.* (1975) and (1976) were considered in the original RAC opinion.

The Agency considers these studies add no additional weight to the findings noted in the key study, nor do they decrease the concern. They were all performed via a non-standard route of exposure, and reported a different set of effects compared to the key study. Nonetheless, they do highlight that the sensitivity to DTPA-induced malformations is less when DTPA is complexed with zinc rather than calcium, supporting the proposed MoA of zinc deficiency.

10.12.3 Short summary and overall relevance of the provided information on adverse effects on development

Original RAC opinion

During RAC's initial assessment, it was concluded that the three DTPA salts were developmental toxicants. This was mainly based on the skeletal effects and reductions in live foetuses observed in the key BASF (1993) study. The DS considered category 2 was warranted, since the findings occurred in the presence of maternal toxicity. The DS also highlighted that the doses required to achieve the same levels of zinc deficiency and hence toxicity would be impossible under foreseeable human exposure.

RAC did not agree with the DS's conclusion. They highlighted that maternal body weight gain was reduced by 16% and body weight was reduced by 7% at the top dose of 1000 mg/kg bw/d (BASF, 1994). Significant increases in retardations occurred at a lower dose of 400 mg/kg bw/d where no maternal toxicity was reported, suggesting no correlation between maternal toxicity and the developmental findings. RAC also noted that any dose-response in the findings might be masked by the significant jump in doses between 400 and 1000 mg/kg bw/d.

RAC supported the proposed mode of action (MoA) that DTPA salts can chelate dietary and systemic zinc, leading to zinc deficiency and related developmental effects, and considered this MoA to be relevant to humans. RAC also suggested that DTPA might chelate other essential ions (copper and iron) which may further explain the developmental toxicity seen.

RAC added that support for developmental toxicity also came from Study 2 (Fukuda and Iida, 1983) which showed incidences of exencephaly, microphthalmia, anophthalmia, total malformed foetuses and foetuses with first and second fused ribs at 536 mg/kg bw/d. Study 3 (Brummett and Mays, 1977) also highlighted that doses of 715 mg/kg bw/d increased the abortion and resorption rate in female pregnant mice. Both of these studies were performed subcutaneously which lowers their reliability.

Overall RAC concluded category 1B for developmental toxicity. They further specified that the route of exposure via other pathways cannot be fully dismissed and hence did not agree with 'oral route only' proposed by the DS.

Summary of the Article 77 (3)c request

Since the RAC opinion, all three substances were considered at the EU Meeting of the Competent Authorities for REACH and CLP (CARACAL) where BASF, Dow and Nouryon requested that RAC review their opinion, on the basis of physiological/kinetic differences between rats and humans. A summary of this request is provided below, taken from the new RAC opinion (ECHA, 2020):

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

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

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

*RAC opinion appears to have missed off additional words here

Summary of the Article 77(3)c revised RAC opinion

Three arguments were considered in the revised opinion; hazard vs risk, susceptibility of pregnant women to DTPA induced zinc deficiency and a comparison to the substance 2-ethylhexanoic acid (2-EHA).

Hazard vs Risk

RAC dismissed the main argument from the original CLH reports and a paper by Arts *et al.* (2018), where they considered that doses of DTPA capable of inducing developmental defects were impossible to achieve in the workplace. RAC highlighted that the CLP regulation is based on the intrinsic hazard; it does not consider exposure (except for concentration limits) or risk.

Susceptibility of pregnant women to DTPA-induced zinc deficiency

BASF, Dow and Nouryon submitted evidence to CARACAL outlining a comparison between human and rat dietary zinc demand. RAC summarised their arguments as follows:

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

RAC noted that the whole-body zinc concentration of the average adult women is approximately 1500mg with ca. 2mg in the plasma and 10% (152 mg) in a rapidly exchanging pool. Outside of these two sources, zinc release may not be fast enough to counteract zinc deficiency (King *et al*., 2000). Taking into account urinary/faecal loss, physiological zinc requirements (2-3 mg) and oral absorption (5%), the daily intake in humans is recommended to be 10 mg with 11-12 mg for pregnant women. Some studies note that increased zinc demand during pregnancy is compensated by upregulated intestinal absorption, a key aspect of zinc homeostasis in humans (Brown *et al*., 2004; Millet *et al*., 2000; Hess *et al*., 1977; King, 2000).

In a review paper by King (2000), epidemiological evidence about zinc deficiency and pregnancy outcomes was considered by RAC. They noted that this evidence was

inconclusive due to several study deficiencies. Nonetheless, studies looking at pregnant women with *acrodermatitis enteropathica*, a rare hereditary disorder affecting zinc absorption, and some animal studies (rats/monkeys) have shown that zinc absorption has to be virtually reduced to zero in order to induce foetal malformations, with moderate reductions in zinc intake negatively affecting foetal growth.

RAC highlighted that zinc supplementation in the BASF (1994) study was high (60mg/kg feed), noting that 25 mg/kg feed was the recommended level for pregnant rats. With this in mind, they considered that 10 consecutive doses of 1000 mg/kg bw/d of DTPA-Na5 must have had a profound effect on maternal zinc balance. To induce such malformations, almost all of the dietary zinc would have to be chelated. Although theoretically possible, based on a DTPA:Zn chelation ratio of 20:1, RAC noted that the dosing regimen would not allow for this. Rats feed mainly at night and in the BASF (1994) study, the rats were dosed with DTPA (via gavage) in the morning, suggesting a lag period between DTPA intake and zinc intake. Therefore, RAC suggested that systemically absorbed DTPA must also play a role in the developmental effects.

A few studies in the original CLH reports were noted, which looked at human DTPA absorption (Resnick *et al*., 1990; Stevens *et al*., 1962). RAC assumed an oral absorption of 4% based on these studies. Using this value, RAC estimated that 400 mg/kg bw/d could deplete the rapidly exchangeable pool of zinc within a few weeks. This was deduced by taking into account the 18 mg urinary loss of zinc from a man intravenously injected with 1-g of Na3CaDTPA (Kalkwarf *et al.,* 1983) and that 4% of 400 mg/kg bw/d dose, in a 60kg women, is 960 mg of DTPA. If 18 mg of zinc is lost/d and the average intake is 10mg zinc/d, assuming all zinc is absorbed, an 8 mg loss of zinc will deplete the rapidly exchangeable pool of zinc (150 mg) in 18.75 days (2-3 weeks). RAC further noted that a zinc deficiency may be present before pregnancy, implicating the early days of embryonic development.

RAC also commented on the length of dosing in the BASF (1994) study indicating that dosing stopped on GD 15 whereas zinc requirement is highest at the later stages of gestation (21 days in total). This combined with a high zinc dosing of 60 mg/kg feed suggested to RAC that if a standard zinc dose (25 mg/kg feed) was administered and dosing had continued throughout gestation, the LOAEL of 400 mg/kg bw/d may have been lower.

RAC didn’t rule out less severe developmental effects happening at lower doses than 400 mg/kg bw/d in humans. Based on the 20:1 chelation ratio, they note that 20-30 mg/kg bw/d of DTPA could still chelate all dietary zinc in humans.

Reiterating their original opinion, RAC noted that the developmental effects could also be due to the chelation of other essential metals (e.g. manganese) and that these additional MoA’s have not been investigated by industry.

Comparison to 2-ethylhexanoic acid (2-EHA)

Industry highlighted that 2-EHA was classified as Repr. 2 for a similar MoA; altered zinc distribution. The classification was proposed under the preceding regulation, the Dangerous Substance Directive (DSD), in a meeting of the commission working group in 1994. The paper looking at the MoA was published 4 years later (Bui *et al*., 1998). According to RAC (2020), where 2-EHA was discussed again, the evidence for this MoA was very limited. Therefore, RAC did not consider the classifications of 2-EHA and DTPA to be related.

Specific concentration limit (SCL)

RAC agreed that an SCL should be set to take into account the low potency of DTPA-induced developmental toxicity. RAC based this value on the procedure described in the 'Guidance on the application of the CLP criteria (V5.0; section 3.7.2.6)' (ECHA, 2017d).

Taking the skeletal malformations seen at 1000 mg DTPA-Na5/kg bw/d in the BASF SE (1994) study, equivalent doses of 1160 and 780 mg/kg bw/d were established for DTPA-K5 and DTPA-H5, respectively. RAC determined ED10 values by linear interpolation of litter-based incidence (mean percentage of affected foetuses per litter) and litter incidence, (see Figure 3, below).

Figure 3. Table showing RAC's ED10 calculations (copied from ECHA, 2023)

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

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

These ED10 values corresponded with that of low potency (3% for a substance classified as 1B). RAC did note that if the animals were fed a diet of 25 mg/kg bw/d then a much lower ED10 may have been achieved. However, they also considered that rats are somewhat more sensitive to the MoA via zinc deficiency, so accepted that low potency was appropriate.

Overall, RAC reconfirmed its proposal to classify as Repr. 1B; H360D. They further concluded an SCL of 3% based on low potency in the BASF (1994) study for all three DTPA salts.

10.12.4 Comparison with the GB CLP criteria

The Agency has considered the information in the EU CLH reports, RAC opinions and EU REACH Registration dossiers, the concerns raised at CARACAL by BASF, Dow and Nouryon, the revised RAC opinion (ECHA, 2020) and subsequent responses from industry and ECHA (documents available on the ECHA website).

In the key study (BASF, 1994), DTPA-Na5 caused developmental toxicity (malformations) in rats at doses of 1000 mg/kg bw/d test material in the absence of severe maternal toxicity. Although this is quite a high dose (equivalent to the limit dose for repeated dose toxicity studies), the Agency notes the low purity of DTPA-Na5 in the test material (43%), and considers it likely that malformations would have been observed at much lower doses had a purer form of DTPA-Na5 been tested. The dose-spacing in the BASF study means there is a large gap between the top dose at which malformations were seen, and the next dose (400 mg/kg bw/d) where no malformations were reported, so information about the dose-response is limited. At 400 mg/kg bw/d, variations (in the ribs) and retardations in skeletal ossification were reported in the absence of maternal toxicity. Furthermore, the Agency agrees with RAC that had the BASF study not used such a high level of zinc supplementation, and dosed the dams for longer (i.e., through to the end of gestation), the reproductive findings may have been more pronounced.

The Agency agrees with RAC that zinc deficiency is a plausible MoA behind the observed developmental toxicity, i.e., reduced dietary uptake of zinc owing to zinc being chelated to DTPA in the gastrointestinal tract, and depletion of existing maternal zinc stores (enhanced by systemically absorbed DTPA chelating with zinc in the plasma and other extracellular fluids, followed by excretion in the urine), leading to zinc deficiency in the developing fetus. The Agency also agrees that this MoA is relevant to humans.

It could be argued that the developmental effects are not caused by an intrinsic property of DTPA or a specific interaction between DTPA and the fetus. Rather, they are a secondary consequence of maternal toxicity. According to the CLP guidance document (Section 3.7; ECHA, 2017d), if an effect (indicative of reproductive toxicity) is a non-specific consequence of another toxic effect then neither category 1 or category 2 are appropriate. However, in the view of the Agency, zinc deficiency cannot just be dismissed as maternal toxicity (indeed, the only maternal effects reported at the top dose were small reductions in body weight gain and body weight – it isn't clear what, if any, adverse effects the zinc deficiency was having on the dams themselves). Furthermore, the severity of the malformations seen in rats at 1000 mg/kg bw/d, are sufficiently concerning to warrant classification in Repr. 1B.

Human relevance

The Agency considers that the MoA (zinc depletion) is relevant to humans. BASF, Dow and Nouryon have provided literature evidence showing that pregnant women are able to

tolerate a degree of zinc depletion in the body due to slower fetal growth and efficient zinc homeostasis in times of deficiency, stating that 'human experimental studies with low zinc diets (2.6-3.6 mg Zn/d) have shown that circulating zinc levels and activities of zinc-containing enzymes can be maintained within the normal range over several months, highlighting the efficiency of zinc homeostasis (FAO/WHO, 2001)'. Some of the key arguments presented from industry are discussed below:

- Based on the daily recommended intake between rats and humans, it was estimated that the increased daily intake of zinc during pregnancy would be between 13-21% and 100% in humans and rats, respectively. This suggests that pregnant rats could be more susceptible to reductions in zinc availability.

- Industry noted that in humans, it would take between 100-200 days to deplete the entire zinc storage, assuming a zinc content of 1.5-2g. In rats, it would only take 17-18 days. However the figure of 100-200 days is based on the entire zinc store, whereas it is known that most of this zinc store is not readily available (King, 2000). Hence a fetal zinc deficiency may occur much sooner. Furthermore, this argument assumes that zinc stores are sufficient prior to the start of pregnancy.

- Industry further noted that the period of major organogenesis in humans is within the first 58d of gestation (280d for total gestation) compared to rats where it is only 15-17d (21d total gestation). In line with bullet point 2, they argued that the period of organogenesis would be protected in humans unlike in rats. Since depletion, theoretically, occurs sooner in rats (5-15d) and during the period of embryonic development, it is possible that rats are more vulnerable to Zn deficiency compared to pregnant women on the same mg/kg bw/d basis. Once again this argument assumes that the entire human zinc store is readily available. In addition, it suggests that major developmental effects do not occur after the organogenesis period. However, zinc sufficiency is essential throughout the entirety of pregnancy.

Another aspect to note is that cases of *acrodermatitis enteropathica* suggest that zinc absorption in humans has to be virtually reduced to zero in order to see fetal malformations. In agreement with RAC, the dosing regimen used in the BASF (1994) study makes it very unlikely that zinc absorption was zero (or close to). The Agency notes industry's argument that rats make use of an anticipatory feeding mechanism during the night. However, even with this in mind, it does not definitively prove that no zinc absorption occurred. This suggests that systemically absorbed DTPA may have also played a role in the observed developmental toxicity. When taking into account the Kalkwarf *et al*. (1983) study, which highlighted that 1g of intravenously injected DTPA caused excretion of 18 mg zinc, it suggests that systemically available DTPA can deplete zinc stores in humans. Therefore, a combination of intestinal and systemic zinc chelation could lead to a zinc deficiency.

Calculations performed by RAC show that the equivalent oral dose of 1g intravenous DTPA is 400 mg/kg bw/d for a 60 kg human. Therefore, excretion of 18 mg zinc, plus a potential reduction in zinc absorption could deplete the 152 mg readily available zinc within 2-3 weeks. Skeletal retardation (reduced ossification) was seen in rats at the same dose in the BASF study (1994), supporting the relevance of this dose for classification. One argument from industry highlighted that a 400 mg/kg bw/d dose would mean an oral intake of 24g (60 kg bw) DTPA per day which is unrealistic. However, this is a risk-based argument and not relevant to hazard classification.

In GB CLP, the purpose of classification is to identify the intrinsic hazards of a substance, therefore arguments relating to workplace exposure are not particularly relevant to the assessment. Furthermore, in agreement with RAC, the Agency considers that reference to 2-EHA provides no weight to the classification of DTPA, as the evidence supporting a zinc-related MoA for this substance is very limited. It should also be noted that the revised RAC opinion for 2-EHA has proposed Repr.1B (H360D); the Agency agreed with this classification.

Taking the original study reports, the arguments presented by industry and RAC's opinions into consideration, the Agency concludes that Repr. 1B for developmental toxicity is warranted. As stated, the developmental effects reported in the BASF (1994) study are statistically significant, severe and not considered to be secondary to maternal toxicity. The proposed MoA of zinc deficiency is plausible and relevant to humans, although other MoA cannot be ruled out.

Specific Concentration limit (SCL)

RAC supported an SCL of 3%, reflecting the apparent low potency of the substance (see section "Specific concentration limit (SCL)" and figure 3, above).

However, it is not clear to the Agency what doses of DTPA-Na5 the rats were exposed to in the prenatal developmental toxicity study. The doses are reported to have been 0, 100, 400 and 1000 mg/kg bw/d, however the test material in the CLH report is reported to have been 43.7% pure. According to the EU REACH registration dossier[2], the test material in the study was 'Trilon C Liquid', which is a solution of DTPA-Na5 in water (purity >43.7%), and the study summary in the EU registration dossier suggests that the doses refer to the amount of Trilon C Liquid administered (rather than the calculated amount of DTPA-Na5). If it is the dose of Trilon C Liquid given to the animals, then at the top dose this would equate to 437 mg/kg bw/d of DTPA-Na5 (assuming the purity percentage is based on weight). In that case, it could be argued that the substance has a medium potency (i.e., the NOAEL would be 175 mg/kg bw/d and the LOAEL would be 437 mg/kg bw/d). On this basis, the Agency concludes that the GCL of 0.3% should apply.

[2] Available at Homepage - ECHA (europa.eu)

10.12.5 Adverse effects on or via lactation

Not considered in this report

10.12.6 Conclusion on classification and labelling for reproductive toxicity

N-carboxymethyliminobis (ethylenenitrilo)tetra(acetic acid) (DTPA) and its pentasodium and pentapotassium salts meet the criteria for classification as **Repr. 1B; H360D (May damage the unborn child) with the GCL of 0.3%**

10.13 Aspiration hazard

Not considered in this report

11. Evaluation of environmental hazards

Not considered in this report

12. Evaluation of additional hazards

Not considered in this report

13. Additional labelling

Not required.

14. References

Arts J., Bade S., Badrinas M., Ball N. and Hindle S. (2018) Should DTPA, an Aminocarboxylic acid (ethylenediamine-based) chelating agent, be considered a developmental toxicant? Regulatory Toxicology and Pharmacology, Volume 97, August 2018, Pages 197-208

ECHA (2017d) Guidance on the application of the CLP criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, version 5.0, ref: ECHA-17-G-21-EN. Available at https://www.echa.europa.eu/

For all other references, please see the EU CLH reports and the EU RAC opinions (available at: https://echa.europa.eu)

CLH (2015) Proposal for Harmonised Classification and Labelling. Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2. Substance name: pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo) tetraacetate. EC number: 205-391-3. CAS Number: 140-01-2. Dossier submitter: DOW Chemical Company Ltd. Version 3. Date: 21 November 2015.

CLH (2016a) Proposal for Harmonised Classification and Labelling. Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2. Substance Name: N-carboxymethyliminobis (ethylenenitrilo)tetra(acetic acid), EC Number 200-652-8, CAS Number 67-43-6. Dossier submitter: Akzo Nobel Functional Chemicals BV. Version 4. Date: 1 April 2016.

CLH (2016b) Proposal for Harmonised Classification and Labelling. Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2. Substance name: Pentapotassium 2,2',2'',2''',2''''-(ethane-1,2- diylnitrilo)pentaacetate. EC Number: 404-290-3 CAS Number: 7216-95-7. Dossier submitter: Akzo Nobel Functional Chemicals BV. Version 4. Date: 1 April 2016.

ECHA (2017a) Committee for Risk Assessment RAC Opinion proposing harmonised classification and labelling at EU level of N-carboxymethyliminobis (ethylenenitrilo)tetra(acetic acid). EC Number: 200-652-8 CAS Number: 67-43-6. Ref: CLH-O-0000001412-86-155/F. Adopted 9 June 2017.

ECHA (2017b) Committee for Risk Assessment RAC Opinion proposing harmonised classification and labelling at EU level of Pentapotassium 2,2',2'',2''',2''''-(ethane-1,2-diylnitrilo)pentaacetate. EC Number: 404-290-3 CAS Number: 7216-95-7. Ref: CLH-O-0000001412-86-157/F. Adopted 9 June 2017.

ECHA (2017c) 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. Ref: CLH-O-0000001412-86-156/F Adopted 9 June 2017

ECHA (2020) Committee for Risk Assessment (RAC) Opinion. Pursuant to Article 77(3)(c) of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals: A reassessment at the request of the European Commission of the developmental toxicity of N-carboxymethyliminobis (ethylenenitrilo)tetra(acetic acid) (DTPA) and its pentasodium and pentapotassium salts EC Number: 200-652-8. Ref: A77-O-0000006841-72-01/F Adopted 11 June 2020

Documents published as part of the EU CLH process: Source: European Chemicals Agency, http://echa.europa.eu/

15. Annexes

No annexes are included in this MCL report.

Further information

This publication is available on the HSE website.

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