CLH report

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

Index Number: **na**

Contact details for dossier submitter:
Dan Thanh Nguyen Phuc,
DOW CHEMICAL COMPANY LTD
Diamond House, Lotus Park Kingsbury Crescent, Middx UB7 0DQ, Staines Middx, United Kingdom
DNguyenPhuc@dow.com

Version number: 3 Date: 21 November 2015

CONTENTS

Part A

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING 5

1.1 SUBSTANCE 5
1.2 HARMONISED CLASSIFICATION AND LABELLING PROPOSAL 5
1.3 PROPOSED HARMONISED CLASSIFICATION AND LABELLING BASED ON CLP REGULATION 7

2 BACKGROUND TO THE CLH PROPOSAL 10

2.1 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING 10
2.2 SHORT SUMMARY OF THE SCIENTIFIC JUSTIFICATION FOR THE CLH PROPOSAL 10
2.3 CURRENT HARMONISED CLASSIFICATION AND LABELLING 11
2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation 11
2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation 11
2.4 CURRENT SELF-CLASSIFICATION AND LABELLING 11
2.4.1 Current self-classification and labelling based on the CLP Regulation criteria 11

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL 11

Part B

Scientific evaluation of the data

1 IDENTITY OF THE SUBSTANCE 12

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE 12
1.2 COMPOSITION OF THE SUBSTANCE 13
1.2.1 Composition of test material 15
1.3 PHYSICO-CHEMICAL PROPERTIES 15

2 MANUFACTURE AND USES 16

2.1 MANUFACTURE 16
2.2 IDENTIFIED USES 16

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES 17

3.1.1 Conclusions on classification and labelling 17

4 HUMAN HEALTH HAZARD ASSESSMENT 17

4.1 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 17
4.1.1 Non-human information 17
4.1.2 Human information 17
4.1.3 Summary and discussion on toxicokinetics 17
4.2 ACUTE TOXICITY 20
4.2.1 Non-human information 20
4.2.1.1 Acute toxicity: oral 20

4.2.1.2 Acute toxicity: inhalation 20
4.2.1.3 Acute toxicity: dermal 20
4.2.1.4 Acute toxicity: other routes 21
4.2.2 Human information 21
4.2.3 Summary and discussion of acute toxicity 21
4.2.4 Comparison with criteria 21
4.2.5 Conclusions on classification and labelling 21
4.3 SPECIFIC TARGET ORGAN TOXICITY – SINGLE EXPOSURE (STOT SE) 21
4.3.1 Summary and discussion of Specific target organ toxicity – single exposure 21
4.3.2 Comparison with criteria 21
4.3.3 Conclusions on classification and labelling 22
4.4 IRRITATION 22
4.4.1 Skin irritation 22
4.4.1.1 Non-human information 22
4.4.1.2 Human information 22
4.4.1.3 Summary and discussion of skin irritation 22
4.4.1.4 Comparison with criteria 22
4.4.1.5 Conclusions on classification and labelling 22
4.4.2 Eye irritation 22
4.4.2.1 Non-human information 22
4.4.2.2 Human information 22
4.4.2.3 Summary and discussion of eye irritation 22
4.4.2.4 Comparison with criteria 22
4.4.2.5 Conclusions on classification and labelling 22
4.4.3 Respiratory tract irritation 22
4.4.3.1 Non-human information 23
4.4.3.2 Human information 23
4.4.3.3 Summary and discussion of respiratory tract irritation 23
4.4.3.4 Comparison with criteria 23
4.4.3.5 Conclusions on classification and labelling 23
4.5 CORROSIVITY 23
4.5.1 Non-human information 23
4.5.2 Human information 23
4.5.3 Summary and discussion of corrosivity 23
4.5.4 Comparison with criteria 23
4.5.5 Conclusions on classification and labelling 23
4.6 SENSITISATION 23
4.6.1 Skin sensitisation 23
4.6.1.1 Non-human information 23
4.6.1.2 Human information 23
4.6.1.3 Summary and discussion of skin sensitisation 23
4.6.1.4 Comparison with criteria 23
4.6.1.5 Conclusions on classification and labelling 23
4.6.2 Respiratory sensitisation 24
4.6.2.1 Non-human information 24
4.6.2.2 Human information 24
4.6.2.3 Summary and discussion of respiratory sensitisation 24
4.6.2.4 Comparison with criteria 24
4.6.2.5 Conclusions on classification and labelling 24
4.7 REPEATED DOSE TOXICITY 24
4.7.1 Non-human information 26
4.7.1.1 Repeated dose toxicity: oral 26
4.7.1.2 Repeated dose toxicity: inhalation 28
4.7.1.3 Repeated dose toxicity: dermal 28
4.7.1.4 Repeated dose toxicity: other routes 29
4.7.1.5 Human information 29
4.7.1.6 Other relevant information 29
4.7.1.7 Summary and discussion of repeated dose toxicity 29
4.8 SPECIFIC TARGET ORGAN TOXICITY (CLP REGULATION) – REPEATED EXPOSURE (STOT RE) 29
4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation 29
4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE 29

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE *30*
4.9 GERM CELL MUTAGENICITY (MUTAGENICITY) 30
4.9.1 Non-human information *31*
4.9.1.1 In vitro data 31
4.9.1.2 In vivo data 31
4.9.2 Human information *31*
4.9.3 Other relevant information *31*
4.9.4 Summary and discussion of mutagenicity *31*
4.9.5 Comparison with criteria *31*
4.9.6 Conclusions on classification and labelling *31*
4.10 CARCINOGENICITY 31
4.10.1 Non-human information *31*
4.10.1.1 Carcinogenicity: oral 31
4.10.1.2 Carcinogenicity: inhalation 31
4.10.1.3 Carcinogenicity: dermal 31
4.10.2 Human information *31*
4.10.3 Other relevant information *31*
4.10.4 Summary and discussion of carcinogenicity *31*
4.10.5 Comparison with criteria *31*
4.10.6 Conclusions on classification and labelling *31*
4.11 TOXICITY FOR REPRODUCTION 32
4.11.1 Effects on fertility *34*
4.11.1.1 Non-human information 34
4.11.1.2 Human information 34
4.11.2 Developmental toxicity *34*
4.11.2.1 Non-human information 34
4.11.2.2 Human information 34
4.11.3 Other relevant information *34*
4.11.4 Summary and discussion of reproductive toxicity *35*
4.11.5 Comparison with criteria *44*
4.11.6 Conclusions on classification and labelling *46*
4.12 OTHER EFFECTS 46
4.12.1 Non-human information *46*
4.12.1.1 Neurotoxicity 46
4.12.1.2 Immunotoxicity 46
4.12.1.3 Specific investigations: other studies 47
4.12.1.4 Human information 47
4.12.2 Summary and discussion *47*
4.12.3 Comparison with criteria *47*
4.12.4 Conclusions on classification and labelling *47*

5 ENVIRONMENTAL HAZARD ASSESSMENT 47

6 REFERENCES 47

7 ANNEXES 53

ANNEX 1 EXPOSURE AND DEVELOPMENTAL TOXICITY HAZARD ASSESSMENT **53**

ANNEX 2 READ ACROSS JUSTIFICATION **68**

READ ACROSS JUSTIFICATION 68

LIST OF ENDPOINTS COVERED 72

PHYSICO-CHEMICAL PROPERTIES (APPENDIX B) 73

TOXICOLOGICAL PROPERTIES (APPENDIX C) 73

MOLECULAR STRUCTURE (APPENDIX A) 73

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo) tetraacetate
EC number:	205-391-3
CAS number:	140-01-2
IUPAC name:	pentasodium 2,2',2",2"',2""-(ethane-1,2-diylnitrilo) pentaacetate
Annex VI Index number:	***na***
Degree of purity:	***100%***
Impurities:	***na***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	No current Annex VI entry exists
Current proposal for consideration by RAC	Acute Tox. 4; H332 STOT. RE 2; H373 Repr. 2; H361d (Oral)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4; H332 STOT. RE 2; H373 Repr. 2; H361d (Oral)

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				Not sufficient for classification
2.2.	Flammable gases				Not sufficient for classification
2.3.	Flammable aerosols				Not sufficient for classification
2.4.	Oxidising gases				Not sufficient for classification
2.5.	Gases under pressure				Not sufficient for classification
2.6.	Flammable liquids				Not sufficient for classification
2.7.	Flammable solids				Not sufficient for classification
2.8.	Self-reactive substances and mixtures				Not sufficient for classification
2.9.	Pyrophoric liquids				Not sufficient for classification
2.10.	Pyrophoric solids				Not sufficient for classification
2.11.	Self-heating substances and mixtures				Not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases				Not sufficient for classification
2.13.	Oxidising liquids				Not sufficient for classification
2.14.	Oxidising solids				Not sufficient for classification
2.15.	Organic peroxides				Not sufficient for classification
2.16.	Substance and mixtures corrosive to metals				Not sufficient for classification
3.1.	Acute toxicity - oral				Not sufficient for classification
	Acute toxicity - dermal				Not sufficient for classification
	Acute toxicity - inhalation	Category 4		na	

3.2.	Skin corrosion / irritation				Not sufficient for classification
3.3.	Serious eye damage / eye irritation				Not sufficient for classification
3.4.	Respiratory sensitisation				Not sufficient for classification
3.4.	Sin sensitisation				Not sufficient for classification
3.5.	Germ cell mutagenicity				Not sufficient for classification
3.6.	Carcinogenicity				Not sufficient for classification
3.7.	Reproductive toxicity	Category 2 (Oral)		**na**	
3.8.	Specific target organ toxicity –single exposure				Not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Category 2		**na**	
3.10.	Aspiration hazard				Not sufficient for classification
4.1.	Hazardous to the aquatic environment				Not sufficient for classification
5.1.	Hazardous to the ozone layer				Not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal word: Warning

GHS08: health hazard

GHS07: exclamation mark

Hazard statements:

H332 Harmful if inhaled;

H373: May cause damage to respiratory tract following prolonged or repeated inhalation exposure;

H361: Suspected of damaging the unborn child if ingested

Proposed notes assigned to an entry: Not applicable

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

There is no current classification in Annex VI of Regulation No. 1272/2008 for pentasodium DTPA. Since 2010, an industry wide self classification for developmental toxicity (Cat. 2) and acute inhalation toxicity (Cat. 4) has been adopted. Following completion of a repeat dose inhalation study in 2014, a proposal for STOT RE classification (Cat. 2) has been adopted and is currently being implemented.

2.2 Short summary of the scientific justification for the CLH proposal

The substance pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo)tetraacetate is a mono constituent substance (origin: organic). This document is specific for classification and labeling of pentasodium DTPA (DTPA-Na5, Cas No. 140-01-2). Where appropriate, data have also been used from DTPA acid (DTPA-H5; CAS no. 67-43-6) and pentapotassium DTPA (DTPA-K5; CAS no. 7216-95-7). Read-across to other uncomplexed or 'empty' DTPA chelates is considered appropriate given they all contain identical functional groups (n=5) that are central to their common mechanism of action. A detailed justification for read-across within the aminocarboxylic acid based chelants chemical category is provided in Annex 2 and takes into account considerations presented in the recently released Read-Across Assessment Framework. The ability of chelants to bind metal ions is the mechanism by which they produce toxicity and available study data covering a range of endpoints indicates a similar behaviour, biologically, for these substances. A proposal for harmonized classification and labeling is also addressed for the DTPA acid and pentapotassium DTPA in separate dossiers and as such, certain data may be common across the three proposals. A possible harmonized classification for DTPA-acid and its salts should however exclude metal salts of DTPA, such as iron DTPA's, because the hazardous properties of the 'non-chelated' or 'empty' DTPA's (DTPA-H5, DTPA-K5 and DTPA-Na5) are clearly different to the metal salts and related to their interaction with ions such as Ca and Zn (see pages 35 and 36 of this document and Annex 2). Those interactions are demonstrated to have no or far less of an effect when DTPA is already complexed with metal ions such as iron and zinc because the affinity for these ions is much stronger than for most other ions such as H, Na or K. Therefore, whilst data from metal salts may be useful in understanding the mode of action of DTPA, direct read-across between 'non-chelated' or 'empty' DTPA's and the metal salts of DTPA is considered not appropriate.

Acute toxicity Cat. 4 is proposed based on an estimated 4h-LC50 value for DTPA of between 1000 and 5000 mg/m^3 based on read across data from Na_2H_2EDTA.

STOT-RE Cat. 2 is proposed based on histopathological findings observed in an OECD 413 guideline and GLP compliant repeat dose inhalation study conducted with Na_2H_2EDTA.

Developmental toxicity Cat. 2, specifically by the oral route, is proposed based on developmental effects observed in an OECD 414 guideline and GLP compliant study. The effects observed are considered secondary to dietary and systemic zinc depletion as a result of the chelating ability of pentasodium DTPA.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

There is no current classification in Annex VI of Regulation No. 1272/2008.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

There is no current classification in Annex VI of Regulation No. 1272/2008

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Acute tox. 4 H332: Harmful if inhaled; STOT RE 2 H373: May cause damage to respiratory tract following prolonged or repeated inhalation exposure; Developmental Toxicant Category 2, H361: Suspected of damaging the unborn child if ingested.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Pentasodium DTPA (Cas No. 140-01-2) is currently marketed throughout the European Community and whilst an industry-wide self classification is in place since 2010, a harmonized classification is considered relevant for other legislation or processes as pentasodium DTPA was included in the PACT-RMOA list of substances by the French Member State in September 2014.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

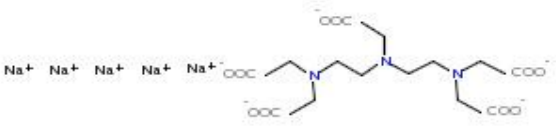
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	205-391-3
EC name:	pentasodium (carboxylatomethyl)iminobis (ethylenenitrilo)tetraacetate
CAS number (EC inventory):	140-01-2
CAS number:	140-01-2
CAS name:	Glycine, N,N-bis[2-[bis(carboxymethyl)amino]ethyl]-,sodium salt (1:5)
IUPAC name:	pentasodium 2,2',2",2"',2""-(ethane-1,2-diylnitrilo) pentaacetate
CLP Annex VI Index number:	NA
Molecular formula:	C14H23N3O10. 5Na
Molecular weight range:	503.2557

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	REMARKS
pentasodium (carboxylatomethyl)iminobis(ethylenenitrilo) tetraacetate	100%		CLH proposal is for pure DTPA only. Impurity profile from each Joint Submitter can be found in their REACH dossier.

Current Annex VI entry:

Table 7: Impurities (non-confidential information)

IMPURITY	TYPICAL CONCENTRATION	CONCENTRATION RANGE	REMARKS
Impurities	n.a.		

Current Annex VI entry:

Table 8: Additives (non-confidential information)

ADDITIVE	FUNCTION	TYPICAL CONCENTRATION	CONCENTRATION RANGE	REMARKS
N.A.				

Current Annex VI entry:

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Solid (or aqueous solution)		
Melting/freezing point	Decomposes without melting		
Boiling point	Decomposes without melting		
Relative density	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		
Surface tension	N/A		
Water solubility	No data		
Partition coefficient n-octanol/water	-3.05		Measured
Flash point	N/A		
Flammability	N/A		
Explosive properties	N/A		
Self-ignition temperature	N/A		
Oxidising properties	N/A		
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	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:		

	pK1: 1.79; pK2: 2.56; pK3: 4.42; pK4: 8.76 and pK5: 10.42. All conducted at 20 C.		
Viscosity	N/A		

2 MANUFACTURE AND USES

2.1 Manufacture

Pentasodium DTPA is synthesised by carboxymethylation of diethylene triamine.

2.2 Identified uses

DTPA is potentially used in a wide number of industries including pulp and paper industries (main use), laundry detergents, cleaners, soaps, and textiles. It can also be used as a setting retarder in the production of plaster, scale remover for substances such as barium sulphate, and as complexing agents of metals used as micronutrients for plants (BASF, 2007). Exposures of DTPA to the general public are minimal. The product is only used in trace amounts in final products (< 2% consumer cleaning products and <0.1% in personal care products), is poorly absorbed dermally, and does not volatilize. Thus consumer exposure, whilst it occurs, would be expected to be very low and significantly lower than workers involved in manufacturing and formulating DTPA.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not the subject of this classification and labeling proposal.

3.1.1 Conclusions on classification and labelling

Not applicable

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

See summary below

4.1.1 Non-human information

See summary below

4.1.2 Human information

See summary below

4.1.3 Summary and discussion on toxicokinetics

Absorption:

Oral:

In studies conducted using rats, dogs and humans, (Dudley et al. 1980a, b; Stevens et al. 1962, Resnick et al. 1990) the oral absorption of DTPA and DTPA salts appears to be very low, with an average intestinal absorption of 3 to 5% across all species.

Dermal:

There are no data available on the dermal absorption potential of DTPA, however in a risk assessment by the European Chemicals Bureau (2004), a structurally related chelating agent, EDTA was reported as having very low dermal penetration potential, with approximately 0.001% absorption through the skin. Considering the larger molecular weight of DTPA compared to EDTA it is believed that the dermal penetration of DTPA will be equally low, i.e. approximately 0.001%.

Inhalation:

There have been a number of studies of the effectiveness of administering aerosolised DTPA complexes to humans via the inhalation route. The substances investigated were various radionuclide complexes of DTPA such as111In-DTPA, 99mTc-DTPA, Pu-DTPA and also the zinc and calcium salts of DTPA. These studies demonstrated that DTPA complexes are absorbed from the respiratory tract into the systemic circulation. The degree of absorption is however dependant on the site of deposition within the respiratory

tract. Dudley et al. (1980b) demonstrated in dogs that the percentage of applied dose absorbed through the respiratory tract increases the further into the respiratory tract the dose is deposited. DTPA deposited high up in the respiratory tract was predominantly swallowed, with approximately 23% absorption from the nasopharyngeal region compared to approximately 90% absorption following instillation into the pulmonary region. A similar pattern was observed in rats (Stather et al. 1976, referenced in Dudley at al. 1980b). In humans, DTPA absorption following the administration of a nebulised spray containing DTPA was estimated to be 20% of the administered dose (Jolly et al. 1972). In this study the aerosol was inhaled through the mouth and mean droplet size was between 0.3 and 2 micro meters, making it more likely that droplets would travel more deeply into the respiratory tract, where absorption is more favourable.

Based on the available data it thus appears that absorption of aerosolised DTPA depends predominantly on the penetration of the droplets into the respiratory tract. The deeper the DTPA is deposited, the more likely it is that it will be absorbed. Considering the study by Jolly et al (1972) where a nebuliser was used to produce very small droplet sizes, it seems that a somewhat worst case estimate for absorption following exposure to an aerosol is approximately 20%.

Exposure to DTPA is also possible via inhalation of the powdered form of the chelating agent. Therefore, considering the potential for absorption via the lung following exposure to inhaled powder, the potential for absorption will depend on the proportion of the inhaled powder that reaches the deeper lung, since much of the material that impacts higher up in the respiratory tract will be carried up into the mouth via the mucocilliary transport. Taking this into account, the ICRP (1994) reported that particles above 10μm are only partially inhaled. Some of the particles are sufficiently large not to be drawn in with an inspired breath (40%). Of the 60% inhaled, 50% are deposited in the extrathoracic air ways and only 10% enter the lung and result in a true inhalation dose. Therefore only 10% of the powder particles less than 10μm in diameter are available for absorption via the lungs, the remaining powder is either not inhaled or deposited higher up in the respiratory tract and eventually swallowed.

Distribution / Excretion:

Following exposure, the portion of the dose that is absorbed and thus available systemically is excreted via the urine very quickly. Intravenous administration of DTPA to man (Stevens et al. 1962) resulted in almost complete excretion via the urine within 24 hours, with a half life of approximately 2 to 4 hours. DTPA does not appear to become sequestered by any particular tissues, and in pregnant rats DTPA does not appear to pass into the fetal circulation (Zylicz et al. 1975). Thus DTPA does not give rise to any concerns regarding bioaccumulation.

Following an oral dose, the unabsorbed material remains in the gastrointestinal tract and is excreted via the faeces. There appears to be little or no excretion of absorbed DTPA via the faeces (Stevens et al 1962).

Effect of DTPA on excretion of metals

There are many studies where the effects of administering DTPA to animals and man on the excretion of essential metals such as calcium, zinc, iron, manganese, magnesium etc. have been studied. Systemic administration of DTPA (intravenous, intraperitoneal, subcutaneous) causes an increase in urinary excretion of zinc, calcium and to a lesser extent iron and manganese. The reason for the increase in the urinary excretion of certain metals following systemic exposure to DTPA is due to its formation of complexes with 'free' metals in the blood and lymph. These complexes are then excreted via the urine, carrying the metals out of the body.

DTPA has a high affinity for zinc and as such, zinc is one of the metals most affected by administration of DTPA. The increased excretion of zinc following prolonged administration of DTPA to humans has manifested as a zinc deficiency, treatable with supplementation of zinc sulphate, or administration of the zinc complex of DTPA.

The removal of metals from the body by DTPA is dependent on a number of factors:

1. The dosing regime. Due to the short half life of DTPA in the body, a single dose is less effective at removing endogenous metals than multiple doses or a continuous transfusion.
2. The availability of unbound or 'free' metals in the circulation. Due to the limited availability of 'free' zinc in the body, the dose of DTPA administered is not directly proportional to the amount of zinc excreted (Havlicek et al. 1967). Small doses will bind more zinc per mole of chelant compared to larger doses.
3. The presence of other metals in the circulation. DTPA has a strong affinity for zinc however it also binds manganese, calcium, iron, sodium, potassium. The presence of higher concentrations of these will therefore affect how much zinc is bound by DTPA
4. The metal complex administered. Zinc complexes of DTPA are more stable and so less likely to cause an increase in excretion of metals. Sodium, Potassium and calcium salts do dissociate more easily and so the chelating agent is released and capable of chelating other metals, increasing their excretion/preventing their absorption

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
rat (male only, Wistar) inhalation: nose only, dust aerosol 30; 300 and 1,000 mg/m^3 10/group: 30 or 300 mg/m^3 20/group: control or 1000mg/m^3 Exposure: 6 hours per day for 5 days OECD Guideline 412 (Repeated dose inhalation toxicity 28/14 Day) Purity 91.7%	Animals receiving 1000mg/m^3 were exposed for 1 day only. Exposure resulted in lethality of 6/20 male animals. Histological examination of the lung revealed congestion, oedema, multifocal heamorrhages and inflammatory infiltration.	Well conducted study in accordance with GLP Test material (CAS number): Na_2H_2 EDTA, 139-33-3 (read-across)	BASF SE (2010)
rat (strain not given) inhalation: vapour 12/sex/dose Exposure: 8 hours per day for 5 days Concentration not given Non-guideline Purity 40 %	No mortalities observed	Test material (CAS number): Pentasodium DTPA, 140-01-2 Non-guideline Non-GLP	BASF SE (1968)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Not the subject of this CLH proposal.

4.2.1.2 Acute toxicity: inhalation

See summary below

4.2.1.3 Acute toxicity: dermal

Not the subject of this CLH proposal.

4.2.1.4 Acute toxicity: other routes

Not the subject of this CLH proposal.

4.2.2 Human information

Not applicable

4.2.3 Summary and discussion of acute toxicity

DTPA acid, potassium and sodium salts are not volatile. Therefore the potential for acute exposure to vapours of these substances is remote. In a study performed using pentasodium DTPA (BASF SE 1968), rats exposed for 8 hours to the vapour generated at room temperature did not suffer any adverse effects. Acute inhalation exposure to the solid form of these materials is self limiting due to the particle sizes of the powders (90% >60 micrometers diameter) which will significantly limit the amount inhaled and delivered to the respiratory tract. Also, workers handling the powdered form are required to wear protection (face masks) and this will further limit the possibility for an acute exposure. If such an exposure were to occur it is not expected that it would be more potent than an oral exposure with respect to systemic toxicity. A structurally-related compound, EDTA-Na2H2 showed limited inhalation toxicity (i.e. a 6-h LC50 value of 1000 mg/m3, corresponding to an estimated 4-h LC50 value between 1000 and 5000 mg/m3) (BASF SE 2010).

The following information is taken into account for any hazard / risk assessment:

Inhalation: 1 acute inhalation study using pentasodium DTPA, 1 using a structurally related compound disodium EDTA.

4.2.4 Comparison with criteria

Pentasodium DTPA is considered to meet the requirements for classification for acute toxicity as described in REGULATION (EC) No 1272/2008.

4.2.5 Conclusions on classification and labelling

The proposed classification for DTPA is Acute Tox 4, H332 Harmful if inhaled.

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

Not the subject of this CLH proposal.

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

Not the subject of this CLH proposal.

4.3.2 Comparison with criteria

Not applicable

4.3.3 Conclusions on classification and labelling

Not applicable

4.4 Irritation

Not the subject of this CLH proposal.

4.4.1 Skin irritation

4.4.1.1 Non-human information

4.4.1.2 Human information

4.4.1.3 Summary and discussion of skin irritation

4.4.1.4 Comparison with criteria

4.4.1.5 Conclusions on classification and labelling

No classification

4.4.2 Eye irritation

Not the subject of this CLH proposal.

4.4.2.1 Non-human information

4.4.2.2 Human information

4.4.2.3 Summary and discussion of eye irritation

4.4.2.4 Comparison with criteria

4.4.2.5 Conclusions on classification and labelling

No classification

4.4.3 Respiratory tract irritation

Not the subject of this CLH proposal.

4.4.3.1 Non-human information

4.4.3.2 Human information

4.4.3.3 Summary and discussion of respiratory tract irritation

4.4.3.4 Comparison with criteria

4.4.3.5 Conclusions on classification and labelling

No classification

4.5 Corrosivity

Not the subject of this CLH proposal.

4.5.1 Non-human information

4.5.2 Human information

4.5.3 Summary and discussion of corrosivity

4.5.4 Comparison with criteria

4.5.5 Conclusions on classification and labelling

No classification

4.6 Sensitisation

Not the subject of this CLH proposal.

4.6.1 Skin sensitisation

4.6.1.1 Non-human information

4.6.1.2 Human information

4.6.1.3 Summary and discussion of skin sensitisation

4.6.1.4 Comparison with criteria

4.6.1.5 Conclusions on classification and labelling

No classification

4.6.2 Respiratory sensitisation

Not the subject of this CLH proposal.

4.6.2.1 Non-human information

4.6.2.2 Human information

4.6.2.3 Summary and discussion of respiratory sensitisation

4.6.2.4 Comparison with criteria

4.6.2.5 Conclusions on classification and labelling

No classification

4.7 Repeated dose toxicity

Table 17: Summary table of relevant repeated dose toxicity studies

METHOD	RESULTS	REMARKS	REFERENCE
rat (Sprague-Dawley) 5/sex/group oral: gavage 0, 83, 333, 1330 mg/kg bw/day Exposure: daily, 28 days OECD Guideline 407 (Repeated dose oral toxicity 28 Day) Purity 43.7%	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 included piloerection, hunched posture, abnormal gait, ptosis, decreased respiratory rate and diarrhea, 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. 333 mg/kg bw/day: Clinical signs of 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. 83 mg/kg bw/day:): no substance related changes observed	2 (reliable with restriction) supporting study experimental result Test material (CAS number): Pentapotassium DTPA, 7216-95-7 (read-across)	Elliot et. al. (1987)

	Pallor seen in all dose groups but was considered related to blood sampling. NOAEL 83 mg/kg bw/day (nominal)		
rat (Wistar) 5/sex/group oral: drinking water 600, 3000 or 12000 ppm Exposure: Daily, 28 Days OECD Guideline 407 (Repeated dose oral toxicity 28 Day) Purity 43.7%	12000ppm: (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. 3000ppm (420 mg/kg bw/day): significantly decreased bodyweight change in males in last test week (approx. 10% lower than controls), increase in ALT in males and decrease in ALP in females 600ppm (75 mg/kg bw/day): no substance related changes observed NOAEL = 75/mg/kg bw/day	1 (reliable without restriction) supporting study experimental result Test material Pentasodium DTPA (CAS number): 140-01-2	BASF (2002)
rat (male only, Wistar) inhalation: nose only, dust aerosol 30; 300 and 1,000 mg/m^3 10/group: 30 or 300 mg/m^3 20/group: control or 1000 mg/m^3 Exposure:6 hours per day for 5 days OECD Guideline 412 (Repeated dose inhalation toxicity 28/14 Day) Purity 91.7%	1000 mg/m^3: Mortality of 6/20 animals following single exposure. Multifocal haemorrhage and inflammatory cell infiltrates in lungs. 300 mg/m^3: Clinical signs of 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 regeneratie hyperplasia of bronchiolar epithelium and mucous cell hyperplasia of large bronchi. Interstitial infiltration	1 (reliable without restriction) supporting study experimental result Test material Na_2H_2 EDTA**,** (CAS number): 139-33-3 (read-across)	BASF SE (2010)

	of eosinophylic granulocytic cells. 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 eosinophylic granulocytic cells. No histopathological findings in recovery animals therefore all lesions in low/mid dose groups considered reversible. LOAEC 30 mg/m^3		
rat (Wistar) 10/sex/group inhalation: nose only, dust 0.5; 3.0 and 15 mg/m^3 Exposure: 6 hours/day, 5 days/week for 13 weeks, total 65 exposures OECD Guideline 413 Subchronic inhalation toxicity study 90 Day) Purity 88.1g/100g	15 mg/m^3: 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. 3.0 mg/m^3: Increased absolute lung weights in females only. No difference in relative lung weight. No other compound related effects observed. 0.5 mg/m^3: no substance related changes observed. NOAEC 3 mg/m^3	1 (reliable without restriction) key study experimental result Test material (CAS number): Na_2H_2 EDTA,139-33-3 (read-across)	BASF (2014)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

There are two standard guideline repeated dose toxicity studies available for DTPA (Elliot et al 1987, BASF SE 2002). The studies were conducted using either the potassium or sodium salt. Overall, the two studies are consistent in the adverse effects identified, particularly with respect to the apparent target organs and the no effect levels. The only significant difference between the two studies is the mortality encountered in the study using gavage administration (Elliot et al 1987). This increased mortality in the males (4/5) and females (1/5) was probably due to a bolus dose effect rather than a

greater toxicity of the potassium salt since the sodium DTPA was administered via the drinking water rather than gavage. With the exception of the high dose group mortality, and the decreased body weight and food consumption in the high and mid dose groups, the other adverse effects identified were relatively minimal (changes in clinical chemistry parameters and some alterations in urine parameters (high dose only)). The liver appears to have been a target organ for toxicity, but these effects may have been due to the decreased bodyweight and stress rather than direct compound related toxicity.

The relatively minimal systemic toxicity observed is also consistent with the low degree of absorption following oral administration and its subsequent rapid excretion (half life of approx 2 hours). DTPA is chemically stable and not reactive or metabolized, thus its toxicity is generally associated with the ability to chelate essential metals. DTPA is known to be capable of producing deficiencies in zinc when administered systemically or orally. The effects in the 2 repeated dose studies in the high dose groups are consistent with the development of a nutritional deficiency, such as a zinc deficiency. The reduction in food intake and associated bodyweight decrease are known to be associated with deficiencies of zinc, as the animals reduce their food intake in an effort to trigger catabolism of their tissues to release more zinc. If these studies had continued for a longer period then it is highly likely that more obvious changes in pathology consistent with a zinc deficiency would appear.

Further support for the toxicity of DTPA being linked to its ability to remove essential metals such as zinc comes from toxicity studies conducted using the zinc salt of DTPA. In a number of comparative studies (predominantly studying developmental toxicity), the zinc salt of DTPA has been significantly less toxic compared to the calcium salt of DTPA.

Due to the nature of DTPA toxicity, it is unlikely that a longer study would identify additional adverse effects or a significantly lower no effect level. There is a finite amount of essential metals in the diet and body. DTPA can only bind to metals during the brief period when it comes into contact (either in the gut or the blood). If a dose of DTPA is insufficient to significantly impact an animal’s intake of essential metals then increasing the number of days of exposure will not have a more severe effect, i.e. there will be a threshold. Thus, rather than leading to a significantly lower no effect level, a longer term study would probably just lead to a greater degree of zinc deficiency thus it would show an increased severity of the effects observed in the shorter study.

There are no long term guideline studies for DTPA, however there is a longer term study available for another salt of DTPA (Planas-Bohne, 1976). In this study Calcium DTPA (approximately 44 mg/DTPA/kg bw) was administered via intraperitoneal injection twice per week to rats for 44 weeks. There were no observed effects on any of the parameters examined (including body weight, clinical signs, pathology, urinalysis, clinical chemistry and hematology). However, by administering DTPA only 2 times per week, there was a recovery period between the doses where the rats would have been able to replace essential nutrients like zinc. Therefore, whilst this study does not indicate any adverse effects following long term exposure to DTPA, if the dose had been administered daily there would probably have been toxicity since it would be equivalent to a daily oral dose of between approximately 400 and 800 mg/kg bw oral dose (assuming 5 - 10% absorption in the gut). This study does however illustrate that the toxicity of DTPA is reversible, since there was apparently sufficient time within this study design for the animals to recoup lost essential elements.

.

4.7.1.2 Repeated dose toxicity: inhalation

To date, two guideline studies have been performed to evaluate the potential toxicity of chelating agents following repeated inhalation exposure. A 5 -day inhalation study with a structurally related compound Na2H2EDTA showed histopathological changes in the respiratory tract at a concentration of 30 mg/m3 (BASF SE 2010). After a recovery period of 14 days, all changes had disappeared. A 90-day inhalation study with the same substance was carried out according to OECD 413 and EC No 440/2008 (BASF SE 2014). Wistar rats, 10 per sex per test group, were exposed (head-nose only), to dust aerosol for 6 hours per day, on 5 consecutive days per week for 13 weeks (65 exposures). The target concentrations were 0.5, 3 and 15 mg/m3. A concurrent control group was exposed to air. On each exposure day a clinical examination was performed before, during and after exposure. Detailed clinical observation was performed at the beginning, midterm and end of the study. Ophthalmology was performed before the beginning of the exposure in all test groups and at the end of the end of the exposure in the control and high concentration group animals. Body weights and food consumption of the animals were determined weekly. At the end of the exposure period, functional observation battery and motor activity tests were performed. On the day after the last exposure, blood was sampled and examined for a range of hematology and clinical chemical parameters as indicated in the guideline. After blood sampling the animals were sacrificed and subject to necropsy (including macroscopic examination of the major internal organs and collection of organ weight data). Selected tissues were processed histopathologically and were evaluated by light microscopy according to the OECD guideline.

When compared with the control group, the following treatment-related adverse findings were noted in Wistar rats after 90 days of inhalation:

High concentration (15 mg/m³)

· Focal hyperplasia of the laryngeal epithelium at the base of the epiglottis in one female animal

· Slight granulocytic infiltrates at the base of the epiglottis of the larynx in two female animals

Mid (3 mg/m³) and low concentration (0.5 mg/m³)

No adverse findings.

4.7.1.3 Repeated dose toxicity: dermal

There are no data on repeated dose dermal exposure. However, due to the very low dermal absorption potential of pentasodium DTPA (predicted <0.001%) it is unlikely any effects would be observed that would result in classification for STOT RE via the dermal route.

4.7.1.4 Repeated dose toxicity: other routes

No data available

4.7.1.5 Human information

No data available

4.7.1.6 Other relevant information

No data

4.7.1.7 Summary and discussion of repeated dose toxicity

See below

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Inhalation exposure of rats to Na2H2EDTA for 90 days (65 exposures) did not lead to any substance related clinical signs of toxicity. Nor were there any effects in clinical chemistry and hematology. Histological examination revealed some effects in larynx at the highest tested concentration of 15 mg/m³. No signs of systemic toxicity were observed up to a concentration of 15 mg/m³. Signs of local toxicity were observed only at the high concentration of 15 mg/m³. Under the current test conditions, the No Observed Adverse Effect Concentration (NOAEC) for local effects was 3 mg/m3, the NO(A)EC for systemic effects was 15 mg/m³.

Inhalation exposure of rats to Na2H2EDTA for 6 hours per day, 5 consecutive days causes concentration dependent lesions in the larynx and lungs that were fully reversible within 14 days. Due to histopahological changes in the low-concentration group a no observed effect level could not be determined.

<u>The following information is taken into account for any hazard / risk assessment:</u>

One 28-day oral gavage study in rats using pentapotassium DTPA, one 28-day oral, drinking water study using pentasodium DTPA, one 90-day and one 5-day inhalation study using disodium EDTA. Additional information comes from published literature on other salts of DTPA such as zinc and calcium.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Classification for repeated dose toxicity is proposed as the CLP criteria for classification for target organ toxicity are considered met. According to REGULATION (EC) No 1272/2008., substances that, on the basis of evidence from studies in experimental

animals can be presumed to have the potential to be harmful to human health following repeated exposure should be classified as STOT RE.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

According to regulation (EC) 1272/2008 substances are classified in Category 1 for target organ toxicity when;

"*Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.*"

Further it goes on to say;

„Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of:

— reliable and good quality evidence from human cases or epidemiological studies; or

— observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations."

Given the available evidence for pentasodium DTPA and the cut-off values provided in the regulation, assignment in Category 1 would not be considered appropriate.

For assignment in Category 2 the regulation goes on to state;

„Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure.

Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations."

For Pentasodium DTPA, given the cut-off values provided, and the available evidence from a structurally related substance, with only slight histopathological effects at the larynx at a concentration of 15 mg/m3, it would be appropriate to apply STOT RE Category 2, H373, specifically for the inhalation route.

4.9 Germ cell mutagenicity (Mutagenicity)

Not the subject of this CLH proposal.

4.9.1 Non-human information

4.9.1.1 In vitro data

4.9.1.2 In vivo data

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

4.9.5 Comparison with criteria

4.9.6 Conclusions on classification and labelling

No classification

4.10 Carcinogenicity

Not the subject of this CLH proposal.

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

4.10.1.2 Carcinogenicity: inhalation

4.10.1.3 Carcinogenicity: dermal

4.10.2 Human information

4.10.3 Other relevant information

4.10.4 Summary and discussion of carcinogenicity

4.10.5 Comparison with criteria

4.10.6 Conclusions on classification and labelling

No classification

4.11 Toxicity for reproduction

Table 20: Summary table of relevant reproductive and developmental toxicity studies

METHOD	RESULTS	REMARKS	REFERENCE
rat (Wistar) 22 pregnant females/group oral: gavage 100; 400 and 1,000 mg/kg body weight Vehicle: water Exposure: day 6 through day 15 post coitum (daily) OECD Guideline 414 (Prenatal Developmental Toxicity Study) Purity 43.7%	1000 mg/kg bw/day: reduced bodyweight and food consumption, dark yellow discolouration of the faeces in all females. Statistically significant lower mean gravid uterus weights, 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 47.4% affected fetuses/litter in controls). 400 mg/kg bw/day: Sttisticlly significant increase in rate of fetuses with skeletal retardations (63.8% affected fetuses/litter vs 47.4% affected fetuses/litter in controls). 100mg/kg bw/day: no substance related effects on dams, gestational parameters or fetuses. NOAEL (maternal toxicity): 400 mg/kg bw/day (nominal) NOAEL (teratogenicity): 100 mg/kg bw/day (nominal)	1 (reliable without restriction) key study experimental result Test material Pentasodium DTPA, CAS number: 140-01-2	BASF SE (1994)
rat (Wistar) No. of animals not specified Subcutaneous 0 µmol/kg body weight (nominal conc. (negative control and another group administered with 0.60 ml of isotonic saline))	NOEL (maternal toxicity): 1080 µmol/kg body weight (no overall effects) NOEL (teratogenicity): 180 µmol/kg body weight ((for Ca-DTPA only), overall effects) NOEL (teratogenicity): 1080 µmol/kg body weight ((for Zn-DTPA only), overall effects)	2 (reliable with restrictions) supporting study experimental result Test material (Common name): Zinc and Calcium salts of DTPA (CAS number. not given)	Fukuda et al (1982)

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 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) equivalent or similar to EPA OPP 83-3 (Prenatal Developmental Toxicity Study) Purity: not specified	A decrease in the survival rates was observed in only the groups injected daily with 1080 µmol/kg body weight (36 human dose) of Ca-DTPA. Abnormalities such as exencephaly, microphthalmia, anophthalmia and fused ribs were observed in groups injected daily with 360, 720 and 1080 µmol/kg body weight (12, 24 and 36 human dose).		
mouse (C57BL/Do) No. of animals not specified subcutaneous 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) equivalent or similar to EPA OPP 83-3 (Prenatal Developmental Toxicity Study) 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 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.	2 (reliable with restrictions) supporting study experimental result Test material (Common name): Zinc DTPA (CAS Number not given)	Brummett et al (1977)

	Days 5-12 5760 µmole Zn-DTPA/kg/day: nine females given 8 daily injections. 5 non-pregnant at autopsy, 3 checked early in gestation had evidence of abortion. 1 gave birth early and ate her pups. None of the fetuses exhibited gross deformities, externally or skeletally except 1 fetus from a dam given 1440 µmole Ca-DTPA/kg/day which had exencephaly.		

4.11.1 Effects on fertility

4.11.1.1 Non-human information

No data available.

In a developmental toxicity study DTPA caused developmental toxicity via an induced zinc deficiency. Such a mode of action is also known to produce effects on male fertility (testicular toxicity) but only in the presence of other signs of systemic toxicity related to a zinc deficiency. A multigeneration study performed using DTPA in the diet would likely result in a zinc deficiency in the animals due to chelation of the dietary zinc by DTPA. As such, this type of study would only demonstrate the toxicity associated with a zinc deficiency rather than that of DTPA. Therefore, for the purposes of REACH registration a reproductive toxicity study was considered unjustified since it would not produce data that cannot already be predicted.

4.11.1.2 Human information

No data available

4.11.2 Developmental toxicity

The results of experimental studies are summarised in Table 20.

4.11.2.1 Non-human information

See extensive discussion below

4.11.2.2 Human information

See extensive discussion below

4.11.3 Other relevant information

See extensive discussion below

4.11.4 Summary and discussion of reproductive toxicity

Effects on fertility

DTPA is a chelating agent with a high affinity for metals such as Zinc, Manganese and Calcium (please refer to the section on toxicokinetics). Zinc is one of the most abundant metals in the human body (2-4g) and is present as a cofactor for a large number of enzymes (between 100 and 300), covering almost all classes of enzyme. As such, deficiencies in zinc can produce a wide array of symptoms including both reproductive and developmental toxicity. If a dietary multi generation reproductive toxicity study were to be performed with DTPA in the absence of any supplementation of essential minerals such as zinc, then it is highly likely that the DTPA would complex with enough of the zinc in the diet leading to an insufficient zinc intake in the animals. This would lead to evidence of male reproductive toxicity (specifically degeneration of the testicular tissue and reduced fertility), developmental toxicity such as terata of the skeletal and viscera and many of the symptoms of zinc deficiency such as alopecia, diarrhea, eye and skin lesions etc. Such a study would therefore not provide evidence of the reproductive or developmental toxicity of DTPA but rather the toxicity associated with a deficiency in zinc.

In the 2 available 28 -day studies where DTPA salts were dosed via gavage or the drinking water (Elliot 1987, BASF 2002) there were some clinical signs of a perturbation in nutrition in the high doses (diarrhea, decreased food consumption, decrease in bodyweight), and in the gavage fed animals there were deaths in the high dose group males. It is very likely that the deaths were associated with diarrhoea caused by chelation of metals such as zinc and calcium in the intestinal tract leading to decreased absorption of food and water loss. In these studies there was no evidence of testicular toxicity in any of the treated groups, indicating that the dosing regime was insufficient to produce sufficient zinc deficiency to lead to testicular toxicity. It is possible that a more prolonged dosing regimen could have produced a more extensive deficiency in zinc, however studies conducted with a similar chelating agent, EDTA (ethylene diamine tetraacetic acid) also failed to produce any evidence of testicular degeneration following dosing via gavage or drinking water (Wynn et al 1970, US Dept. of Health, Education & Welfare 1977, Kawamata et al 1980).

DTPA is poorly absorbed both orally and via dermal application and is unlikely to be absorbed significantly via inhalation due to its high particle size (>10 microns diameter) when in powdered form and low volatility when in solution. It is not metabolised and is excreted with a very short half life (2 hours) in humans and rats. With the exception of being able to complex metal ions, chelating agents are of low chemical reactivity as evidenced by their lack of genotoxicity and skin sensitising potential. As such, it is unlikely that the chelating agent itself is a proximate toxicant, but rather that its ability to bind metal ions is responsible for observed toxicity (as indicated above). Therefore if a study were conducted where metal ions (for example zinc) were supplemented sufficiently it is unlikely that any systemic toxicity, including developmental or reproductive effects would be observed. This is supported by developmental toxicity studies conducted using the zinc salt of DTPA where no developmental (or systemic) toxicity was observed in mice injected intraperitoneally at doses in excess of 1000 mg/kg bw/day (Brummett et al 1977), in contrast to the calcium salt of DTPA where the same doses caused significant increases in developmental and systemic toxicity in mice. Additional support comes from studies (and treatments) conducted in humans and

animals where the zinc salt of DTPA was administered with no evidence of systemic toxicity at therapeutic doses (Kalkwarf et al 1983; Sato 1993).

In conclusion, it is plausible that a standard dietary multigeneration study conducted with DTPA would identify evidence of reproductive and developmental toxicity. However such toxicity would be due to an induced deficiency in zinc and any reproductive or developmental effects would be observed only in the presence of, and secondary to parental toxicity. Reproductive toxicity effects secondary to a zinc deficiency should not be considered relevant for classification if it can be demonstrated that occupational or consumer exposure to DTPA would not result in a deficit in an individual's zinc status.

As part of a classification and labelling justification prepared for developmental toxicity it has been demonstrated that exposure to DTPA through worker and consumer uses would be insufficient to produce a deficiency in zinc in the workforce or consumer population (See Annex I). Since reproductive toxicity would require a zinc deficiency to be induced as a first step in the toxicity, it is very unlikely that this would occur.

Developmental toxicity

In a standard OECD Guideline teratogenicity study DTPA administration caused developmental toxicity (BASF 1994). In this study the dams in the high dose group exhibited reduced food consumption between GD 6-10, reduced bodyweight at GD17 and GD20, and reduced bodyweight gain during GD6-8 and GD15-17 ($p < 0.05$). Overall, bodyweight gain during and after cessation of treatment was lower in this group than in controls. Faeces were discoloured (dark yellow) in this group during treatment, but reverted to normal during the post-treatment phase. There were no differences between the other treatment groups and the control group. Gravid uterus weights in the dams of the high dose group were lower than control, as were the live litter sizes and bodyweights of both male and female fetuses. There was also an 11.5% reduction in adjusted bodyweight gain of the dams, although this was not statistically significant. There were no differences between the other treatment groups and the control group. There were no treatment-related findings observed following external or visceral examination of the fetuses. There were, however, significant increases in select malformations and variations of the skeleton in the high-dose group, and select retardations in both the high- and mid-dose groups, compared to control incidences.

Skeletal malformations were manifested as missing thoracic and lumbar vertebrae and bipartite sternebrae. Variations noted were shortened or absent 13^{th} rib and rudimentary cervical ribs. The only retardations found were incomplete ossification of the skull and sternebrae. Whilst these findings are a clear indication that DTPA is capable of producing terata at high oral doses, the relevance of this effect to man is questionable. It is considered probable that the developmental toxicity is occurring secondary to a maternal zinc deficiency, i.e. maternal toxicity. In order to reproduce this toxicity in a pregnant human, DTPA would have to be administered in such as way that the zinc status would be negatively impacted throughout pregnancy. This would require significant oral doses of DTPA (See Annex 1). The support for the hypothesis that DTPA produces a zinc deficiency comes from an understanding of three key issues:

1. Does DTPA induce Zn deficiency in the maternal organism?

2. Is Zn deficiency per se a trigger for embryo-/fetotoxicity?
3. What is the relevance of this mechanism for the effects observed with DTPA in the teratogenicity study?

Does DTPA induce Zn deficiency in the maternal organism?

In the OECD guideline teratogenicity study on DTPA (BASF 1994) there was no assessment of zinc status in either the maternal or fetal organisms. Therefore from the results of this study alone it is not possible to conclusively state that the zinc status was affected by DTPA administration. However by considering the physical properties of DTPA and the results of other studies conducted with it or structurally similar chemicals there is enough evidence available to support the conclusion that DTPA administration is capable of altering zinc status. Therefore it is plausible that this occurred in the teratogenicity study in question.

DTPA and Zinc

DTPA is a chelating compound used both industrially and pharmacologically to bind metals. DTPA predominantly binds divalent metals including zinc, iron, manganese, calcium, and monovalent ions metals such as sodium and potassium. Of these it binds most strongly to zinc (binding affinity Log K 18.3) compared to calcium (Log K 10.7) or potassium and sodium (Log K 0.9). Therefore in biological matrices where zinc is present, DTPA will have a strong tendency to dissociate from metals such as calcium, sodium or potassium in favour of binding zinc. Also, compared to other chelating agents such as PDTA (propylene-diamino-tetraacetic acid) and EDTA (ethylene-diamino-tetraacetic acid), DTPA has a greater affinity for zinc. Therefore one might expect any biological activity relating to the chelation of zinc to be more pronounced with DTPA than other chelating agents, although bioavailability will also play a significant role.

It should also be recognized that the capacity of DTPA to bind zinc in the gut and in the systemic circulation will still be affected by the presence of other metals and the availability of zinc to bind (See Annex 1).

DTPA and Zinc excretion

Having established that DTPA has a high affinity for zinc, the effect of DTPA administration to experimental animals and humans on zinc status can be considered. There are numerous studies examining the effects of administration of the calcium salt of DTPA (CaDTPA) to animals on the excretion of zinc (urinary and fecal). (Cantilena and Klassen, 1982; Cohen and Guilmette, 1976; Domingoet al., 1988, Planas-Bohneet al., 1975 and 1976; Tandonet al., 1984). In these studies the routes of administration were either; intraperitoneal, intravenous or subcutaneous. The dosing regimes used in these studies include; single doses, multiple doses in one day, multiple daily doses, and

continuous infusion. The results of all of these studies have shown that administration of CaDTPA resulted in an increased excretion of zinc relative to control regardless of the route of administration. Similar work conducted using the zinc salt of DTPA has demonstrated that administration of this compound did not cause an increase in the excretion of zinc (after taking into account the zinc that is part of the test substance). This work has also identified that the calcium form of DTPA is far more toxic than the zinc form. The investigators commenting that the chelation of endogenous metals such as zinc and perhaps manganese and subsequent increased excretion is responsible for the toxicity (Planas-Bone et al1976).

Further evidence of the potential of DTPA to cause an increase in zinc excretion comes from work in mice and rats to identify an effective antidote for acute zinc intoxication (Llobet et al 1988; Domingo et al 1988). This work compared a number of chelating agents including DTPA and EDTA. In both studies mice (or rats) were administered lethal doses of zinc via intraperitoneal injection and then administered doses of chelating agents either immediately or 10 minutes later via intraperitoneal injection. The antidotal effectiveness was assessed by comparing the degree of zinc induced mortality. Llobet et al (1988) also assessed the excretion of zinc in the feces and urine. In both studies the calcium salt of DTPA was most protective against acute zinc toxicity. It also caused an increase in zinc excretion in the urine and feces.

DTPA and Zinc deficiency

Data in humans show that intravenous administration of DTPA (the calcium salt) is capable of causing an increase in the excretion of zinc, in some cases leading to a deficiency. In one case study, (Proksch and Koumllmel, 1985), a patient treated for manganese poisoning with CaEDTA and CaDTPA exhibited zinc deficiency syndrome with acrodermatitis enteropathica-like skin changes. This resolved following oral administration of zinc aspartate. In a second study (Kalkwarf et al., 1983) levels of trace metals were assessed in the urine samples of a worker contaminated in the 1976 Americium incident. This worker had been treated over the course of 3 years with different forms of DTPA (CaNaDTPA or ZnNaDTPA) in an effort to reduce the radio nuclide contamination. Analysis of the workers urine identified that of all the trace metals assessed, zinc was the only one excreted at much higher levels than ‘normal’ and the peaks in zinc excretion appeared to correspond to treatments with CaNaDTPA. The treatment schedule also included intermittent zinc supplementation. It was noted that this supplementation or administration of ZnNaDTPA compensated for the loss of zinc caused by CaNaDTPA.

With the exception of the more recent toxicity studies conducted with sodium or potassium DTPA, all previous experimental investigations of DTPA (calcium or zinc salts) have been conducted using non-oral routes of administration. Therefore there is very little information on the effect of oral administration of DTPA (gavage or dietary) on the general health (including zinc status) of animals and man. However in a 28 day study in rats (Elliot et al.,1987), gavage administration of the potassium salt of DTPA at

doses up to 1330 mg/kg bw/day resulted in clear signs of toxicity including an increase in mortality (at the highest does). In the mid and low dose animals (83 and 333 mg/kg) there were no mortalities and with the exception of a reduced feed consumption in some mid dose males, there were no clinical signs observed. In the highest dose group, there were several mortalities in both sexes (4/5 male, 1/5 female). Prior to death, the male animals displayed hunched posture, abnormal gait, diarrhea, piloerection, yellow-brown staining of fur and decreased respiration rate. High dose animals also consumed less food and watery contents in the caecum were noted in 3/4 high dose females at necropsy. These effects are consistent with those observed following a similar treatment regime with EDTA and other structurally similar chelating compounds. Owing to the low oral absorption of DTPA, it is probably exerting its effects, such as binding dietary zinc, in the gut, thus causing an absorption-mediated deficiency of zinc, rather than a deficiency due to increased urinary elimination. Whilst these data do not show that oral dosing of DTPA is capable of causing a zinc deficiency, it does demonstrate the consistency in toxicity with other chelating compounds such as EDTA which are generally believed to cause a zinc deficiency (Swenerton and Hurley 1971; EDTA RAR 2004) leading to developmental toxicity, albeit at much higher doses (>1000mg/kg).

In summary, the available data demonstrate that

- DTPA is capable of forming stronger complexes with the essential element zinc than other metals such as calcium, sodium and potassium.
- DTPA administered to both animals and man is capable of increasing zinc excretion and in some cases inducing a deficiency.
- Administration of the zinc salt of DTPA does not cause increased zinc excretion and is less toxic than other forms of DTPA.
- Oral administration of potassium DTPA produces effects consistent with those seen with other structurally related chelating agents; i.e. in vivo DTPA appears to act in a similar manner to those chelating agents.

Is Zn deficiency per se a trigger for embryo-/fetotoxicity?

The following text is an extract from Rogers et al. (1985) that gives a good summary of the spectrum of effects of zinc deficiency on the developing fetus.

“The production of congenital malformations in a mammal by maternal Zn deficiency was first reported in 1966 by Hurley and Swenerton in the Sprague-Dawley rat (Hurley and Swenerton, '66). Since this initial report, there has been considerable work on the teratogenic effects of Zn deficiency and the mechanisms involved. Virtually every developing organ system has been shown to be adversely affected by maternal Zn deficiency; the types of defects produced by Zn deficiency are many, and they occur with high frequencies. Fetuses of Zn-deficient rats have been reported to have brain defects, eye defects, cleft palates, and skeletal defects, as well as gross malformations of the cardiovascular, respiratory, and urogenital systems (Hurley and Swenerton, '66; Hurley,

'69; Mills et al., '69; Hurley and Shrader, '72; Warkany and Petering, '72; Apgar, '72). In addition to these structural defects, the biochemical development of the lung (Vojnick and Hurley, '77) and pancreas (Robinson and Hurley, '81a, b) is also adversely affected by gestational Zn deficiency in the rat. ”

Subsequently it is very well understood that a nutritional deficiency in zinc in the maternal organism can produce very severe consequences in the developing fetus. However an important consideration is that the extent, duration and timing of zinc deficiency during pregnancy will affect the severity and localization of the effects on the developing fetus (Hurley et al., 1971; Record et al., 1985; Hickory et al., 1979). This is important because less extensive effects are associated with a mild or fluctuating zinc deficiency during pregnancy, and the timing during pregnancy will determine the organ systems/skeletal structures affected (Hurley et al., 1971; Record et al., 1985; Hickory et al., 1979). The effects of short term/transitory zinc deficiency highlight the fact that in rats the maternal organism does not seem to be capable of compensating for a sudden drop in zinc intake by mobilizing zinc stores (Hurley et al., 1971); in fact it seems that during periods of zinc deficiency the maternal liver sequesters zinc via induction of the zinc-binding protein, matallothionein, thus restricting the supply of zinc to the embryo/fetus (Rogers et al., 1985). King (2000) notes that in humans and animals, transfer of sufficient zinc to the fetus is dependent on maintenance of normal maternal serum zinc concentrations, therefore interfering with the maternal zinc status is the first step in producing a zinc deficiency in the developing fetus. Importantly, teratogenic effects have resulted from a decrease in the transfer of zinc to the embryo, even in the absence of detectable decreases in absolute zinc levels (Daston et al., 1991; Keen et al., 2003; Leazer et al., 1992; Tauberneck et al., 1994). Thus it appears possible to induce teratogenicity in the absence of overt visible maternal toxicity.

In the studies mentioned above examining zinc deficient diets and developmental toxicity it is often reported that maternal feed consumption and bodyweight gain were reduced compared to control animals. However where a pair fed control group was also included, the restriction in food intake was shown to cause some decrease in maternal bodyweight gain but it did not result in any developmental toxicity (Rogers et al., 1985). These findings implicate zinc deficiency, not reduced feed consumption as the causative agent responsible for developmental toxicity.

In summary, there is a significant body of literature on the effects on the developing fetus of deficiencies in nutrients such as zinc. From this database it is very clear that zinc plays such an important role in so many of the processes involved in the growth and development of the fetus that a deficiency in this nutrient has serious consequences. Subsequently any substance capable of negatively affecting the zinc status of the maternal organism is likely to have adverse effects on the developing fetus resulting in varying degrees of malformations. These malformations will also depend on the duration and severity of the deficiency.

What is the relevance of this mechanism (zinc deficiency) for the effects observed with DTPA in the teratogenicity study?

DTPA and evidence of zinc deficiency mediated teratogenicity

Fisher et al (1975) administered ZnDTPA (6 mice at 360 micromol/kg bw and 6 mice at 2900 micromol/kg bw), CaDTPA (6 mice at 360 micromol/kg bw and 12 mice at 2900 micromol/kg bw) or saline solution (12 mice) to female mice (strain C57BL/Do) via daily subcutaneous injections. These doses are equivalent to 199 or 1600mg ZnDTPA /kg bw and 179 or 1441 mg CaDTPA/kg bw. 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 micromol/kg bw CaDTPA there were no viable offspring observed. Only one stillborn pup was observed but it appeared grossly normal. In the 360 micromol/kg bw CaDTPA group there were no adverse effects on reproduction or developmental parameters. Both dose levels of ZnDTPA were reported to be 'completely harmless' to the mothers and the pups.

Fisher et al. (1976) administered a range of doses of CaDTPA 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 on either days 2-6, 7-11 or 12-16 during pregnancy. The mice received injections of 0, 720, or 1440 micromoles/kg bw (days 2-6 or 7-11) or doses of 0, 720, 1440, or 2880 micromoles/kg bw (days 12-16). These doses are equivalent to 0, 357, 715 and 1430 mg CaDTPA/kg bw. The dams were sacrificed on day 18 of gestation and the fetuses examined for morphologic alterations. CaDTPA (357 or 715 mg/kg bw) 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 control) when dosed from day 12 to 16 whereas 1430 mg/kg bw did. Dosing with 715 mg/kg bw produced malformations in fetuses in all dosing period groups. The types of malformation and number of fetuses affected varied however with the dosing schedule. The malformations observed were typical of those associated with zinc deficiency (exencephaly with ablepharia, exencephaly, spina bifida aperta, cleft palate). Polydactyly was also observed when 715 and 1430 mg/kg bw were administered from days 12 to 16. The authors noted that this may not be a true effect of CaDTPA as the strain of mouse used has a background incidence of 1.5% for this malformation. However this type of malformation is still consistent with zinc deficiency and so should not be ruled out all together. Dosing with 357 mg/kg bw only produced malformations when dosed from days 2 to 6 and these malformations were consistent with those observed with 715 mg/kg bw dosed for the same period.

Brummett and Mays (1977) investigated the teratogenicity of the zinc salt of DTPA in the mouse using a similar protocol to that used by Fisher et al., (1976). Pregnant mice were subcutaneously dosed with ZnDTPA daily either from days 2-6 or 7-11 during gestation. The strain of mice (C57BL/Do) was the same as that used by Fisher et al (1975 and 1976). The doses of ZnDTPA used were either 0, 5720 or 11520 micromoles/kg bw; these doses are equivalent to 0, 3163 and 6371 mg/kg bw. Due to the hypertonic nature of the test material an additional group of mice were treated with a solution of sodium chloride (1380 micromole NaCl/ml) at the same ion concentration, osmolality, pH and volume as the high dose ZnDTPA treatment. A CaDTPA dose group (1440 micromole/kg bw = 715 mg/kg bw) dosed daily on days 7-11 was also included in this study. The pregnant mice were euthanized on day 18 of gestation and the fetuses removed and examined for gross malformations, visceral malformations and skeletal malformations. Dosing with ZnDTPA in this study did not result in any malformations of the fetuses

although 6371 mg/kg (days 2-6 and 7-11) and 3163 mg/kg (days 7-11) caused an increase in embryo toxicity relative to controls (aborted litters or resorptions). However administration of the hypertonic saline solution also caused an increase in aborted litters and resorbed fetuses relative to control. The only malformed fetus observed was in the CaDTPA group which had exencephaly. Considering the previous studies on CaDTPA it seems likely that had it been dosed in this study from days 2-6 then the malformations observed would have been far more extensive. This seems to suggest that it could take a few days for DTPA to induce a zinc deficiency and that it might last for a few days after dosing has ceased since organogenesis peaks in the mouse between days 7 and 11 of gestation, thus the sensitivity to zinc induced malformations should be greatest during this time period.

In a follow up study to that reported by Brummett and Mays (1977), Calder et al. (1978) dosed mice daily via subcutaneous injection 4 days after mating began until birth or until 29 injections had been administered. Two forms of ZnDTPA were used in this study, one commercial batch with no NaCl present and another made in the lab containing NaCl. No gross malformations were reported in this study although it appears only an external exam was performed. The authors report that 6371 mg/kg bw ZnDTPA (commercial grade) was not toxic to either dams or pups although it did produce a statistically significant drop in pup weight. Doses of 1560 or 3163 mg/kg bw ZnDTPA (commercial or lab grade) did not significantly alter any of the parameters examined (pup weight, pups/litter, abortion rate) relative to control. The study also followed the progress of the dams and the pups for their remaining lifespan to understand whether there were delayed effects on fertility or viability. At the time of this study report there was no evidence of any impairment in the fertility of the mature pups or the viability of their offspring however no additional details are given.

The above studies appear to demonstrate that CaDTPA is capable of causing fetotoxicity and malformations consistent with zinc deficiency and that the frequency and type of these malformations is dependent on the dosage and dosing period during pregnancy. Conversely ZnDTPA dosed at significantly higher dose levels for equivalent dosing periods does not appear to cause malformations. It does however result in increased fetotoxicity albeit at extremely high dose levels. The explanation given by the investigators as to why there is a difference in teratogenicity between the calcium and zinc salts of DTPA is that the toxicity is due to the chelation of essential metals such as zinc and manganese (consider the data on increased excretion of zinc following DTPA administration) and that the zinc salt of DTPA cannot chelate any additional zinc. CaDTPA on the other hand will release the calcium and bind zinc in the body increasing its excretion and producing a zinc deficient state.

The support for the mechanism of maternal zinc deficiency being responsible for teratogenicity of chemicals capable of depleting zinc comes from work where a zinc depleting agent was dosed in conjunction with a zinc supplemented diet. Doses that previously caused teratogenicity were found to be non teratogenic in the presence of sufficient zinc (Swenerton and Hurley (1971). Considering this work, it is plausible that if the diet used in the BASF study using sodium DTPA (BASF 1994) had been supplemented with zinc as done by Swenerton and Hurley (1971) then the developmental toxicity would have been prevented. This would also be consistent with the difference in the toxicity of calcium DTPA compared with zinc DTPA.

Whilst the data above demonstrate that DTPA is capable of producing malformations when not dosed as the zinc salt, there has been some question about the types of

malformations observed in the developmental toxicity study conducted on sodium DTPA (BASF 1994). In that study sodium DTPA was dosed daily to pregnant rats via gavage at doses of 100, 400 and 1000 mg/kg bw, gestation days 6 to 15. The effects observed in the fetuses in this study were not as severe as those observed with subcutaneous dosing of CaDTPA, or a complete zinc deficiency. It has also been noted that missing vertebrae is not a malformation commonly associated with zinc deficiency and therefore perhaps there is another mechanism of action. However missing vertebrae resulting from a zinc deficiency has been reported in the past (Swenerton and Hurley 1966) as have delays in ossification, missing ossification centers, bipartite sternebrae and many other skeletal deformities (Rogers et al., 1985; Stevens et al., 1962; Hurley et al., 1971; Jankowski et al., 1995; Record et al., 1985, 1986; Ferreira et al., 1989). As mentioned previously, zinc deficiency potentially can affect a broad range of developmental processes, with the specific malformations manifested being mainly a function of exposure timing, duration and dose. Assuming that a functional zinc deficiency requires at least few days of dosing, the zinc deficiency likely occurred during a stage of embryogenesis when axial skeleton patterning (i.e., the vertebrae and its derivatives) is being established. Thus, a causal association between DTPA-induced zinc deficiency and missing vertebrae is entirely plausible.

Perhaps when considering the less severe effects observed in the BASF study it should be acknowledged that the route of administration can affect the degree and frequency of malformations observed. Chelating agents dosed in the diet rather than via gavage will have a larger window of opportunity to interact with essential metals in the gut and subsequently preventing their absorption by forming a complex that is less able to be absorbed in the gut and increasing excretion. Therefore dietary dosing will probably be more effective at producing a zinc deficiency than gavage dosing. Thus perhaps the use of gavage dosing in the BASF study was in part responsible for the less severe malformations observed.

In summary,

- The zinc salt of DTPA is significantly less teratogenic/fetotoxic than the calcium salt. This is most likely due to the induction of a maternal zinc deficiency by CaDTPA resulting from an increase in zinc excretion.
- It is apparent therefore that the salt form of DTPA which is dosed to pregnant animals is directly related to the teratogenicity potential and this in turn is related to the potential for chelation of zinc. Thus salt forms of DTPA that can chelate zinc will be potentially teratogenic i. e. those salts where the DTPA-metal complex has a lower dissociation constant than ZnDTPA.
- The concept of a mode of action involving zinc deficiency is further supported by the data on other chemicals that can deplete zinc, where zinc supplementation negated the teratogenicity of a dose known to be teratogenic.
- The dosing route plays a very important role in the elicitation of a teratogenic response, dietary dosing being more effective than gavage dosing and subcutaneous/intravenous or intraperitoneal dosing being more effective than oral dosing.
- The duration of dosing and timing during pregnancy are also important factors influencing the type, severity and frequency of malformations.

Conclusions

The difference in the teratogenicity of the calcium and zinc salts of DTPA indicates that DTPA itself is unlikely to be having a direct teratogenic effect on the developing fetus. If DTPA were directly teratogenic then the salt form should not significantly influence the teratogenicity particularly following intravenous or subcutaneous dosing.

DTPA has a strong affinity for zinc when compared to other metals such as sodium, potassium and calcium. It is also not absorbed well from the intestinal tract following oral dosing in humans and thus absorption in rats is also likely to be poor. Therefore it is plausible that oral dosing of pregnant rats with DTPA will lead to zinc being bound in the gut and excreted in the faeces. Absorbed DTPA will either be bound already to zinc or will bind endogenous zinc (or other metals) in the body before being excreted.

The potential increase in zinc excretion associated with oral dosing with DTPA may then cause a reduction in the zinc available to the mother and the developing fetuses. Considering that pregnant rats receiving sub optimal levels of zinc do not appear to be able to mobilize tissue stores of zinc to ensure adequate supply to the fetus (conversely the effect is perhaps further exacerbated by increased sequestration due to increased maternal metallothionein production), the decrease in zinc intake/increased zinc excretion will result in an insufficient supply of zinc to the fetus. An insufficient zinc supply on the developing fetus produces malformations and increased fetotoxicity.

The timing of this drop in zinc availability during pregnancy will determine the degree of teratogenicity. Due to the relatively limited spectrum of effects observed in the BASF study it is probable that the gavage dosing of DTPA did not cause a complete deficiency in zinc throughout pregnancy but instead zinc levels fluctuated, deficient levels coinciding with certain stages in fetal development such as skeletal development and bone ossification.

In conclusion there are adequate data to support the hypothesis that the teratogenicity resulting from gavage dosing of rats with DTPA is a result of an induced deficiency of zinc in the mother which subsequently impacts the fetus.

The following information is taken into account for any hazard / risk assessment:

OECD 414 pre-natal developmental toxicity study in rats using Pentasodium DTPA

4.11.5 Comparison with criteria

Pentasodium DTPA does not meet the requirements for classification for reproductive toxicity as described in REGULATION (EC) No 1272/2008.

Developmental toxicity classification:

The chelating agent DTPA appears to be developmentally toxic following high oral doses in rats. DTPA complexes with essential metals such as zinc in the gut, preventing these from becoming bio-available. At high doses this deprivation of essential nutrients leads to

toxic effects such as developmental toxicity due to the importance of zinc in the healthy development of a growing fetus. As a result of this it is considered appropriate to classify DTPA for developmental toxicity. In determining the appropriate classification an assessment of the relevance of the hazard to humans has been made. This assessment takes two parts; 1) estimation of exposures to the workforce (the group with the highest exposure levels) and 2) an assessment of how these exposures may affect the zinc status of the workforce, specifically a pregnant worker.

Taking conservative assumptions into account, the estimated worker exposures to DTPA are low. Due to the much lower levels of DTPA in formulated products that may be available to the consumer, consumer exposure levels would be significantly lower than worker exposure levels. The effect of estimated worker exposures to DTPA under normal working conditions or following an accidental acute exposure would not be considered to be detrimental to a workers (including pregnant women) zinc status. Thus it is highly unlikely that a consumer's zinc status would also be negatively affected, considering the significantly lower level of exposure likely in the general population. Thus exposure to DTPA would not result in the development of a zinc deficiency.

According to REGULATION (EC) No 1272/2008.,

"Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B)"

There is no human data to show evidence of developmental toxicity and therefore classification in category 1A is considered not appropriate.

Further the regulation goes on to say;

„the classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate"

Within the framework of a weight of evidence approach it is demonstrated in a comprehensive effects and exposure assessment that the occurrence of developmental toxicity is secondary to primary maternal zinc deficiency. Furthermore in humans it would require unrealistic exposure situations and thus is extremely unlikely, therefore Classification of DTPA in Category 1B according to the regulation (EC) No 1272/2008 is considered not appropriate.

In addition the regulation goes on to say;

"Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.
Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."

Given the primary toxicity of DTPA administration is reduced uptake of dietary zinc and depletion of existing maternal zinc stores, classification of DTPA may be considered inappropriate as the effects observed can be considered as secondary toxicities. However, classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women and in the context of the information provided it would seem appropriate to assign Category 2 classification to DTPA with a generic concentration limit (GCL) ≥3%. It is also proposed that the route relevant to this classification be specified as 'Oral'. This is supported by the very minimal absorption following dermal exposure and the limited possibility for sufficient inhalation exposure to generate this toxic effect.

4.11.6 Conclusions on classification and labelling

Classification as Category 2. GCL ≥3%. Oral route only

4.12 Other effects

Not the subject of this CLH proposal.

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Not the subject of this CLH proposal

4.12.1.2 Immunotoxicity

Not the subject of this CLH proposal

4.12.1.3 Specific investigations: other studies

4.12.1.4 Human information

4.12.2 Summary and discussion

4.12.3 Comparison with criteria

4.12.4 Conclusions on classification and labelling

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not the subject of this CLH proposal

6 REFERENCES

Allard et al. (1996). Absence of 14CO2 evolution from 14C-labelled EDTA and DTPA and the sediment/water partition ratio. Chemosphere, Vol. 33, No. 4, pp. 577-583, 1996.

Allen, J. A., Brooker, P. C, Birt, D. M. (1987). Analysis of metaphase chromosomes obtained from CHO cells cultured in vitro and treated with GLYCINE N-N BIS [2-[BIS(CARBOXYMETHYL) AMINO] ETHYL] - PENTA POTASSIUM SALT. Testing laboratory: Huntingdon Research Centre Ltd., UK. Report no.: AKZ 232/87980. Owner company: Akzo Chemie UK Ltd, UK. Report date: 1987-10-13.

Anders Svenson et al. (1989). Aqueous photolysis of the iron (iii) complexes of NTA, EDTA and DTPA. Chemosphere, Vol. 18, Nos.9/10, pp 1805-1808, 1989.

BASF AG (1988). Wachstumshemmtest in Anlehnung an Bringmann-Kuehn. Testing laboratory: BASF Department of Ecology. Report no.: 9/0998/88/w2. Report date: 1988-10-05.

BASF AG (1989a). Bestimmung der akuten Wirkung von Trilon C fluessig gegenueber dem Wasserfloh Daphnia magna Strauss. Testing laboratory: Department of ecology, BASF AG. Report no.: 1/1135/2/89-1135/89. Owner company: BASF AG. Report date: 1989-07-20.

BASF AG (1989b). Algentest. Testing laboratory: BASF Department of Ecology. Report no.: 2/0998/88. Owner company: BASF. Report date: 1989-09-07.

BASF SE (1968a). Ergebnis der gewerbetoxikologischen Vorprüfung. unpublished report. Testing laboratory: Department of Toxicology. Report no.: XVIII/26. Owner company: BASF SE. Report date: 1968-03-29.

BASF SE (1968b). Ergebnis der gewerbetoxikologischen Vorprüfung. unpublished report. Testing laboratory: Department of Toxicology. Report no.: 1968. Owner company: BASF SE. Report date: 1968-03-29.

BASF SE (1982a). Akute Hautreizwirkung/Aetzwirkung gemaess OECD. unpublished report. Testing laboratory: Department of Toxicology. Report no.: 81/395. Owner company: BASF SE. Report date: 1982-01-20.

BASF SE (1982b). Akute Reizwirkung am Auge des Albino-Kaninchens gemaess OECD. unpublished report. Testing laboratory: Department of Toxicology. Report no.: 81/395. Owner company: BASF SE. Report date: 1982-01-20.

BASF SE (1989). Report on the Stuy of Trilon C flüssig in the Ames Test (Standard Plate Test and Preincubation Test with Salmonella typhimurium). unpublished report. Testing laboratory: Department of Toxicology. Report no.: 40M1021/884392. Owner company: BASF SE. Report date: 1989-03-16.

BASF SE (1993). Maximization Test for the sensitizing potential of Trilon C flüssig in guinea pigs. unpublished report. Testing laboratory: Department of Toxicology. Report no.: 30H0812/892379. Owner company: BASF SE. Report date: 1993-07-08.

BASF SE (1994). Study of the Prenatal Toxicity of Trilon C liquid in Wistar Rats After Oral Administration (Gavage). unpublished report. Testing laboratory: Department of Toxicology. Report no.: 30R0812/89117. Owner company: BASF SE. Report date: 1994-11-18.

BASF SE (2002). Trilon C flüssig - Repeated dose oral toxicity study in Wistar rats; administration in the drinking water for 4 weeks. unpublished report. Testing laboratory: Experimental Toxicology and Ecology. Report no.: 32S0812/89116. Owner company: BASF SE. Report date: 2002-09-04.

BASF SE (2014), Trilon BD – 90-Day inhlation study in Wistar Rats dust exposure unpublished report. Testing laboratory: Experimental Toxicology and Ecology. Report no.: 5010628/09127. Owner company: BASF SE. Report date: not given

Brummett et al (1977). Teratological Studies of Zn-DTPA in mice.

Dufkova, Vera (1984). EDTA in algal culture media. Arch. Hydrobiol. Suppl. 67, 4 pp. 479-492.

Elliott P. H., Smyth C., Crook D., Gibson W. A., Hadley J. C., Gopinath C. (1987). Twenty-eight day oral toxicity study in rats with GLYCINE N-N BIS [2-[BIS(CARBOXYMETHYL) AMINO] ETHYL] - PENTA POTASSIUM SALT. Testing laboratory: Huntingdon Resarch Centre Ltd, UK. Report no.: AKZ 231/ 871020. Owner company: Akzo Chemie UK, Ltd., UK. Report date: 1987-11-23.

Fukuda et al (1982). Toxicological Study on the Safety of DTPA as a drug (I) Teratological Study in the rat. Hoken Butsuri, 18, 37-42 (1983).

Gardner, J. R. (1987a). Acute oral toxicity of Armogard S495. Testing laboratory: Huntingdon Research Centre Ltd., UK. Report no.: 8725D/AKZ 226/AC. Owner company: Akzo Chemie UK Ltd. Report date: 1987-02-13.

Gardner, J. R. (1987b). Acute dermal toxicity to rats of glycine N-N-BIS[2-[BIS(CARBOXYMETHYL) AMINE] ETHYL]-PENTA POTASSIUM SALT. Testing laboratory: Huntingdon Research Centre Ltd., UK. Report no.: 8713OD/AKZ 227/AC. Owner company: Akzo Chemie (UK) Ltd. Report date: 1987-02-24.

Hinck et al. (1997). Resistance of EDTA and DTPA to aerobic biodegradation. Wat. Sci. Tech. Vol. 35, No.2-3, pp.25-31, 1997.

Kawamata, K. et al (1980). Comparative toxicity studies of Na2EDTA and CaNa2EDTA. Jpn. J. Pharmacol., 30 (Suppl) 234P (1980).

Kettunen-Knuutila et al. (2004). Degradation of EDTA and DTPA in chlorine dioxide bleaching conditions. Water Science and Technology. Vol. 50, No. 3, pp. 141-144, 2004.

Kynoch, S. R. & Parcell, B. I. (1987). Delayed contact hypersensitivity in the guinea pig with GLYCINE N-N BIS[2-[BIS(CARBOXYMETHYL) AMINO] ETHYL] - PENTA POTASSIUM SALT. Testing laboratory: Huntingdon Research Centre Ltd., UK. Report no.: 87833D/AKZ 230/SS. Owner company: Akzo Chemie UK, Ltd., UK. Report date: 1987-07-20.

Kynoch, S. R. (1984). Acute oral toxicity to rats of glycine, N-N bis[2-[bis(carboxymethyl) amino]ethyl]-penta potassium salt. Testing laboratory: Huntingdon Research Centre, UK. Report no.: 84753D/AKZ 172/AC. Owner company: Akzo Chemie UK Ltd. Report date: 1984-09-27.

Liggett, M. P. & Parcell, B. I. (1984a). Irritant effects on rabbit skin of GLYCINE, N-N- BIS [2-[BIS (CARBOXYMETHYL) AMINO] ETHYL] - PENTA POTASSIUM SALT. Testing laboratory: Huntingdon Research Centre, UK. Report no.: 84643D/AKZ 170/SE. Owner company: Akzo Chemie UK Ltd., UK. Report date: 1984-08-24.

Liggett, M. P. & Parcell, B. I. (1984b). Irritant effects on the rabbit eye of GLYCINE, N-N BIS [2-[BIS(CARBOXYMETHYL) AMINO] ETHYL] - PENTA POTASSIUM SALT. Testing laboratory:

Huntingdon Research Centre, UK. Report no.: 8469D/AKZ 171/SE. Owner company: Akzo Chemie UK, Ltd., UK. Report date: 1984-09-04.

Liggett, M. P. & Smith, P. A. (1987a). Irritant effects of GLYCINE N-N BIS[2-[BIS(CARBOXYMETHYL) AMINE] ETHYL] - PENTA POTASSIUM SALT. Testing laboratory: Huntingdon Research Centre, Ltd., UK. Report no.: 87123D/AKZ 228/SE. Owner company: Akzo Chemie UK Ltd., UK. Report date: 1987-02-24.

Liggett, M. P. & Smith, P. A. (1987b). Irritant effects on the rabbit eye of GLYCINE N-N BIS [2-[BIS(CARBOXYMETHYL) AMINO] ETHYL] - PENTA POTASSIUM SALT. Testing laboratory: Huntingdon Research Centre Ltd, UK. Report no.: 87160D/AKZ 229/SE. Owner company: Akzo Chemie UK Ltd., UK. Report date: 1987-03-06.

Lucke-Huhle, C. (1976). Proliferation-dependent cytotoxicity of diethylenetriaminepentaacetate (DTPA) in vitro. 349-54. [Health physics].

M. Douglas (1987a). Assessment of ready biodegradability of glycine N-N bis [2-[Bis (carboxymethyl) amine] ethyl]-pentapotassium salt. toxfils 9434. Testing laboratory: Huntingdon Research Centre Ltd. Report no.: AKZ 233 (a) /87623-3. Owner company: Akzo Nobel Chemicals. Report date: 1987-07-23.

M. Douglas (1987b). The acute toxicity of Glycine N-N Bis[2-[Bis(carboxymethyl) amine]ethyl]-pentapotassium salt to Rainbow trout. Testing laboratory: Huntingdon research centre. Report no.: Akz233/87721. Owner company: AkzoNobel. Report date: 1987-06-27.

M. Douglas (1987c). The acute toxicity of Glycine N-N Bis[2-[Bis(carboxymethyl) amine]ethyl]-pentapotassium salt to Daphnia Magna. Testing laboratory: Huntingdon research centre. Report no.: akz233/87720. Owner company: AkzoNobel. Report date: 1987-06-29.

MacDonald, R. S. (1998). Zinc deprivation of murine 3T3 cells by use of diethylenetrinitrilopentaacetate impairs DNA synthesis upon stimulation with insulin-like growth factor-1 (IGF-1). 1600-5. [The Journal of nutrition].

May, K. (1990). Armogard S462: Assessment of mutagenic potential in histidine auxothrops of Salmonella Typhimurium (the Ames test). Testing laboratory: Life Science Research Ltd., UK. Report no.: 90/AKL084/0441. Owner company: Akzo Chemicals Int. BV, Amersfoort, the Netherlands. Report date: 1990-06-05.

Means et al. (1980). Relative degradation rates of nitrilotriacetic acid, EDTA and diethylenetriaminepentaacetic acid and environmental implications. Environmental Pollution (Series B) I (1980) 45-60.

Metsarinne S et al (2004). Biological and photochemical degradation rates of diethylenetriaminepentaacetic acid (DTPA) in the presence and absence of Fe(III). Chemosphere, 55, (2004) pg 379-388.

Metsarinne et al. (2004). Biological and photochemical degradation rates of diethylenetriaminepentaacetic acid (DTPA) in the presence and absence of Fe(III). Chemosphere 55 (2004) 379–388.

Meylan, W. M. and Howard, P. H. (2009). AOP v1.92. Meylan, W. M. and Howard, P. H. 1993. Computer estimation of the atmospheric gas-phase reaction rate of organic compounds with hydroxyl radicals and ozone. Chemosphere 26: 2293-99.

P. F. Kingston (1983). Toxcicity of six chemicals to the Brown shrimp (crangon crangon). Testing laboratory: Herion-Watt University. Report no.: (IOE-Mar1983). Owner company: AkzoNobel. Report date: 1983-03-01.

Pitter et al. (2001). Biodegradabality of ethylenediamine-based complexing agents and related compounds. Chemosphere 44 (2001) 823-826.

Planas-Bohne, F. (1976). Toxicological Studies on DTPA. Diagn Treat Inc Radionuclides Proc Int Semin 1975 505-515,1976 Tax - Rattus, Heiligenberg.

R. A. van Dam, J. T. Ahokas, D. A. Holdway (1999). Chronic Toxicity of Diethylenetriamine Pentaacetic

Acid to Crimson-Spotted Rainbowfish (Melanotaenia fluviatilis): Effects on Reproduction, Condition, and Ethoxyresorufin O-Deethylase Activity. Ecotoxicology and Environmental Safety 43: 74-82.

Richold, M., Jones, E., Fenner, L. A. (1984). Ames metabolic activation test to assess the potential mutagenic effect of KDTPA. Testing laboratory: Huntingdon Reserach Centre plc., UK. Report no.: AKZ 173/84716. Owner company: Akzo Chemie UK, Ltd., UK. Report date: 1984-08-29.

Rodriguez et al. (1999). Chemical degradation of EDTA and DTPA in a totally chlorine free (TCF) effluent. Wat. Sic. Tech. Vol. 40. No. 11-12, pp. 267-272, 1999.

Runge, V. M (2005). Subchronic toxicity of the gadolinium chelates. Acad Radiol. 2005, May; 12 Suppl 1: S6-9. [Academic radiology].

Smith, V. H. (1980). Early effects of inhaled Ca-DTPA on the rat lung. 9-16. [Toxicology letters].

Srivastava RC, Dwivedi PP, Behari JR, Athar M (1986). Evaluation of LD50 of some polyaminocarboxylic acids used as chelating drugs in metal intoxication. Toxicology letters, volume 32, pages 37-40.

Sterner, W. & Chibanguza, G. (1983a). Test for primary skin irritation of Dissolvine D40. Testing laboratory: IBR Forschungs-GmbH, Walsrode, Germany. Report no.: 1-3-403-83. Owner company: Akzo Nobel Functional Chemicals. Report date: 1983-09-01.

Sterner, W. & Chibanguza, G. (1983b). Draize animal mucous membrane irritation test of Dissolvine D40. Testing laboratory: IBR Forschungs-GmbH, Walsrode, Germany. Report no.: 1-3-409-83. Owner company: Akzo Nobel Functional Chemicals. Report date: 1983-08-01.

Stevens E, Rosoff B, Weiner M, Spencer H. (1962). Metabolism of chelating agent diethylenetriamine pentaacetic acid (C14 DTPA) in man. Proc. Soc. Exp. Biol. Med.; Vol. 111 pages 235-238.

Sykora et al. (2001). Biodegradability of ethylenediamine-based complexing agents. Wat. Res. Vol. 35, No. 8, pp. 2010–2016, 2001.

The Dow Chemical Company (1957a). Results of Range Finding Toxicological Tests on Penta Sodium Salt of Diethylenetriamine penta acetic acid. Unpublished data of The Dow Chemical Company. Testing laboratory: The Dow Chemical Company. Report no.: T57.23-43-1. Owner company: The Dow Chemical Company. Study number: K-19427-001.

The Dow Chemical Company (1957b). RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON DIETHYLENE TRIAMINE PENTAACETIC ACID: PENTASODIUM SALT (VERSENEX 80). Testing laboratory: The Dow Chemical Company. Report no.: T-5723 -43-2. Owner company: The Dow Chemical Company. Study number: K -19427-002.

The Dow Chemical Company (1957c). RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON PENTA SODIUM SALT OF DIETHYLTRIAMIWE PENTA ACETIC ACID. Unpublished data of The Dow Chemical Company. Testing laboratory: The Dow Chemical Company. Report no.: T57.23-43-1. Owner company: The Dow Chemical Company. Study number: K-19427-001.

The Dow Chemical Company (1957d). RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON DIETHYLENE TRIAMINE PENTAACETIC ACID: PENTASODIUM SALT (VERSENEX 80). Unpublished data of The Dow Chemical Company. Testing laboratory: The Dow Chemical Company. Report no.: T-57.23-43-2. Owner company: The Dow Chemical Company. Study number: K-19427-002.

The Dow Chemical Company (1958a). Results of Rangefinding Toxicological Tests on Diethylene Triamine Pentaacetic Acid (Versenex Acid) . Testing laboratory: The Dow Chemical Company. Owner company: The Dow Chemical Company. Study number: K-018702-002. Report date: 1958-01-15.

The Dow Chemical Company (1958b). Results of range finding toxicological tests on diethylene triamine pentaacetic acid: Pentasodium salt (VERSENEX - 80). Unpublished data of The Dow Chemical Company. Testing laboratory: The Dow Chemical Company. Report no.: T57.23-43-3. Owner company: The Dow Chemical Company. Study number: K-19427-3.

The Dow Chemical Company (1958c). Results of skin irritation tests on Versene 100, Versenol 120,

Versenex 80, Fe-3-liquid and Fe-3-Specific. Unpublished data of The Dow Chemical Company. Testing laboratory: The Dow Chemical Company. Report no.: T-57-23-0-20. Owner company: The Dow Chemical Company. Study number: K-194247-004.

The Dow Chemical Company (1958d). RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON DIETHYLENE TRIAMINE PENTAACETIC ACID: PENTASODIUM SALT (VERSENEX - 80). Unpublished data of The Dow Chemical Company. Testing laboratory: The Dow Chemical Company. Report no.: T-57.23-43-3. Owner company: The Dow Chemical Company. Study number: K-19427-003.

The Dow Chemical Company (1961a). RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON VERSENEX 80 (DIETHYUNE TRIAMINE PENTAACETIC ACID: PENTA SODIUM SALT - 40% AQUEOUS SOLUTION). Unpublished data of The Dow Chemical Company. Testing laboratory: The Dow Chemical Company. Report no.: T-57.23-43-5. Owner company: The Dow Chemical Company. Study number: K-19427-05.

The Dow Chemical Company (1961b). Results of range finding toxicological tests on Versenex 80 (diethylene triamine pentaacetic acid: penta sodium salt - 40% aqueous solution). Unpublished data of The Dow Chemical Company. Testing laboratory: The Dow Chemical Company. Report no.: T-57.23-43-5. Owner company: The Dow Chemical Company. Study number: K-19427-005.

The Dow Chemical Company (1976a). DOT test for corrosiveness to skin on Versenex 80 with 1.5% excess NaOH. Unpublished data of The Dow Chemical Company. Testing laboratory: The Dow Chemical Company. Report no.: TXT: 3079.0. Owner company: The Dow Chemical Company. Study number: K-19427-007.

The Dow Chemical Company (1976b). DOT TEST FOR CORROSIVENESS TO SKIN ON VERSENEX 80 WITH 1.9% EXCESS NaOH. Unpublished data of The Dow Chemical Company. Testing laboratory: The Dow Chemical Company. Report no.: TXT: 3079.2. Owner company: The Dow Chemical Company. Study number: K-19427-009.

The Dow Chemical Company (1976c). DOT TEST FOR CORROSIVENESS TO SKIN ON VERSENEX 80 WITH 2% EXCESS NaOH. Unpublished data of The Dow Chemical Company. Testing laboratory: The Dow Chemical Company. Report no.: TXT: 3079.1. Owner company: The Dow Chemical Company. Study number: K-19427-008.

The Dow Chemical Company (1976d). Analysis of the Versene Famly Chelates in the Aquatic Environment. Unpublished Data of the Dow Chemical Company. Testing laboratory: The Dow Chemical Company. Report no.: ES-0101. Owner company: The Dow Chemical Company. Study number: T3.02-000-000-078. Report date: 1976-10-06.

The Dow Chemical Company (2002). XUS-40881.00 EXPERIMENTAL CHELATING AGENT (DTPA): A DERMAL SENSITIZATION STUDY IN HARTLEY ALBINO GUINEA PIGS MODIFIED BUEHLER DESIGN. Testing laboratory: The Dow Chemical Company. Owner company: The Dow Chemical Company. Study number: DR-0380-7532-001. Report date: 2002-12-09.

The Dow Chemical Company (2005). EVALUATION OF THE READY BIODEGRADABILITY OF DIETHYLENETRIAMINEPENTAACETIC ACID (DTPA) USING THE OECD GUIDELINE 301F: MANOMETRIC RESPIROMETRY TEST. Testing laboratory: The Dow Chemical Company. Report no.: 031024. Owner company: The Dow Chemical Company. Study number: K-018702-003. Report date: 2005-12-08.

US Department of Health Education and Welfare (1977a). Bioassay of trisodium ethylenediaminetetraacetate trihydrate (EDTA) for possible carcinogenicity. Carcinogenesis Technical Report Series No. 11 (1977). Testing laboratory: Litton Bionetics, Inc., Kensington, USA.

US Department of Health Education and Welfare (1977b). Bioassay of trisodium ethylenediaminetetraacetate trihydrate (EDTA) for possible carcinogenicity. Carcinogenesis Technical Report Series No. 11 1977. Testing laboratory: Litton Bionetics, Inc., Kensington, USA.

Van Dam et al (1996). Comparative Acute and Chronic Toxicity of DTPA and Ferric-complexed DTPA to Daphnia carinata. Arch. Environ. Contam. Toxicol. 31, 433-443 (1996).

Van Dam et al (1999). Investigating mechanisms of diethylenetriamine pentaacetic acid toxicity to the cladoceran, Daphnia carinata. Aquatic toxicology 46 (1999), 191-210.

Wynn, J. E. et al (1970). The Toxicity and Pharmacodynamics of EGTA: Oral Administration to Rats and Comparisons with EDTA. Toxicology and Applied Pharmacology 16, 807-817 (1970).

References for Developmental toxicity Discussion

Apgar, *Journal of Nutrition* 102, 343-348, 1972

BASF AG, Department of Toxicology. 1994. Study of the prenatal toxicity of Trilon C liquid in Wistar rats after oral administration (gavage). Project No. 30R0812/89117), 18 November 1994

Brummett and Mays, *Health Physics*, 33, 624-626, 1977.

Calder *et al., Health Physics*, 36, 524-526, 1979.

Cantilena and Klaassen, *Toxicology and Applied Pharmacology* 63, 344-350, 1982

Cohen and Guilmette, *Bioinorg Chem* 5(3), 203-210, 1976

Daston *et al., Toxicology ad Applied Pharmacology*, 110(3), 450-463, 1991

Domingo *et al., Veterinary and Human Toxicology*, 30(3), 224-228, 1988b

Domingo *et al., Veterinary and Human Toxicology*, 30(6), 524-527, 1988a

Elliott *et al.*, Twenty-eight day oral toxicity study in rats with glycine, N-N-bis[2-[bis(carboxymethyl) amino]ethyl]- pentapotassium salt. *Huntingdon Research Centre report AKZ 231/871020-3*, dated November 23, 1987

European Chemicals Bureau, *European Union Risk Assessment Report: Edetic Acid (EDTA) Priority list 1, Volume 49*, 2004

Ferreira *et al., Teratology* 39, 181-194, 1989

Fisher *et al., Teratology*, 14, 123-128, 1976.

Fisher *et al., Health Physics*, 29, 780-782, 1975.

Hickory *et al., Journal of Nutrition*, 109, 883-891, 1979.

Hurley and Shrader, *International Review of Neurobiology, C.C. Pfeiffer, ed. Academic press, New York, Supplement.* 1, 7-51, 1972

Hurley and Swenerton, *Proceedings of the Society for Experimental Biology and Medicine*, 123(3), 692-696, 1966.

Hurley *et al., Teratology* 4, 199-204, 1971

Hurley *et al., American Journal of Clinical Nutrition* 22, 1332-1339, 1969

Jankowski *et al., Teratology* 51, 85-93, 1995

Kalkwarf *et al., Health Physics* 45(4), 937-947 1983

Keen *et al., Journal of Nutrition* 133(2), 1597S-1605S, 2003

Kimmel, *Toxicology and Applied Pharmacology* 40, 299-306, 1977

Kimmel and Sloan, *Teratology* 12, 330-331, 1975

King, *American Journal of Clinical Nutrition* 71 (suppl), 1334S–43S, 2000

Leazer *et al., Teratology*, 45(5), 481, 1992

Llobet *et al., Archives of Toxicology* 61(4), 321-323, 1988

Mills *et al., American Journal of Clinical Nutrition* 22, 1240-1249, 1969

Planas-Bohne and Olinger, *Health Physics* 31, 165-166, 1976

Proksch and Koumllel, *Deutsche medizinische Wochenschrift.* 110(25), 1001-1003, 1985

Record *et al., Teratology* 32, 397-405, 1985

Record *et al., Teratology* 33, 311-317, 1986

Robinson and Hurley, *Journal of Nutrition* 111, 858-868, 1981a

Robinson and Hurley, *Journal of Nutrition* 111, 869-877, 1981b

Rogers *et al., Teratology*, 31, 89-100, 1985.

Stevens *et al., Proceedings of the Society for Experimental Biology and Medicine*, 111 (2), 235-238, 1962.

Swenerton and Hurley, *Science,* 173, 62-64, 1971.

Tandon *et al., Environmental Research* 35(1), 237-245, 1984

Tauberneck *et al., Reproductive Toxicology*, 8(1), 24-40, 1994

Vojnik and Hurley, *Journal of Nutrition* 107, 862-872, 1977

Warkany and Petering, *Teratology* 5, 319-334, 1972

WHO. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. *WHO Food Additives Series,* No 5, 1974

7 ANNEXES

ANNEX 1

DTPA (Diethylenetriaminepentaacetic Acid) Exposure and Developmental Toxicity Hazard Assessment

The following information is provided in the context of weight of evidence assessment to ascertain whether, following use of pentasodium DTPA, the reprodevelopmental effects observed in animal studies could manifest themselves under real-life situations. The scenarios described represent worst-case exposure and should be considered as supporting evidence for correct interpretation of the most appropriate developmental classification for DTPA in line with Section 1.1.1.5 of Annex I of regulation (EC) No 1272/2008., which states;*' For the purpose of classification for health hazards (part 3) route of exposure, mechanistic information and metabolism studies are pertinent to determining the relevance of an effect in humans. When such information, as far as there is reassurance about the robustness and quality of the data, raises doubt about relevance in humans, a lower classification may be warranted.'*

Summary

The chelating agent Diethylenetriaminepentaacetic acid (DTPA) has been discovered to induce developmental toxicity when administered at high doses to pregnant rats.

DTPA complexes with essential metals such as zinc in the gut, preventing these from becoming bio-available.

At high doses this deprivation of essential nutrients leads to toxic effects such as developmental toxicity due to the importance of zinc in the healthy development of a growing fetus. As a result of this it is considered appropriate to classify DTPA for developmental toxicity. In determining the appropriate classification an assessment of the relevance of the hazard to humans has been made. This assessment takes two parts; 1) estimation of exposures to the workforce (the group with the highest exposure levels) and 2) an assessment of how these exposures may affect the zinc status of the workforce, specifically a pregnant worker.

Taking conservative assumptions into account, the estimated worker exposures to DTPA are low. Due to the much lower levels of DTPA in formulated products that may be available to the consumer, consumer exposure levels would be significantly lower than worker exposure levels.

The effect of estimated worker exposures to DTPA under normal working conditions or following an accidental acute exposure would not be considered to be detrimental to a workers (including pregnant women) zinc status. Thus it is highly unlikely that a consumer's zinc status would also be negatively affected, considering the significantly lower level of exposure likely in the general population. Thus exposure to DTPA would not result in the development of a zinc deficiency.

According to the text of the Classification, Labelling and Packaging directive, where data from animals provide clear evidence that a substance is capable of producing reproductive or developmental toxicity, this substance can be classified as category 1B. However, within the framework of a weight of evidence approach it is demonstrated in a comprehensive exposure assessment that the occurrence of developmental toxicity in humans would require unrealistic exposure situations and thus is extremely unlikely ***therefore Classification of DTPA in Category 2 according to the regulation (EC) 1272/2008 is considered to be appropriate***.

1. Introduction and Approach

Diethylenetriaminepentaacetic Acid (DTPA) is a chelating agent used in a number of industries. In a study of the compound (BASF 1994) high doses of DTPA were shown to cause developmental toxicity in the rat. This report presents an assessment of the risk of developmental toxicity from current uses of DTPA in support of an appropriate hazard classification.

DTPA is used in a wide number of industries including pulp and paper industries (main use), laundry detergents, cleaners, soaps, and textiles. It is also used as a setting retarder in the production of plaster, scale remover for substances such as barium sulphate, and as complexing agents of metals used as micronutrients for plants (BASF, 2007). Exposures of DTPA to the general public are minimal. The product is only used in trace amounts in final products (< 2% consumer cleaning products and <0.1% in personal care products), is poorly absorbed dermally, and does not volatilize. Thus consumer exposure, whilst it occurs, would be expected to be very low and significantly lower than workers involved in manufacturing and formulating DTPA. For this reason this assessment focuses on workers and the potential for exposures to DTPA. The exposure assessments for the three potential routes of exposure, (i.e. oral, dermal and inhalation), have been developed using conservative exposure assumptions reflecting the limited data available on work exposures.

As the induction of zinc deficiency, caused by the chelating properties of DTPA, is considered to be the mode of action responsible for the developmental toxicity this assessment determines:

- potential occupational exposure to DTPA during manufacturing and formulating
- if the occupational exposure to DTPA has the potential to cause zinc deficiency adequate to produce adverse health effects in workers and, in particular, pregnant women

2. Exposure Assessment

As the availability of DTPA exposure data in the workplace is limited, the exposure assessment has used exposure models to estimate work place exposures. In addition as patterns of exposure to EDTA and DTPA are very similar, the exposure scenarios used in the EDTA EU Risk Assessment have been applied to assess exposure to DTPA (European Chemicals Bureau 2004).

Oral exposures in the workplace

It is generally assumed that oral exposure to industrial chemicals in the workplace can be discounted (Technical Guidance Document) and DTPA is no exception, making it unlikely that any oral exposure will occur during manufacturing or formulation processes. However, for the purpose of this evaluation the possibility of some small contamination of food occurring within the workplace is considered as part of the exposure assessment. In the absence of data on the potential oral intake of dusty chemicals in the workplace, an exposure level of 25 mg/day is assumed. This assumption is based on a US EPA estimate that the daily adult unintentional soil intake would fall within the range of 0 to 50 mg/day (US EPA 1997). For the purpose of this assessment the midpoint of this range was used. For a 70 kg worker this corresponds to an oral dose of 0.35 mg/kg bw/day.

It should be understood that an estimated exposure of up to 25 mg/day is still likely to be a gross overestimate of actual worker exposure through the dietary route.

Absorption following oral exposure is approximately 5% (Stevens et al. 1962, Bondesson et al., 2007) therefore the actual systemic dose to DTPA following oral exposure in the workplace is 1.25mg/day.

In summary assuming oral ingestion of 25 mg DTPA:

Amount remaining in the Gastrointestinal Tract (GIT) is 23.75 mg/day

Systemic exposure is 1.25 mg/day

Dermal exposures in the workplace

Data on dermal absorption of DTPA are not available. However, data on similar chelating agents suggest that the rate of absorption will be low. Dermal penetration data for EDTA has been reported by the European Chemicals Bureau, (2004) as 0.001% absorption. Unpublished data from BASF (2007) reported 0.1% dermal absorption for Nitrilotriacetic acid (NTA). As DTPA has a higher molecular weight than EDTA but a similar log K_{ow} it is proposed that dermal penetration of DTPA will be equivalent to or less than that of EDTA, i.e. approximately 0.001%.

Given this very low dermal penetration, systemic exposure via dermal exposure to DTPA is not considered to significantly add to a combined workplace exposure estimate. However to ensure dermal exposures are in fact insignificant a worst case estimate for dermal exposure of DTPA has been developed based on the assessment of EDTA by the EU (European Chemicals Bureau, 2004). In the dermal exposure estimate for EDTA 5 mg/cm^2/day dermal exposure and an exposed area of 840 cm^2 was considered appropriate. Using these values, the theoretical worst case dermal exposure for DTPA is 4,200 mg/person/day.

Taking into account the dermal absorption, the systemic dose for a 70 kg person would be

= 4,200 mg/day x 0.001% / 70 kg

= 0.0006 mg/kg bw /day

Inhalation exposures in the workplace

DTPA is sold either as a liquid or a solid (a crystalline solid powder). The manufacture and major industrial use of liquid forms of DTPA are not anticipated to form aerosols. The main use for DTPA is in the paper and pulp industry and for this use it is supplied as a liquid. Due to the enclosed nature of the production process, the low volatility of liquid DTPA and the use of suitable personal protective equipment by the work force (goggles, gloves and respiratory protection) the potential for exposure to liquid DTPA during production and use in the paper and pulp industry is considered to be minimal. A similar conclusion was reached in the EDTA EU Risk Assessment report (European Chemicals Bureau 2004).

There are some applications (agricultural spraying) where aerosols of DTPA-containing solutions might be formed, however the concentration of DTPA in these solutions are typically very low (< 1%). In addition, due to the hazards posed by other components of the solutions (pesticides and fertilizers) it is well accepted that workers applying these products wear goggles, gloves and respiratory protection (European Chemicals Bureau, 2004). Thus exposures to aerosols containing DTPA are not expected to be significant.

Inhalation of DTPA powder in the air is likely to be the most significant source of inhalation exposure in the workforce. Although DTPA powder is manufactured in an enclosed process there is potential for inhalation exposure when the powder is transferred into containers for transport and when the powder is transferred from transport containers into formulating vessels. The manufacture and use of DTPA is very similar to EDTA and the EDTA EU Risk Assessment report (European Chemicals Bureau, 2004) also identified the potential for exposure to the powdered form of that chelating agent.

The data in Table 1 presents data on particle size distribution from three DTPA powder producers (Akzo Nobel, personal communication 2008, DOW, personal communication 2008, Dabeer, personal communication 2008). Of these three manufacturers, Akzo is the only company that manufactures DTPA Acid powder in the EU. Dow manufactures in the US and Latin America, whilst Dabeer no longer produces the DTPA acid powder. Therefore this data on particle size is considered to be representative of the DTPA powder in use Globally.

Table 1. Particle Size Distribution in DTPA Powder

AkzoNobel Data			Dow Data		Dabeer Data		BASF Data	
Size (µm)	Percentage by weight		Size (µm)	Average Percentage (by weight)	Size (µm)	Average percentage (by weight)	Size (µm)	Average percentage (by weight)
	Maximum Percentage	Minimum Percentage						
355	25	10	>355	10.0	> 800	1.5	< 1589	100.0
250	30	10	250 - 355	13.5	800-200	5.5	< 1002	99.3
180	20	5	180 - 250	15.0	200-150	17.3	< 502	94.6
125	15	10	125 - 180	10.0	150-100	52.0	< 200	76.6
90	20	5	90 - 125	6.5	100-71	23.7	< 100	51.5
63	20	5	53 - 90	17.2	71-63	0.2	< 50	30.9
< 63	25	5	< 53	29.0	63-40	0.0	< 20	12.7
< 10	4	0					< 10	5.3
							< 4	1.3
							< 2	0.1

These data suggest that the powder is dominated by particles greater than 63 µm with majority of particles being >10 µm. A report by ICRP (1994) concluded particles above 10 µm are only partially inhaled. Some of the particles are sufficiently large not to be drawn in with an inspired breath (40%). Of the 60% inhaled 50% are deposited in the extrathoracic airways. Only 10% enter the lung and result in a true inhalation dose.

Using the ICRP report (1994) as a guide this analysis assumes 90% of the inspired particles will be deposited in the extrathoracic airways and eventually swallowed. The remaining 10% are assumed to be deposited in the lung and completely absorbed.

During manufacturing DTPA dust levels are kept under control using local exhaust ventilation and workers handling the packing of DTPA are also required to wear gloves, goggles and respiratory protection (Dow MSDS: Versenex* DTPA acid Chelating Agent, BASF MSDS: Trilon C and Trilon CS). Whilst the actual concentrations of DTPA in the air are not monitored as a standard, the total dust concentration in the air is kept below 2 mg total dust per m^3 air (personal communication AkzoNobel). The DNEL LT local effects for DTPA has been set at 1.5 mg/m3 (respirable particles). In analogy with the dust limits (viz. 10 mg/m3 for inhalable dust and 3 mg/m3 for respirable dust), a DNEL LT for inhalable particles could be set at 5 mg/m3. Therefore, while dust samples will contain a variety of particles, for this assessment a conservative assumption is that the dust is inhalable DTPA and that inhalation exposure of 2 mg/m^3 represents the situation in the EU and globally.

To estimate exposure during formulating DTPA, (e.g. pouring DTPA powder into mixing vessels or other processing machinery), the same assumptions used in the EDTA EU Risk assessment (European chemicals Bureau, 2004) have been applied, i.e. a 1 hour daily exposure to an airborne DTPA powder concentration of approximately 5 mg/m^3 in the absence of ventilation. This leads to an average 8 hour TWA exposure of 0.6 mg/m^3. Although gloves, goggles and respiratory equipment are required when handling DTPA for the purposes of this exposure assessment it is conservatively assumed respiratory protection is **not** worn.

Inhalation exposure to DTPA during manufacture assumes an air concentration of 2 mg/m^3 and inhalation of 10 m^3 air over an 8 hour work period giving a Total Exposure to DTPA of 20 mg/day. Of the 20 mg approximately 90% is swallowed (giving an oral dose 18 mg/day of which 5% will be absorbed into the systemic circulation) while 10% enters the deep lung and is assumed to be 100% absorbed. Thus systemic exposure is a combination of 0.9 mg from the GIT (i.e. 5% of the ingested dose) and 2 mg from the lung.

Thus:

Amount of DTPA entering systemic circulation is 2.9 mg/day

Amount of DTPA remaining in GIT is 17.1 mg/day

To estimate inhalation exposure when formulating DTPA it is assumed the air concentration over the course of the day is 0.6 mg/m^3. Considering the inhalation of 10 m^3 over the course of the working day, the total exposure would be 6 mg/day. Of the 6 mg approximately 90% is swallowed (giving an oral dose 5.4 mg/day of which 5% will be absorbed into the systemic circulation) while 10% enters the deep lung and is assumed to be 100% absorbed. Thus systemic exposure is a combination of 0.27 mg from the GIT (i.e. 5% of the ingested dose of 5.4 mg) and 0.6 mg from the lung.

Thus:

Amount of DTPA entering systemic circulation is 0.87 mg/day

Amount of DTPA remaining in GIT is 5.13 mg/day

Estimated Combined Inhalation and Oral exposure During Manufacture of DTPA

Total dose remaining in the GIT = direct oral exposure + indirect following inhalation exposure

= 23.75 mg + 17.1 mg

= approximately 41 mg/day

= 0.58 mg/kg bw/day (assuming 70 kg bodyweight)

Total systemic exposure =(Total oral exposure*absorption) + (inhalation exposure*absorption)

= 1.25 mg + 2.9 mg

= 4.15 mg/day

= 0.03 mg/kg bw/day

As the exposure to DTPA during manufacturing are higher than during formulating these values have been used in the health assessment described below. Also as dermal exposures are trivial these have been excluded from the assessment.

4. Industrial exposures to DTPA: Health Evaluation

As indicated previously, the actual hazard associated with DTPA is its ability to bind metals such as zinc reducing bio-availability. Therefore in order to fully evaluate the risk associated with DTPA exposure to workers it is necessary to understand how exposures to DTPA in the workplace could affect zinc status in workers. This Health Evaluation examines:

- the average dietary zinc intake by workers
- impact of occupational exposure to DTPA on zinc status and
- if the resulting reduction in the bio-availability of dietary zinc is likely to be associated with an adverse health outcome

Human Zinc intake

The average zinc content of a healthy adult is between 1.5 and 2 g (Bedawal *et al.*, 1991). Past surveys have shown that pregnant women consume an average of 10 mg Zn/d (Swanson and King, 1983) although other data from the UK indicate that the zinc intake of women in general (not specific to pregnant women) is approximately 7 mg per day (NDNS, 2002). In 27 reported studies, dietary zinc intakes of non-vegetarians ranged from 5.7 to 22 mg/d; the intakes of vegetarians ranged from 5 to 12.6 mg/d with a mean of approximately 8 mg/d (King, 2000). The reason for the lower intake in vegetarians is the absence from the diet of meat, which is a significant source of zinc.

Overall it appears that an average zinc intake for a female vegetarian would be between 5 and 8 mg/day.

In general, approximately 25% of the dietary intake of zinc appears to be absorbed, although there are a number of factors that can influence this (King, 2000). For example, absorption is reduced by the presence in the diet of excess calcium, fibre or phytate, smoking and alcohol abuse (King, 2000). It is also apparent that zinc absorption from the gut is increased in individuals consuming less zinc in the diet or when the person has lower zinc status (Cousins, 1986). Excretion of zinc is also decreased in individuals with a lower intake of zinc (King, 2000). A zinc intake of between 2.7 and 5 mg/day from sources other than animal protein (for example a vegetarian diet, or diet high in cereals) is considered insufficient to maintain zinc homeostasis (Prasad, 1985a, b). Presumably this daily zinc intake from a diet containing animal protein would also be considered low but due to the greater bio-availability of the zinc in this type of diet it would still suffice for maintenance of zinc homeostasis. It should be noted that many estimations of what would be a sufficient intake to maintain zinc homeostasis are based on studies to assess the amount of zinc intake necessary to exactly match the amount of zinc excreted (Hambridge, 2003). However, as indicated above, zinc absorption and excretion are not constant, and vary with an individual's zinc status, therefore assessments of zinc requirement are not necessarily absolute and thus the estimate given above of an 'insufficient' zinc intake is a range and not a single limit.

It has been identified that during pregnancy the requirement for zinc increases above that required in a non-pregnant state (King, 2000). Since the zinc intake of women during pregnancy does not appear to increase significantly any additional requirement for zinc probably comes from adjustments in maternal zinc homeostasis, i.e. a decrease in the amount of zinc excreted, a mobilisation of maternal zinc stores, and/or an increase in the amount of zinc taken up from the diet. The latter of these mechanisms is likely the most significant (King, 2000). Thus, while the increased requirement for zinc during pregnancy might make such a group more susceptible to substances altering the availability of zinc, this group is also in an adaptive state, and likely better able to respond to an unexpected decrease in zinc availability.

There might be a concern that the vegetarian (and vegan) population having a diet high in fibre but low in animal protein may represent a susceptible population for zinc depletion. However a balanced vegetarian (or vegan) diet should provide sufficient nutrition during pregnancy, albeit with lower levels of some minerals such as zinc.

At present there is no advice (in Europe and the US) to pregnant women proposing the level of zinc in the diet should be monitored or supplemented, rather advice focuses on the importance of a well balanced diet in general, with specific advice on folic acid, vitamin B12 and iron. In populations where zinc deficiency is more prevalent (due to poor nutrition as a result of poverty, or eating habits that include consumption of significant amounts of clay and cereals) trials to assess the benefits of supplementing the diet with zinc (Shah *et al.*, 2006) have been inconclusive and in general there appears to

be little or no benefit to zinc supplementation during pregnancy in zinc deficient populations.

With respect to the workforce, it is generally accepted the worker population is 'healthier' than the general population, for instance, in epidemiological studies the 'healthy worker effect' often has to be taken into account as a confounder when comparing workers to the general population. It thus seems defensible to assume that the average worker (pregnant or not), is healthy with a balanced diet containing zinc in the mid range of 5 – 8 mg/day, i.e. approximately 6.5 mg zinc/day. While this health assessment includes pregnant women, in reality the number of women working with DTPA is low and the number of pregnant women even lower. As such considering the effect of an industrial exposure to DTPA on pregnant women is a fairly conservative approach.

Estimation of Effect of DTPA Exposure on Zinc Status

In a review by Domingo (1998), the developmental toxicity of DTPA was demonstrated to be linked to its ability to bind essential metals such as zinc and so the toxic effects observed are due to an induced deficiency in essential metals such as zinc rather than the DTPA itself. If exposure to DTPA does not negatively impact an individual's zinc status then it will not cause developmental toxicity. In order to determine how DTPA would affect an individual's zinc status it is therefore necessary to consider how much zinc is expected to bind to a given dose of DTPA. The assumption being that zinc bound to DTPA is no longer bio-available and the more that becomes bound to DTPA the more severe the effect on the individuals zinc status.

The zinc-DTPA complex consists of 1 mole of DTPA and 1 mole of zinc. This is equivalent to 6 mg of DTPA and 1 mg zinc. A worst case estimate of the amount of zinc that a dose of DTPA could bind would therefore assume that for every 6 mg of DTPA dosed, 1 mg of zinc will be chelated. There are however many factors in a biological system influencing the amount of zinc-DTPA complex formed. For example DTPA complexes with other metal ions and the amount of each complex is affected by the presence of other metals, the pH and the concentration of DTPA (increasing the concentration will increase the probability that each metal complex will be formed). In the gut, DTPA will therefore bind to whatever metal ions are present and favoured by the pH conditions; if there is very little zinc in the gut or there are other metals present to which it also binds then the DTPA will be less likely to bind zinc. To illustrate this, consider a balanced female diet, containing around 2600 mg potassium, 750 mg of calcium, 230 mg magnesium, 10 mg iron, 1 mg copper and 7 mg zinc (NDNS, 2002), whilst only iron and copper have a greater affinity for DTPA than zinc, the significantly larger amounts of calcium, magnesium and potassium available will mean that DTPA is far more likely to interact with these elements than zinc. Any DTPA that becomes systemically available will face a similar situation, but will also have to compete with the various metal transport proteins in the body, such as metallothionein. Therefore it seems unrealistic to assume that 1 mole of DTPA will bind 1 mole of zinc.

Support for this argument comes from two pieces of information. The first is from the BASF developmental toxicity study (BASF 1994) in which, gavage doses of 100, 400 and 1000 mg/kg bw/day DTPA resulted in no, mild and moderate developmental toxicity respectively. These doses are equivalent to approximately 30, 130 and 330 mg DTPA/day (assuming average bodyweight of 300g during dosing period). Assuming 1 mole of DTPA will bind 1 mole of zinc, then 30, 130 and 330 mg/day DTPA should be capable of binding approximately 5, 22 and 55 mg/zinc per day. Under such circumstances all three doses would be capable of binding the 1.5 mg/day zinc in the diet (based on 60 mg zinc /kg diet, average food consumption of 25 g diet/day) and thereby produce evidence of a zinc deficiency. The fact only the top dose has any significant effect on the daily zinc intake indicates significantly more than 1 mole of DTPA is necessary to prevent the uptake or utilisation of 1 mole of zinc.

The second piece of information comes from data of an individual treated with DTPA following an exposure to the isotope ^{241}Americanum (Kalkwarf et al., 1983). It was discovered through the analysis of the urinary excretion of essential metals, that following an intravenous injection of 1g of the calcium salt of DTPA, zinc excretion in the urine was significantly increased compared to normal, with approximately 18 mg of zinc excreted in the urine (other metals were not significantly affected). The number of moles of zinc excreted per mole of DTPA was calculated to be approximately 0.13, i.e. 7 moles (42mg) of DTPA bind approximately 1 mole (1 mg) of zinc.

Exposure to DTPA can affect zinc status in two ways:

1. Chelating zinc in the GIT thereby reducing the availability of zinc for absorption.
2. Systemic DTPA can bind zinc already absorbed into the circulatory system, carrying it out of the body in the urine.

Both considerations are described below.

Effect of DTPA in the GIT (combined oral and inhalation exposure) on Zinc Status

As calculated above the total amount (combination of oral and inhalation exposure) of DTPA present in the GIT of a worker is 41 mg/day or 0.58 mg/kg bw/day. Based on a ratio of 7 moles (42 mg) of DTPA being required to bind 1 mole zinc, the maximum amount of zinc which could be bound to DTPA present in the GIT is, at most, 1 mg of the 6.5 mg of zinc present in the diet.

Effect of Systemic Exposure to DTPA (combined oral and inhalation) on Zinc Status

The total estimated worker systemic exposure following a combination of oral and inhalation exposure is 4.15 mg/day or 0.03 mg/kg bw/day. Based on a ratio of 7 moles of DTPA dosed to 1 mole of urinary zinc excretion, it can be estimated that a systemic intake of 4.15 mg of DTPA would cause a urinary excretion of approximately 0.1 mg zinc.

Health Evaluation

The calculations described above indicate that occupational exposure to DTPA during manufacturing process can lead to reductions in the bioavailability of zinc both by reducing amount of dietary zinc available for absorption from the GIT and by binding zinc already absorbed in the systemic circulation. As described earlier there is on average 6.5 mg of zinc present in the diet of which approximately 25% is absorbed from the GIT into body. Of the 6.5 mg of zinc in the diet the worst case scenario is that 1 mg might bind to DTPA in the GIT. This however leaves 5.5 mg available for absorption and as only 25% is usually absorbed there is still an excess of zinc. Also at this time it is perhaps worth remembering the estimated exposures to DTPA are grossly exaggerated using a combination of both oral and inhalation exposures even though any oral exposure is most unlikely to occur during manufacturing or formulating the chemical. In reality it is unlikely that exposure to DTPA has any impact on the uptake of zinc from the GIT. Additionally, the ability of DTPA in the gut to bind to zinc is dependent on timing of DTPA exposure and food intake. Since DTPA exposure is likely to be a low level throughout the day with occasional peaks, there will be limited times where the DTPA can interact with dietary zinc; consider the greater toxicity of the similar chelating agent EDTA when dosed via the diet, versus dosing via gavage (Kimmel, 1977).

To examine the possible effect of DTPA on systemic zinc it is assumed dietary intake is 6.5 mg of zinc and absorption from the GIT is 25% giving a systemic level of about 1.63 mg zinc per day. A loss of 0.1 mg zinc in the urine would effectively reduce the net intake of zinc to approximately 1.53 mg/day. This is equivalent to a person reducing their average zinc intake from 6.5 mg/day to approximately 6.12 mg/day, which is still a sufficient dietary intake.

In considering the small effect of DTPA on zinc status and the fact that such small changes can be compensated quite readily by adjustments in zinc uptake and/or excretion it is seems unlikely that even using grossly exaggerated estimation of DTPA exposure will have any health impact for pregnant and non-pregnant workers alike.

Acute exposure situation

It is possible that a worker could get a large, one time, and accidental exposure to DTPA following an accident in the workplace. In order to assess the risk associated with such an exposure, consider the following scenarios;

1) Cleaning of a powder spillage by an external enterprise/contractor, exposure 8h to 50 mg/m³, respiratory protection not worn (Schneider *et al.*, 2007), i.e. 10 m^3 air inhaled over 8 hours, resulting in 500 mg/worker/d inhaled. Of this 90% is swallowed, of which 5% is absorbed, i.e. 22.5 mg. The remaining 50 mg reach the deep lung, of which 100% is absorbed. This leads to a total systemic exposure of 72.5 mg.

2) A worker not wearing respiratory protection is exposed orally to a DTPA mixture following a spillage (splash) (Schneider *et al.*, 2007). This leads to an exposure of approximately 2 g of mixture (a higher exposure level is unlikely since the worker would be aware of the exposure and spit the liquid out). The most concentrated DTPA formulation on the market in the EU and US is a 40% aqueous solution. The worker would therefore be exposed to 0.8 g of DTPA, of which 5% would be absorbed through the gut. Thus systemic exposure would be approximately 40 mg DTPA.

Considering the approximate ratio of 7 moles DTPA to 1 mole of zinc, these 2 exposure scenarios could lead to loss of zinc from the body of up to 2 mg. Considering the total body zinc store is between 1500 and 2000 mg, and the daily intake of zinc from the diet should supply between 1.5 and 2 mg zinc per day, this loss of approximately 2 mg should not have a significant impact on the zinc status of a worker and would be easily recoverable within 1 to 2 days. Thus, even a large accidental exposure such as those described above is unlikely to have an effect on zinc status.

Conclusion

In conclusion, based on the ability of the body to compensate for changes in zinc status and the minimal amount of zinc that could be affected by DTPA, it is unlikely that exposure to DTPA in the workplace will adversely affect an individual's zinc status. Therefore, since there is clear mechanistic information that raises strong doubt about the occurrence of the developmental toxicity in humans, classification in Category 2 (according to regulation (EC) 1272/2008) is considered appropriate.

Classification proposal:

According to the Classification, Labelling and Packaging Directive:

Category 2 developmental toxicant

References

BASF 2007. Trilon® C types, Trilon CS, Trilon C Liquid, Trilon C Powder, Organic chelating agents used to control the concentration of metal ions in aqueous systems. Technical Information TI/EVD 1153 e January 2007

BASF AG, Department of Toxicology. 1994. Study of the prenatal toxicity of Trilon C liquid in Wistar rats after oral administration (gavage). Project No. 30R0812/89117), 18 November 1994

Bedawal *et al.,* (1991). Effects of zinc deficiency ad toxicity on reproductive organs, pregnancy and lactation: a review. *Trace Elements in Medicine,* **8 (2)**, 89-100

Bondessen *et al.,* (2007). Site of deposition and absorption of an inhaled hydrophilic solute. *British Journal of Pharmacology,* **63(6)**, 722-731

Cohen and Guilmette (1976). Biological effects of the enhanced excretion of zinc after Calcium Diethylenetriaminepentaacetate Chelation therapy. *Bioinorg Chem* **5**(3), 203-210,

Cousins (1986). Towards a molecular understanding of zinc metabolism. *Clinical Physiological Biochemistry,* **4**, 20-30

Durbin and Schmidt (1989). Predicting the Kinetics of Chelating Agents in Man from Animal Data, *Health Physics*, **57 (Sup. 1)**, 165-174

Domingo (1998). Developmental toxicity of metal chelating agents. *Reproductive toxicity*, **12(5)**, 499-510

European Chemicals Bureau, *European Union Risk Assessment Report: Edetic Acid (EDTA) Priority list 1, Volume 49*, 2004

Hambridge, (2003). Human Zinc Homeostasis; Good but not Perfect. *Journal of Nutrition*, **133**, 1438S-1442S

Hurley and Swenerton, (1966). Congenital malformations resulting from zinc deficiency in rats. *Proceedings of the Society for Experimental Biology and Medicine,* **123(3)**, 692-696

ICRP. 1994. International Commission on Radiological Protection. *Human Respiratory Tract Model for Radiological Protection*. ICRP 66. Oxford: Pergamon Press.

Kalkwarf *et al.* (1983). 1976 Hanford americium exposure incident: urinary excretion of trace metals during DTPA treatments, *Health Physics* **45**(4), 937-947

Kimmel (1977). Effect of Route of Administration on Toxicity and Teratogenicity if EDTA in the Rat. *Toxicology and Applied Pharmacology* **40**, 299-306

King (2000). Determinants of maternal zinc status during pregnancy. *The American Journal of Clinical Nutrition,* **71(suppl)**, 1334S-1343S

NDNS (2005). The National Diet and Nutrition Survey; Adults aged 19 to 64. Volume 5: Summary Report. *Her Majesties Stationary Office*, ISBN 0 11 621570 4

Prasad (1985a). Clinical manifestations of Zinc deficiency. *Annual Review of Nutrition,* **5**, 342-363

Prasad (1985b). Clinical, endocrinologic, and biochemical effects of zinc deficiency. *Special topics in endocrinology and metabolism*, **7**, 45-76

REACh 2008. Chapter R.8: Characterization of dose/concentration-response for human health GUIDANCE ON INFORMATION REQUIREMENTS AND CHEMICAL SAFETY ASSESSMENT "Technical Guidance Documents in support of the New EU Chemicals Legislation (REACH) – V: Development of a Technical Guidance Document for preparing the Chemical Safety Assessment (REACH Implementation Project 3.2-2)" Service Contract Number CCR.IHCP.C432365.X0. 10. March 2008

Rogers *et al.*, (1985). Zinc Deficiency in Pregnant Long-Evans Hooded Rats: Teratogenicity and Tissue Trace Elements *Teratology*, **31**, 89-100

Shah D, Sachdev HP (2006). Zinc deficiency in pregnancy and Fetal outcome. *Nutrition Reviews,* **64 (1)**, 15-30

Sikov MR, Smith VH, and Mahlum DD, (1975). Teratological effectiveness and fetal toxicity of DTPA in the rat. *Pacific Northwest Laboratory Annual Report BNWL-1950 PTI*, pg. 138-141

Stevens *et al.*, (1962). Metabolism of chelating agent diethylenetriamine pentaacetic acid (C14 DTPA) in man. *Proceedings of the Society for Experimental Biology and Medicine,* **111 (2)**, 235-238

Swanson and King (1993) Reduced serum zinc concentration during pregnancy. *Obstet Gynecol,* **62**, 313–8.

USEPA 1997. Exposure factors Handbook Office of Research and Development, Exposure Factors Handbook, Volume I, EPA/600/P-95/002a, August 1997

ANNEX 2

Justification in support of read across within the Aminocarboxylic acid-based chelants chemical category

According to REACH Practical guide 6: How to report read-across and categories, REACH TGD, Chapter R.6: QSARs and grouping of chemicals, and Read-Across Assessment Framework (RAAF)

INTRODUCTION

Within REACH, the obligation to conduct tests with vertebrate animals should be considered as a last resort, only after exhausting all potential sources of information on the physical and (eco)toxicological properties of chemicals (EU 2006). Article 13 of REACH requires that use must be made whenever possible by alternatives to vertebrate animal tests, through the use of alternative methods as *in vitro* methods, (Q)SARs or from structurally related substances via grouping or read-across.

Annex XI of REACH offers the option to evaluate specific endpoints by read-across. According to Chapter R.6 (QSARs and grouping of chemicals) of REACH technical guidance documents (TGD), read-across can be applied for "s*ubstances whose physico-chemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity*". Application of the read-across concept requires that physico-chemical properties, human health effects and environmental effects or environmental fate may be predicted from data from one or more reference substances to make a prediction of the endpoint for the target chemical. This avoids the need to test every substance for every single endpoint. In this report a justification for read across within the aminocarboxylic acid-based metal chelants group is presented according to the requirements stipulated in Chapter R.6 (QSARs and grouping of chemicals) of the REACH TGD.

The Read-Across Assessment Framework (RAAF; ECHA, 2015) document has been prepared to facilitate improvements in the use of read-across aimed at fulfilling the requirements of the REACH regulation.

Any read-across approach must be based on structural similarity between the source substance(s) (reference substances for which tests have been conducted) and target substance(s) (other substances that have not been tested). However, structural similarity alone is not sufficient to justify the possibility to predict properties of the target substance(s) by read–across and a read-across hypothesis needs to be provided. In the current document structural similarities based on structural formula are indicated in Appendix A1; the read-across hypothesis is indicated in Section 2.

READ ACROSS JUSTIFICATION

Aminocarboxylic acid-based chelants chemical category

The acid (H^+) or salt (Na^+, K^+, NH_4^+) chelates are considered uncomplexed chelates and are different from metal chelates as they are bound to a metal ion. The uncomplexed chelates are called 'empty' chelates. When no hydrogens have been substituted (EDTA acid), the chelant exists as an inner salt or zwitterion.

The following molecular structural formulas are applicable to this category:

EDTA: $(HOOCCH_2)_2NCH_2CH_2N(CH_2COOH)_2$,

DTPA: $(HOOCCH_2)_2NCH_2CH_2\ N(CH_2COOH)CH_2CH_2N(CH_2COOH)_2$

The 'empty' chelates used in this category are the following:

CAS no	EC no	Abbreviation
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 the aminocarboxylic acid-based chelant category possess similar molecular structures that contain common functional groups (see Appendix A). 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. The diethylenetriamine structures contain five acetic acid groups (DTPA); the ethylenediamine structure has four acetic acid groups (EDTA).

Therefore all category members have identical functional groups. It is the presence of multiple carboxylic acid groups on the amine that provides chelants with their unique metal ion chelating or sequestering properties. This common property is the important feature to consider in assessing the aquatic and mammalian toxicity of chelants and in justifying their consideration as a category.

Because of this unique metal ion chelating property, metal EDTA and DTPA chelates have also been used for comparison consisting of 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^{+} as counter ion (see further for the hypothesis):

CAS no	EC no	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

A common mechanism of action for the chelant category based on structural and chemical similarity is the fundamental basis for a category approach for these closely related chemicals. The ability of chelants to remove and add ions to solution is the mechanism whereby these chemicals produce toxicity. Environmental fate and ecological and mammalian toxicity profiles are consistent within the category. Category members have demonstrated high stability to hydrolysis. Category members emitted to waterways will remain dissolved in this environmental compartment. If emitted to soil or sediment, category members will exhibit high water solubility and soil mobility. This behavior is based on the presence of multiple carboxylate anion groups in the molecular structure, and is supported by the demonstrated high water solubility and negligible vapor pressure of category members.

With regard to environmental biodegradation, several of the members of the category have been tested in actual laboratory studies with similar and predictable results from standard laboratory tests, in general being found not to be readily biodegradable. However, results of recent studies indicate that EDTA, EDTA-CaNa2 and EDTA-Na2H2 can biodegrade under certain conditions.

The substantial body of evidence that chelants are not directly toxic to aquatic and mammalian organisms but exert their influence by affecting mineral balance, together with the fact that the backbone structures of the chelants in the category have similar affinities for metals supports the inclusion of these chelants in a category. Subtle differences in toxicity due to the presence of calcium, magnesium, manganese, ferric (or ferrous) iron, copper or zinc can be explained by their affinity towards these metals and their ability to supply metals to organisms.

According to the chemical equilibrium and kinetic properties of metal-ligand complexes, a certain portion of a free metal ion is always present in solution. This is particularly important for aquatic systems. Uncomplexed chelants like EDTA and DTPA would be expected to add H+ ions to media (which would lead to decreased pH), and would chelate metals present in their milieu based on affinity (see Table below). The highest affinity of EDTA and DTPA is for Fe^{3+}, the lowest affinity for Mg^{2+}. The order of affinity is the same for EDTA and DTPA, with DTPA showing higher values because DTPA has five acetic groups for binding whereas EDTA has only four. Note that this table has a logarithmic scale, indicating e.g. that DTPA has a 10-times higher affinity for Fe^{2+} than for Mn^{2+}.
The Fe^{3+} and Cu^{2+}-containing chelants would not be expected to significantly affect mineral balance at low concentrations because the affinity for ferric ion or copper is stronger than for most other ions. Also, the EDTA-Zn(NH4)2 and EDTA-ZnNa2 compounds would be expected to have less of an effect than EDTA-(NH4)2 or EDTA-H4 on zinc balance. The magnesium and calcium-containing chelants would be expected to be of intermediate toxicity (between EDTA-H4 and EDTA-Fe or EDTA- Zn-containing chelants), since they would not affect pH as much as the acids and would provide essential ions that are not toxic in amounts that would be supplied by the chelants, but also would chelate essential ions such as Zn^{2+} and Fe^{2+} or Fe^{3+}. Data show that the toxic profile of metal chelants in this category generally follows this pattern, and can be predicted by the type of ion that the chelant is complexed with and its affinity for the particular ion.

Stability constants (Log K values)

Metal 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

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

Based on RAAF (ECHA, 2015), regarding possible scenarios, scenario 4 applies to compare EDTA with DTPA. These are structurally related substances because they have a similar backbone and their structures differ only in the number of functional groups (viz. 4 acetic acid groups attached to the nitrogens for EDTA and five of such groups for DTPA). Both substances are hardly absorbed and are not further (bio)transformed. Data (see Table above) indicate that their potency towards binding metals increases with the number of functional groups (4 for EDTA and 5 for DTPA). The information on other properties reported in the data matrix (binding of Zn so that insufficient Zn is bioavailable) presents an overall consistent quantitative pattern throughout the category. In the repeated dose toxicity studies, similar effects were observed but at higher doses of DTPA due to the presence of more functional groups.

Also based on RAAF (ECHA, 2015), when comparing the various empty and metal EDTA's and DTPA's, scenario 6 is applicable, i.e. exposure to different substances causes qualitatively and quantitatively similar effects via a common mechanism. The metal EDTA's and DTPA's are structurally similar substances containing a similar backbone; the structures differ in the ion(s) bound (e.g. H^+, Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, and/or Fe^{3+}), meaning that the amount of Zn bound will depend on both the structure, viz. DTPA has a higher affinity for Zn than EDTA; but also the counter ion(s) present, viz. EDTA-FeNa and DTPA-FeNaH will not easily exchange with Zn (and thus bind Zn instead of Fe) because of their much higher affinity for Fe^{3+} than for Zn^{2+}. In contrast, EDTA-CaNa2 or DTPA-CaNa3 would easily exchange their Ca for Zn so that insufficient zinc will be bioavailable. In this respect, at high levels (at levels inducing Zn deficiency) EDTA-H4 and DTPA-H5 are more hazardous than EDTA-Fe or DTPA-Fe (see also next sections).

Using the category approach, read across has been performed from the appropriate tested members to those without available data. The toxicity of the counter-ion is considered for read-across but may not be the deciding factor in read-across. In general, with the exception of copper, a conservative approach is used whereby read-across will be from the most toxic substance to that without data.

Two 'empty' chelates have previously been assessed in the OECD HPV program (SIAM 18): EDTA-H4 (CAS No. 60-00-4) and EDTA-Na4 (CAS No. 64-02-8). The data can be viewed at http://www.oecd.org/env/hazard/data/. Data for counter ions can be viewed in the OECD HPV assessments for several calcium salts, zinc salts and iron salts, and the ammonia category found at: http://www.oecd.org/env/hazard/data/

LIST OF ENDPOINTS COVERED

For all human health endpoints the relevant information obtained on the 'empty' chelates EDTA-H4, EDTA-Na4, EDTA-Na2H2, EDTA-Na3H, DTPA-H5, DTPA-Na5, or DTPA-K5 will be used for read-across.

Use is also made – where appropriate – of relevant information obtained on the metal chelates EDTA-FeNa, EDTA-MnNa2, EDTA-CaNa2, EDTA-CuNa2, DTPA-CaNa3, and DTPA-FeNaH.

PHYSICO-CHEMICAL PROPERTIES (APPENDIX B)

The members of this category are (or can be produced) as solid granular materials in the pure or neat state with molecular weights that range from about 300 to 500 and possess similar physical/chemical properties.

The metal ammonium chelates (EDTA-Zn(NH4)2, EDTA-Cu(NH4)2, EDTA-Mn(NH4)2, and DTPA-Fe(NH4)2) and the metal potassium chelate (EDTA-MnK2) are generally produced or sold as aqueous solutions (see below).

As metal-organic salts, or inner salts, all category members decompose before melting upon sufficient heating (generally at temperatures >150-300°C). Therefore true melting points are not applicable. Chelants that are metal salts do not exist as discrete neutral molecules, and therefore cannot volatilize, exert appreciable vapour pressure, or boil. Therefore, vapour pressure and boiling point data are not applicable for such chelants and are generally not determined. Henry's law constants are also expected to be negligible. Chelants that exist as neutral molecules (not metal salts) can exert vapour pressure, but in this case the vapour pressure is exceedingly low. All category members are soluble in water, most of these are very soluble (>10 g/L). They are all insoluble in organic solvents, therefore also possessing negative partition coefficients (log K_{ow}).

The relative densities are generally around 1.5-1.8, and particle size distributions show large values, viz. generally 30-50% is smaller than 100 microns (inhalable), but only up to 5% is smaller than 5 microns (respirable).

Self-ignition temperatures are generally between 200-400°C or even higher than 400°C. The metal chelants are not flammable, and they do not have explosive or oxidizing properties.

The chelates in aqueous solutions (metal ammonium and potassium chelates), generally freeze at ca. -20°C and their boiling point is between 100 and 110°C. The density is around 1.2-1.3 g/cm3. Their vapour pressure is close to that of water at 20°C (2.3 kPa). The viscosity is between ca. 5-15 mPa.s for the metal ammonium chelates solutions whereas that for water is 0.894 mPa.s (at 25°C).

TOXICOLOGICAL PROPERTIES (APPENDIX C)

Toxicokinetics, Metabolism and Distribution

Oral

Studies with the 'empty' chelate EDTA-Na2H2 and EDTA-CaNa2 indicate that these complexes are poorly absorbed in mammals after oral administration (Foreman and Trujillo, 1954; Foreman *et al*., 1953; Yang and Chan, 1964). For each of these materials, approximately 1-5% and 90-99 % of the administered dose was detected in urine and feces, respectively, within 24-48 hours. Experiments with EDTA-CaNa2 in rats and man showed that at the low pH of the stomach, calcium dissociates from EDTA, leading to

precipitation of EDTA in the stomach and re-dissolution at pH levels encountered in the small intestine (Foreman *et al*., 1953; Foreman and Trujillo, 1954). Because ions complexed to EDTA dissociate from EDTA in the GI tract, and EDTA is poorly absorbed, mammalian toxicity data for all EDTA-containing chelants in the category are expected to be similar, with subtle differences arising from the ability of the complexes to dissociate under pH's encountered in the GI tract. Therefore, the toxicity of these molecules would be dictated by the indirect ability of the molecules to sequester and or supply cations rather than direct toxicity of the molecules EDTA and DTPA.

With regard to EDTA-FeNa, the iron remains complexed with the EDTA under the acidic conditions in the stomach. The strength of the complex is reduced as the pH rises in the upper small intestine, allowing release of some of the iron for absorption (Heimbach *et al*., 2000). Once ingested, the absorption of iron is regulated through the same physiological mechanisms as for other forms of dietary iron (Heimbach *et al*., 2000) and would include uptake of uncomplexed iron from the lumen of the gut as needed by the body, and transported to the blood and plasma coupled to transferrin. Following oral treatment of rats with either iron EDTA or iron DTPA, a slightly higher amount of iron was excreted in the urine following treatment with iron EDTA (9.9%) when compared to iron DTPA (6.2%; Rubin and Princiotto, 1960). Zinc absorption was significantly increased and retention and elimination enhanced in rats fed a zinc deficient diet fortified with EDTA-FeNa compared with the same diet without EDTA-FeNa (Hurrell *et al*., 1993). Enhanced zinc absorption was found from low bioavailability diets supplemented with EDTA-FeNa (Davidsson *et al*., 1994). In this same study, there was no effect on calcium absorption and no effect on retention of zinc or calcium. In another study, manganese absorption and urinary excretion were unchanged in adults receiving a diet fortified with either EDTA-FeNa or ferric sulphate (Davidsson *et al*., 1998).

In studies with rats, dogs and humans, the oral absorption of DTPA and DTPA salts is low with an average intestinal absorption across species of 3 to 5% (Dudley *et al*., 1980ab; Resnick *et al*., 1990; Stevens *et al*., 1962). Following exposures, the absorbed dose is rapidly excreted via the urine. The excretion of DTPA, however, is almost exclusively via the feces. The passage of the DTPA through the gut varies between individuals; however, there is almost complete excretion of the chelant 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).

The available evidence indicates that the limited amount of absorbed EDTA- and DTPA-complexes are not or are scarcely metabolized and are excreted as chelated complexes via the urine following glomerular filtration and tubular secretion.

Inhalation

Aerosolised DTPA complexes of DTPA including ^{111}In-DTPA, ^{99m}Tc-DTPA, Pu-DTPA, and also the zinc and calcium salts of DTPA, have been administered to either dogs or rats via the inhalation route (Ballou, 1978; Dudley et al., 1980ab; Dahlback, 1990). These studies demonstrated that DTPA complexes are absorbed from the respiratory tract into the systemic circulation but that the degree of absorption is dependent on the site of deposition and the way of breathing (nose or mouth). In dogs, the percentage of applied dose absorbed increases the further in the respiratory tract that the dose is deposited

(Dudley *et al*., 1980b). DTPA deposited high up in the respiratory tract was predominantly swallowed, with approximately 23% absorption from the nasopharyngeal region compared to approximately 90% absorption following instillation into the pulmonary region. A similar pattern was observed in rats (Stather *et al*. 1976, referenced in Dudley *et al*. 1980b). In humans, DTPA absorption following the inhalation of a nebulised spray containing DTPA (mean droplet size 0.3-2.0 µm) was estimated to be 20% of the administered dose (Jolly *et al*., 1972). Similar absorption patterns are expected for EDTA.

Dermal

After application of ^{14}C-labelled EDTA-CaNa2 to the skin of healthy young adult men, no radioactivity was detected in the blood, and that found in urine accounted for a maximum of only 0.001% of the administered dose (Foreman and Trujillo, 1954). There are no data available on the dermal absorption potential of DTPA but dermal absorption potential is not considered to differ from EDTA.

Other Routes of Exposure

Following parenteral administration of ^{14}C-labelled EDTA-CaNa2 in rats, 95 to 98% was eliminated in urine unchanged within six hours. Peak plasma levels were found 50 minutes after administration with <0.1% of the material oxidized to $^{14}CO_2$ and with no amount of the substance retained in any organ (Foreman *et al*., 1953). Almost all radioactivity was excreted within 12 to 16 hours.

In man, i.v. administration of DTPA-Na5 resulted in almost complete excretion within 24 hours with a half-life of 2 to 4 hours with little or no excretion via the feces (Stevens *et al*., 1962).

Systemic administration of DTPA (intravenous, intraperitoneal, subcutaneous) caused an increased urinary excretion of zinc, calcium and to a lesser extent iron and manganese. The increased urinary excretion of certain endogenous metals following systemic exposure to DTPA is due to its formation of complexes with ‘free’ metals in the blood and lymph. These complexes are then excreted via the urine (Bohne *et al*., 1976; Cantilena and Klassen, 1982; Cohen and Guilmette, 1976; Domingo *et al*., 1988; Havlicek, 1967; Tandon, 1982). Zinc is one of the metals most affected by administration of DTPA with zinc deficiency manifested following prolonged administration of DTPA.

Overall

Toxicokinetic data with category members are available. By the inhalation route, aerosolized DTPA and its salts are absorbed from the respiratory tract into systemic circulation but the degree of absorption is dependent on the site of deposition, i.e. deposition in the upper respiratory tract will finally result in oral ingestion and absorption via the oral route is expected to be low. Dermal application of radiolabeled 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. EDTA and its

salts are eliminated from the body, 95% via the kidneys and 5% by the bile, along with the metals and free ionic calcium which was bound in transit through the circulatory system. In whatever salt EDTA is administered, it is likely to chelate metal ions *in vivo*. This also applies to DTPA.

Acute Toxicity

Studies are available on many empty and metal chelates for acute toxicity via the oral route, and for several substances on the inhalation and dermal routes of exposure.

Oral exposure

Administration of the 'empty' chelate EDTA-Na4 resulted in an LD50 value of 1750 mg/kg bw.

In rats, except for copper EDTA (viz. EDTA-CuNa2: LD_{50} 890 mg/kg bw, and EDTA-CuK2 and EDTA-Cu(NH4)2: LD_{50} between 300 and 2000 mg/kg bw for both substances), oral LD_{50} values of all other metal chelates tested were > 2000 mg/kg bw indicating low acute oral toxicity. At higher doses approaching the LD_{50} values, clinical signs consisting of dyspnea, diarrhea and spastic gait were observed.

Inhalation exposure

With regard to the 'empty' chelates, a single 6-h inhalation exposure of male rats to a respirable dust aerosol of 1 mg/L EDTA-Na2H2 resulted in 30% mortality (BASF, 2010). It is assumed that this effect is due to chelation of calcium in the lungs. Based on these results it was concluded that inhalation exposure to an 'empty' but relatively strong chelating agent like EDTA-Na2H2 (log K value for Ca = 10.7) induced more severe effects when compared to inhalation exposure to a metal-chelate. As such all 'empty' strong chelates like EDTA-H4, EDTA-Na4, and DTPA-H5, DTPA-Na5 and DTPA-K5 (log K value for Ca = 10.8) are also considered to be harmful by inhalation, and need to be classified in GHS Cat. 4 (i.e. 4-h LC50 between 1 and 5 mg/L).

Acute inhalation exposures to dusts of several of the metal chelates (EDTA-FeNa, EDTA-MnNa2, EDTA-CaNa2, EDTA-CuNa2, DTPA-FeHNa and DTPA-CaNa3) were generally without effect in rats (Appendix C), indicating a much lower affinity to bind (additional) Ca than for the empty chelates.

Dermal exposure

A limited number of acute dermal toxicity studies was carried out as dermal absorption of empty and metal chelates is considered to be poor. Dermal administration of the 'empty' chelate DTPA-K5 resulted in an LD50 value > 2000 mg/kg bw.

Studies carried out with EDTA-FeNa and EDTA-Fe(NH4)(NH4)OH also showed LD_{50} values >2000 mg/kg bw (Appendix C).

Skin and eye irritation

With regard to the 'empty' chelates, eye irritation (*in vivo*), but no skin irritation, was seen with EDTA-H4, EDTA-Na4, DTPA-H5, and DTPA-K5. The irritancy potential is related to the pH of the individual acid or salt. Thus, more acidic members of the category such as monosodium EDTA or EDTA acid, and the more basic members such as tetra sodium salt of EDTA, have inherently greater irritancy potential.

Many of the aminocarboxylic acid-based metal chelants are not irritating to skin and eyes, except for copper EDTA. Skin irritation (*in vitro*) was observed with EDTA-Cu(NH4)2 and EDTA-CuK2, borderline eye irritation *(in vitro)* was seen with EDTA-CuK2, and eye irritation (*in vivo*) with EDTA-CuNa2. Thus copper-EDTA (viz. EDTA-CuNa2, EDTA-CuK2 and EDTA-Cu(NH4)2) showed borderline eye and/or skin irritation.

Thus the classification of the metal chelates (except copper) should be 'not irritating to the skin or eyes'.

Skin sensitization

With regard to the empty chelates, skin sensitization studies have been carried out with EDTA-Na2H2, DTPA-H5, DTPA-Na5, and DTPA-K5 (Appendix C).

Skin sensitization studies have also been carried out with the following metal chelates: EDTA-FeNa, EDTA-CuNa2, and DTPA-FeHNa (Appendix C).

It was concluded that both the aminocarboxylic acid-based metal and 'empty' chelates are not skin sensitisers based on these studies in mice and guinea pigs.

Repeated-dose Toxicity

Oral exposure

Reliable data are available for oral repeated-dose studies with the 'empty' chelates EDTA-Na2H2, EDTA-Na3H, DTPA-K5, and DTPA-Na5, and with the metal chelates EDTA-Ca2Na2, EDTA-FeNa, EDTA-MnNa2, EDTA-CuNa2, and DTPA-FeNaH (Appendix C).

Although the target organ was considered to be the 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. Under physiologically relevant conditions, the salts of various category members will ionize based on the dissociation constants of the parent chelate and thus all salts of a particular parent, such as EDTA or DTPA, are assumed to chelate metal ions *in*

vivo based on the inherent chelating strength of the parent chelate molecule. As in the case of zinc, deficiency is presumed to exhibit a threshold effect, and both dose and duration of exposure become important factors in the overall toxicity observed with longer-term administration.

With regard to the 'empty' EDTA chelates, a NOAEL of 1125 mg/kg bw EDTA-Na2H2 was observed in an oral 1-month study (Kawamata, 1980). In a 13-week repeated-dose toxicity study, rats (both sexes) fed EDTA-Na2H2 (0, 1, 5, or 10%) showed mortality at the highest dose. In addition, there was decreased food consumption (emaciation at 10%) and diarrhea at doses of 5% (ca. 2500 mg/kg bw/day) and above. The NOAEL was 1% (ca. 500 mg/kg bw/day; Wynn, 1970). A 7-wk oral study with EDTA-Na3H showed a NOAEL of 453 mg/kg bw (NTIS, 1977). In a 2- year dietary study in rats and mice (both sexes) also with EDTA-Na3H (0, 3750 or 7500 ppm) a NOAEL of 7500 ppm (ca. 500 mg/kg bw/day in rats and ca. 938 mg/kg bw/day in mice; highest dose tested) was determined (NTIS, 1977). Overall, also NOAELs were generally around 500 mg/kg bw.

With regard to the 'empty' DTPA chelates, a 28-day repeated-dose oral gavage study with DTPA-K5 was carried out in rats at 0, 83, 333 or 1330 mg/kg bw/day. Mortality was observed at 1330 mg/kg bw/day. Other effects reported included increased serum potassium levels, decreased body weights, clinical signs and diarrhea. Less severe effects were observed at 333 mg/kg bw/day. The NOAEL was 83 mg/kg bw/day (Elliott, 1987). In a 28-day drinking water study, rats received 0, 600, 3000 or 12,000 ppm DTPA-Na5. Body weight reductions and histopathological changes of the urinary tract were observed at 12,000 ppm and 3000 ppm. The NOAEL was 600 ppm (ca. 75 mg/kg bw/day; BASF, 2002). Overall, the NOAELs for the 'empty' DTPA chelates were close to 100 mg/kg bw.

With regard to EDTA and its metal salts, in a 2-year dietary study, rats fed EDTA-CaNa2 at 0, 50, 125 or 250 mg/kg bw/day showed no effect on behaviour, appearance, growth, longevity or hematology up to one year. After 1 year, there was a downward trend in hematology parameters. There were no gross pathologic findings, changes in organ weights or treatment-related lesions in any organ that was examined. The NOAEL was 250 mg/kg-bw/day (highest dose tested; Oser, 1963). The same authors also reported a one-year dietary study in dogs resulting in a NOAEL of 338 mg/kg bw (highest dose tested; Oser, 1963). In a 31-day dietary study, female rats fed EDTA-CaNa2 (0, 0.3, 1.0, 3.0 or 5.0%) showed only a slightly decreased body weight gain at 5.0% (approximately 3636 mg/kg bw/day) which was therefore considered to be a NOAEL (Dow, 1955). Another 1-month study with this substance showed a LOAEL of 2750 mg/kg bw/day (Kawamata, 1980). In 31- and 61-day studies, male rats fed EDTA-FeNa up to 84 mg/kg bw/day had decreased plasma sodium and calcium concentrations but did not exhibit any organ toxicity. The NOAEL was considered to be 84 mg/kg bw/day (highest dose tested). Iron accumulated in the liver, spleen and kidneys in a dose-related manner but this did not result in excess iron in other tissue or in iron toxicity (Appel, 2001). In a 39-day study, rats received EDTA-FeNa at a level of 1200 mg Fe per kg diet. Taking into account a consumption of ca. 25 g per day, and a mean weight of ca. 250 g during the study, rats received 30 mg Fe per day or 120 mg Fe per kg bw per day. This corresponds to 367/56 x 120 = 800 mg EDTA-FeNa per kg bw/day. At this level no changes in growth rate were seen (Yeung, 2005). In a 3-month oral gavage study in rats with EDTA-MnNa2 at levels of 150, 500 and 1500 mg/kg bw, the NOAEL was 500 mg/kg bw because of decreased BW, increased water consumption, increased kidney weight and renal histopathology at 1500 mg/kg bw (Wolterbeek, 2010). A similar study with EDTA-CuNa2 in rats at the same dose levels showed significant mortality at 1500 mg/kg bw and

although the dose level was reduced to 1050 mg'kg bw after about one week mortality still occurred. At the next lower level of 500 mg/kg bw, kidney, liver, and spleen effects were observed. A NOAEL slightly below 150 mg/kg bw was established due to limited liver and kidney effects at this level (Lina, 2013). Overall, NOAELs were around 500 mg/kg bw or higher except for EDTA-CuNa2 that showed a NOAEL close to 150 mg/kg bw.

With regard to DTPA and its metal salts, in a 3-month oral gavage study in rats with DTPA-FeNaH at levels of 150, 500 and 1500 mg/kg bw, the NOAEL was 500 mg/kg bw because of soft faeces, decreased body weight gain, prolonged prothrombin time, increased haemoglobin concentration, decreased ALAT activity and chloride concentration, decreased ALP activity and increased relative weights of kidneys and liver at 1500 mg/kg bw (Wolterbeek, 2011). Thus the NOAEL for the metal DTPA chelate was 500 mg/kg bw as for most EDTA compounds.

Inhalation exposure

Inhalation of the 'empty' chelate EDTA-Na2H2 in male rats exposed to 30, 300 or 1000 mg/m^3 6 hours/day for up to 5 days produced adverse effects at all concentration levels in lungs and larynx. Mortality was observed at 1000 mg/m^3 following a single 6-h exposure. The histopathological effects observed in animals exposed at 30 and 300 mg/m^3 for 5 days were fully reversed after a 14-day recovery period (BASF, 2010). In a following 90-day inhalation toxicity study according to OECD guideline 413 (BASF, 2014), rats were exposed to 0.5, 3 or 15 mg/m3 during 6 hours/day, 5 days/week. A mild inflammation in the respiratory tract was observed in females exposed to 15 mg/m3, which was considered a LOAEC. No adverse effects were observed at 0.5 and 3 mg/m3. It is assumed that the local effects observed are due to chelating properties of the material impacted at typical critical sites. Calcium and possibly zinc may have been leached from intercellular junctions and other membranes or connective tissue with the sequel of a precipitated cell shedding, subsequent replacing activities, and metaplasia. Based on a comparison of the effects observed in the 5-day and 90-day inhalation studies, the local effect is assumed to be mainly concentration-related, hence the impact of exposure time should be low at subcritical concentrations. Although the number of exposure days was a factor 13 higher than in the 5-day range-finder study, the local effects at 15 mg/m3 in the 90-day inhalation study were mild compared to the 5-day range-finder study in which more severe effects were observed at 30 mg/m3. Based on these results it was concluded that repeated inhalation exposure to an 'empty' chelate like EDTA-Na2H2 resulted in more severe effects when compared to inhalation of a metal-chelate, as was also the case in the acute inhalation toxicity studies.

Repeated inhalation toxicity studies with metal chelates are limited. A 12-day inhalation toxicity study with DTPA-CaNa3 at levels of 0, 420, 880 and 1300 mg/m3 (2 h/day) and 1180 mg/m3 (4 h/day) showed focal and reversible pulmonary histiocytosis in the rat. At the level of 420 mg/m3, the incidence and severity of the histiocytosis was comparable to that in the chamber- and aerosol control group; this level, therefore, was considered a NOAEC (Smith, 1980).

It should also be noted that in the studies indicated above, rats were exposed to aerosols consisting of respirable particles (i.e. MMAD <<10 microns), whereas humans will not be exposed to such small particles in significant amounts in view of the large particle size

of the powders manufactured (only up to 5% < 10 microns diameter). In addition, when in solution, exposure will also be limited because of the low vapour pressure except when nebulized. However, also in the latter case, it is not expected that excessive amounts of small droplets (< 10 microns) will be generated. As such most particles/droplets will end up in the upper respiratory tract and will mainly be cleared to the gut via the mucociliary escalator, and finally poorly absorbed as absorption from the gut is low (up to 5% for EDTA).

Genetic Toxicity

With regard to the 'empty' chelates, available data from *in vitro* genotoxicity studies [EDTA-Na2H2**,** EDTA-Na3H, DTPA-Na5, and DTPA-K5; Appendix C] indicate that these materials generally do not induce gene mutations or chromosomal aberrations. In the available *in vivo* genotoxicity studies with EDTA-Na2H2 there was also no evidence of genotoxicity except for signs of aneuploidy (Zordan, 1990; Russo, 1992).

Available data from *in vitro* genotoxicity studies for metal chelates [EDTA-FeNa, EDTA-MnNa2, EDTA-CaNa2, EDTA-CuNa2, and DTPA-FeHNa; Appendix C] indicate that these materials generally do not induce gene mutations or chromosomal aberrations *in vitro*. Although there have been some positive findings reported *in vitro* for some category members, these positive effects have been generally attributed to the threshold mechanisms of pH changes and the chelation of critical nutrient metals such as zinc rather than direct DNA reactivity. In the available genotoxicity studies with the metal chelates there was no evidence of genotoxicity except for signs of aneuploidy in *in vitro* studies with EDTA-FeNa (de Vogel, 2010), DTPA-FeHNa (Usta, 2013b), and EDTA-CuNa2 (Usta, 2013a). In the *in vitro* micronucleus tests with these substances with a treatment period of 20 h (continuous treatment without S9 -mix), the substances were positive at relatively high levels, inducing aneugenic but no clastogenic effects. This long treatment period together with the high concentrations of chelant may have resulted in exchange and substantial binding of essential elements such as zinc. Heimbach (2000) concluded that the lack of effects by the Zn-EDTA salt in contrast to effects induced by Ca-, Na- and Mn-salts of EDTA, provided evidence that zinc is required for the initiation or continuation of DNA synthesis and maintaining cell function. As such, the significance of mutations produced by EDTA-FeNa, DTPA-FeHNa or EDTA-CuNa2 at non-physiological concentrations in an *in vitro* screening system or following *in vivo* administration using the non-physiological ip injection route (injection close to the gonads) is difficult to extrapolate for relevance to humans. Therefore, the overall 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.

It is understood that EDTA and DTPA are capable of binding zinc, and under some circumstances *in vitro* or *in vivo*, this chelation of zinc can limit its availability to dividing cells where it is used as a co-factor for enzymes involved in DNA synthesis.
Based on the assumption of a threshold mode-of action for aneugens, it was concluded that EDTA and DTPA and their (metal) salts are not mutagenic for humans and do not present a genotoxic hazard.

Carcinogenicity

An oral two-year study with the 'empty' chelate EDTA-Na3H in mice and rats (NTIS, 1977) and an oral 2-year study with the metal chelate EDTA-CaNa2 in rats (Oser, 1963) indicated no evidence of carcinogenicity. The amino carboxylic acid-based (metal) chelants (EDTA, DTPA, and HEDTA) category members are not expected to be carcinogens based on the absence of any pre-neoplastic or neoplastic lesions in the repeated dose toxicity studies carried out with several (metal) chelants of these groups.

Toxicity to Reproduction (Fertility and Developmental Toxicity)

Fertility

A chronic study with the 'empty' chelate EDTA-Na3H that included histological examination of gonadal tissues for evidence of adverse effects showed no adverse effects on reproductive organs (NTIS, 1977).

Reproductive toxicity studies for metal chelates are available in which the potential for reproductive effects from exposure to EDTA-CaNa2, EDTA-MnNa2, EDTA-CuNa2, and DTPA-FeHNa has been examined (Appendix C). In a non-guideline chronic study, no adverse clinical, histological, hematological or reproductive effects were found over 4 generations in rats fed a diet of 0, 50, 125 or 250 mg/kg bw/day of EDTA-CaNa2. The NOAEL for reproductive toxicity was 250 mg/kg bw/day (highest dose tested; Oser, 1963). The three other metal chelating agents, EDTA-MnNa2, EDTA-CuNa2 and DTPA-FeNaH were recently tested using an extended OECD 422 protocol, i.e. the pre-mating period, which should be at least 2 weeks, was extended to 10 weeks and sperm analyses were included. At 1500 mg EDTA-MnNa2/kg bw decreased sperm motility was observed (Wolterbeek, 2010), whereas at 1500 mg DTPA-FeHNa male fertility effects consisted of decreased relative weight of epididymides, a decrease in sperm motility and epididymal sperm reserve (Wolterbeek, 2011). Although these effects on male fertility were seen at the highest dose tested (1500 mg/kg bw), it did not result in effects on reproduction as there were no changes in reproductive performance in animals of these groups. No reproduction effects occurred at the level of 500 mg/kg bw which was considered a NOAEL for fertility for both EDTA-MnNa2 and DTPA-FeHNa. Reproduction effects were also absent at levels of 150 and 500 mg EDTA-CuNa2/kg bw; at the next higher level of 1500/1050 mg/kg bw significant mortality occurred before mating took place (Lina, 2013).

EDTA and DTPA are chelating agents with a high affinity for metals such as zinc, manganese and calcium. Zinc is one of the most abundant metals in the human body (2-4 g) and is present as a cofactor for a large number of enzymes (between 100 and 300), covering almost all classes of enzyme. As such deficiencies in zinc can produce a wide array of symptoms including reproductive toxicity.

As can be seen from the stability constants in Section 2, the chelating agents EDTA and DTPA have the highest preference for Fe3+, followed by Cu2+, Zn2+, Fe2+ and then Mn2+. The gut enterocytes have the ability to convert Fe3+ to Fe2+ prior to absorption, so the Fe3+ is released, then converted to Fe2+ for which DTPA has a lower affinity so it preferentially binds other ions for which it has a higher affinity, such as zinc. Thus, the release of the Fe3+ leaves the DTPA open to bind zinc either in the gut or subsequent to

absorption (the absorption of DTPA is estimated to be ca. 5%, as with EDTA). This zinc chelation at a high level of 1500 mg/kg bw may then cause the effects on male reproductive organs. The lower affinity of EDTA for Mn2+ when compared to Zn2+ may also explain the male reproductive effect observed at 1500 mg EDTA-MnNa2/kg bw. For EDTA-CuNa2 no effects on male fertility were observed at 500 mg/kg bw; however, as indicated, effects on male fertility could not be examined at the higher level tested because of premature mortality. With regard to EDTA-CaNa2, the highest dose tested was only 250 mg/kg bw.

If dietary multi generation reproductive toxicity studies were to be performed with other members of these metal chelating agents group (and especially in case of the 'empty' chelates) in the absence of any supplementation of essential minerals such as zinc, then it is highly likely that the chelating agents, if administered at high levels, would complex with enough of the zinc in the diet leading to an insufficient zinc intake in the animals. This would lead to evidence of male reproductive toxicity (specifically degeneration of the testicular tissue and possible reduced fertility), developmental toxicity such as terata of the skeletal and viscera (see further) and many of the symptoms of zinc deficiency such as alopecia, diarrhea, eye and skin lesions etc.. Such studies would therefore not provide evidence of the reproductive toxicity of the chelating agents but rather the toxicity associated with a deficiency in zinc.

In the two available 28-day studies where two 'empty' DTPA chelates (DTPA-K5 and DTPA-Na5) were dosed via gavage or the drinking water, there were some clinical signs of a perturbation in nutrition at the high doses (diarrhea, decreased food consumption, decrease in bodyweight), and in the gavage fed animals there were deaths in the high dose group males (Elliott, 1987; BASF 2002). It is very likely that the deaths were associated with diarrhea caused by chelation of metals such as zinc and calcium in the intestinal tract leading to decreased absorption of food and water loss. In these studies, although of short duration, there was no evidence of testicular toxicity in any of the treated groups, indicating that the dosing regimen was insufficient to produce sufficient zinc deficiency to lead to testicular toxicity. It is possible that a more prolonged dosing regimen could have produced a more extensive deficiency in zinc.

Overall, it is concluded that at high testing levels, reproductive toxicity is due to an induced deficiency in zinc and any reproductive effect would be observed only in the presence of, and secondary to parental toxicity. Reproductive toxicity effects secondary to a zinc deficiency should not be considered relevant for classification if it can be demonstrated that occupational or consumer exposure to these chelating agents would not result in a deficit in an individual's zinc status. As part of a classification and labeling justification prepared for developmental toxicity it has been demonstrated that exposure to these chelating agents through worker and consumer uses would be insufficient to produce a deficiency in zinc in the workforce or consumer population. Since reproductive toxicity would require zinc deficiency to be induced as a first step in the toxicity it is very unlikely that this would occur.

Developmental Toxicity

With regard to the 'empty' chelates developmental toxicity data are available for EDTA-H4, EDTA-Na2H2, EDTA-Na3H, EDTA-Na4, and DTPA-Na5 (Appendix C). Data from these multigeneration and prenatal developmental toxicity studies also suggest that

developmental effects are observed in the presence of maternal toxicity and are related to reduced plasma zinc concentrations. These effects are independent of whether the acid or sodium salts are applied. Studies on developmental toxicity showed a specific fetotoxic and teratogenic potential of EDTA-Na2H2; a LOAEL of ca. 1000 mg/kg bw/day was determined. When females were fed the 2% EDTA-Na2H2 (ca. 1000 mg/kg bw/day) diet throughout pregnancy, reproduction was impaired only slightly. All rats had living young at term and litter size was normal, although the young were slightly smaller than controls. However, 7% of the fullterm young were malformed, while none of the control fetuses showed gross congenital malformations. When females were fed the 3% EDTA-Na2H2 diet (ca. 1500 mg/kg bw) throughout pregnancy, reproduction was so severely disturbed that none of the mated females had grossly visible implantation sites at term. When the 3% EDTA-Na2H2 diet was fed from days 6 to 14 of gestation or from day 6 of gestation to term, almost alI of the mated females had implantation sites, but nearly half of these sites had dead or resorbed fetuses. Females fed the 3% EDTA-Na2H2 diet from days 6 to 21 had less than half the normal number of young per litter, and full-term young had a mean body weight of only 1.8 g as compared with 5.3 g in controls; furthermore, 100% of the young were grossly malformed. In contrast, females given 1000 ppm of dietary zinc along with the same 3% EDTA-Na2H2 for the same period of time during gestation had essentially normal reproduction and none of the young were malformed (Swenerton, 1971). The pattern of malformations comprised cleft palate, severe brain deformities, eye defects, micro- or agnathia, syndactyly, clubbed legs and tail anomalies. These effects were exhibited in studies using maternally toxic dose levels. The mechanism resulting in developmental effects is found to occur via zinc depletion resulting in zinc deficit (Hurley, 1971).

In a non-guideline prenatal developmental toxicity study, rats were administered EDTA-H4 (967 mg/kg bw), EDTA-Na2H2 (1243 mg/kg bw), EDTA-Na3H (1245 mg/kg bw), or EDTA-Na4 (1374 mg/kg bw) by oral gavage on gestation days 7-14. Clinical effects included diarrhea and a reduction of body weight gain during the treatment period in dams. There was no effect of treatment on litter size, post-implantation loss, sex ratio, fetal body weight, or mortality. There was no effect of treatment on the incidence of fetal abnormalities; the NOAEL for developmental toxicity, therefore, were the doses tested (Schardein, 1981).

In a prenatal developmental toxicity study, rats were administered via oral gavage 0, 100, 400 or 1000 mg/kg bw/day DTPA-Na5. At 400 mg/kg bw/day there was a statistically significant increase in the total number of fetuses with skeletal variations and retardations in fetuses (shortened or absent 13th rib, rudimentary cervical ribs, delays in ossification). At 1000 mg/kg bw/day, in addition to effects observed in the mid dose, there was a reduction in litter size and an increase in the number of skeletal malformations (missing thoracic and lumbar vertebrae and bipartite sternebrae) but no visceral or external malformations were present. This dose also produced a reduction in maternal body weight gain (adjusted). The NOAEL for maternal toxicity was 400 mg/kg bw/day and for developmental toxicity 100 mg/kg bw/day (BASF, 1994).

Developmental toxicity data are available for the metal chelates EDTA-CaNa2, EDTA-MnNa2, EDTA-CuNa2, DTPA-FeHNa, DTPA-CaNa3 and DTPA-ZnNa3 (Appendix C).

In a non-guideline prenatal developmental toxicity study, rats were administered EDTA-CaNa2 (1340 mg/kg bw) by oral gavage on gestation days 7-14. Clinical effects included diarrhea and a reduction of body weight gain during the treatment period in dams. There was no effect of treatment on litter size, post-implantation loss, sex ratio, fetal body

weight, or mortality. There was no effect of treatment on the incidence of fetal abnormalities; the NOAEL for developmental toxicity, therefore, was the dose tested (Schardein, 1981).

Three other (metal) chelating agents, EDTA-MnNa2, EDTA-CuNa2 and DTPA-FeNaH were recently tested using an extended OECD 422 protocol, i.e. the pre-mating period, which should be at least 2 weeks, was extended to 10 weeks, then animals were mated, and pups were necropsied on day 4 after birth. At 1500 mg EDTA-MnNa2/kg bw there was a decreased number of females with live born pups, a decreased number of (live) pups, and increased postimplantation loss (Wolterbeek, 2010), whereas at 1500 mg DTPA-FeHNa/kg bw no developmental toxicity was observed (Wolterbeek, 2011). The NOAEL for developmental toxicity was 500 mg/kg bw for EDTA-MnNa2 and 1500 mg/kg bw for DTPA-FeHNa. Developmental effects were also absent at a level of 500 mg EDTA-CuNa2/kg bw; at the next higher level of 1500/1050 mg/kg bw significant mortality occurred before mating took place (Lina, 2013).

As already discussed, EDTA and DTPA are chelating agents with a high affinity for metals such as zinc. Deficiencies in zinc can produce a wide array of symptoms including both reproductive and developmental toxicity. Like with reproduction toxicity, if developmental toxicity studies were to be performed with chelating agents from this category in the absence of any supplementation of essential minerals such as zinc, then it is highly likely that the chelating agents would complex with enough of the zinc in the diet leading to an insufficient zinc intake in the animals. This would lead to evidence of developmental toxicity such as terata of the skeletal and viscera and many of the symptoms of zinc deficiency such as alopecia, diarrhea, eye and skin lesions etc.. Such studies would therefore not provide evidence of the reproductive or developmental toxicity of the chelating agents but rather the toxicity associated with a deficiency in zinc.

EDTA and DTPA are poorly absorbed both orally and via dermal application and are unlikely to be absorbed significantly via inhalation due to their large particle size (up to 5% < 10 micron diameter) when in powdered form and low volatility when in solution. Such compounds are not metabolised and are excreted with a short half life in humans and rats. With the exception of being able to complex metal ions, chelating agents are of low chemical reactivity as evidenced by their lack of genotoxicity (clastogenicity) and skin sensitising potential. As such, it is unlikely that the chelating agent itself is a proximate toxicant, but rather that its ability to bind metal ions is responsible for the observed toxicity (as indicated above). Therefore if one were to conduct a study where metal ions (for example zinc) were supplemented sufficiently it is unlikely that any systemic toxicity, including developmental or reproductive effects would be observed. This is supported by developmental toxicity studies conducted using the zinc salt of DTPA where no developmental (or systemic) toxicity was observed in rats injected subcutaneously at doses up to 565 mg/kg bw/day (Fukuda, 1983; highest dose tested) or in mice treated in the same way (Brummett, 1977). In the latter study in mice, a level of 3000 mg/kg bw/day showed developmental effects only when injected during days 5 -12 of gestation but not when injected on days 2 -6 or on days 7 -11. This was compared to the calcium salt of DTPA where DTPA-CaNa3 caused significant increases in developmental toxicity at 179 mg/kg bw in rats (but not at 90 mg/kg bw; Fukuda, 1983) and at 700 mg/kg bw in mice (only dose level tested; Brummett, 1977). As such DTPA-CaNa3 showed developmental toxicity at lower levels when compared to DTPA-ZnNa3 which supports the notion that - because the affinity of DTPA is much higher for Fe than

for Zn, and much higher for Zn than for Ca - that DTPA-FeHNa is much less developmentally toxic than DTPA-CaNa3 due to less binding of Zn.

Additional support comes from studies (and treatments) conducted in humans and mice where the zinc salt of DTPA was dosed with no evidence of systemic toxicity at therapeutic doses (Kalkwarf et al 1983; Sato 1997).

Overall, members of the aminocarboxylic acid-based (metal) chelants category would be expected to exhibit developmental effects only in the presence of a zinc deficiency which is, however, not expected under normal nutritional conditions.

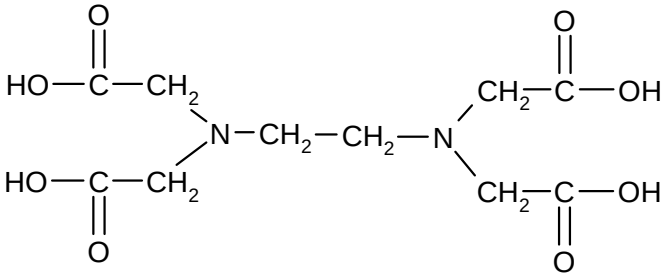
Neurotoxicity

Behavioural and functional testing of neurotoxic effects was carried out in rats in extended oral OECD 422 studies (ca. 3-month duration) with the metal chelates EDTA-MnNa2, EDTA-CuNa2, and DTPA-FeHNa (Appendix C). None of these substances showed neurotoxic effects at levels up to 1500 mg/kg bw/day. Overall, members of the aminocarboxylic acid-based metal chelants category would not be expected to exhibit neurotoxic effects.

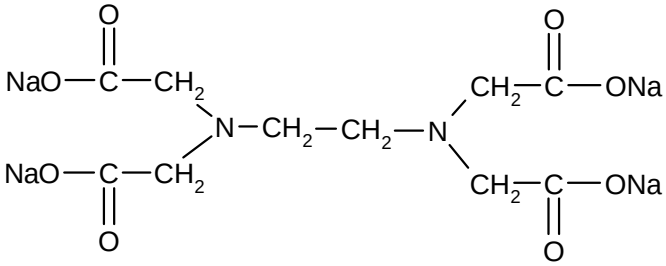
MOLECULAR STRUCTURE (APPENDIX A)

Appendix A1. Details 'empty' chelates (category members)

EDTA-H4

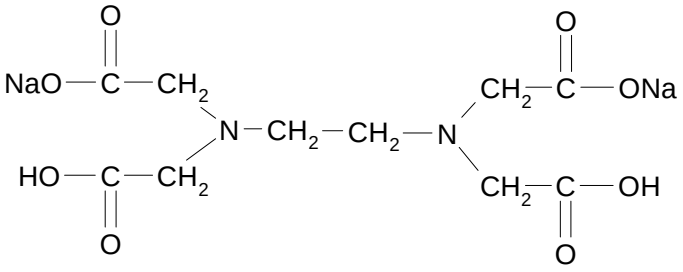
Chemical name	Ethylenediaminetetraacetic acid
IUPAC name	2,2',2",2"'-(ethane-1,2-diyldinitrilo)tetraacetic acid
EC number	200-449-4
CAS Number	60-00-4
Molecular structure	
Molecular formula	C10 H16 N2 O8
Molecular weight	292.2

EDTA-Na4

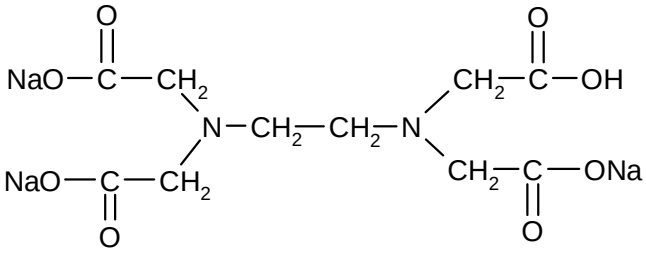
Chemical name	Ethylenediaminetetraacetic acid, tetrasodium salt
IUPAC name	Tetrasodium 2,2',2",2"'-(ethane-1,2-diyldinitrilo)tetraacetate
EC number	200-573-9
CAS number	64-02-8 (anhydrous); 13235-36-4 (tetrahydrate)
Molecular structure	
Molecular formula	C10 H12 N2 O8.Na4
Molecular weight	380.2 (anhydrous) 452.2 (tetra hydrate)

EDTA-Na2H2

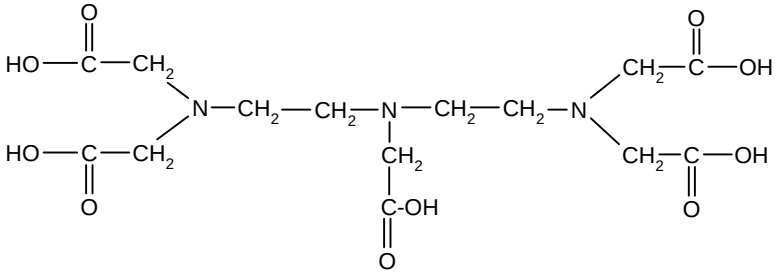
Chemical name	Disodium dihydrogen ethylenediaminetetraacetate

IUPAC name	Disodium dihydrogen 2,2',2",2"'-(ethane-1,2-diyldinitrilo)tetraacetate
EC number	205-358-3
CAS Number	139-33-3 (anhydrous); 6381-92-6 (dihydrate)
Molecular structure	
Molecular formula	C10 H14 N2 O8.Na2
Molecular weight	336.2 (anhydrous); 372.2 (dihydrate)

EDTA-Na3H

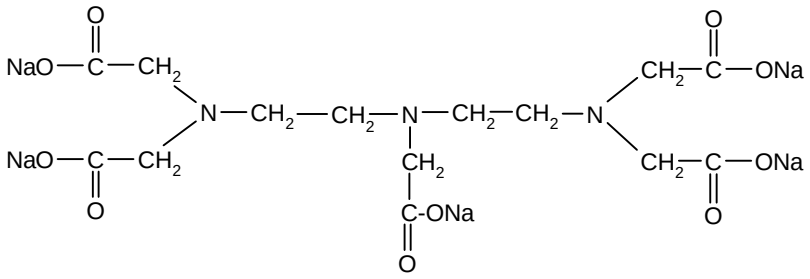
Chemical name	Trisodium hydrogen ethylenediaminetetraacetate
IUPAC name	Trisodium hydrogen 2,2',2",2"'-(ethane-1,2-diyldinitrilo)tetraacetate
EC number	205-758-8
CAS Number	150-38-9
Molecular structure	
Molecular formula	C10 H13 N2 O8.Na3
Molecular weight	358.2

DTPA-H5

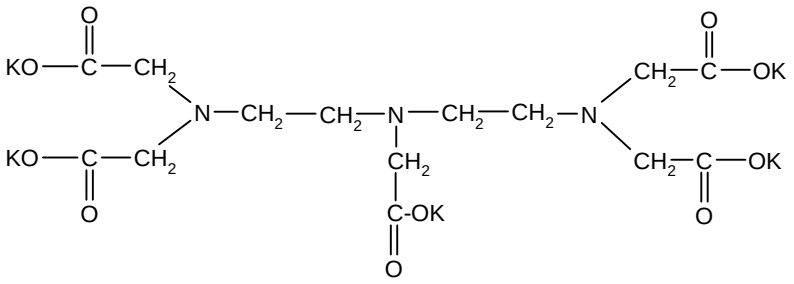
Chemical name	Diethylenetriaminepentaacetic acid
IUPAC name	2-[bis[2-(bis(carboxymethyl)amino)ethyl]amino]acetic acid
EC number	200-652-8
CAS Number	67-43-6
Molecular structure	
Molecular formula	C14 H23 N3 O10
Molecular weight	393.3

DTPA-Na5

Chemical name	Diethylenetriaminepentaacetic acid, pentasodium salt
IUPAC name	Pentasodium 2,2',2",2"',2""-(ethane-1,2-diylnitrilo)pentaacetate
EC number	205-391-3
CAS Number	140-01-2

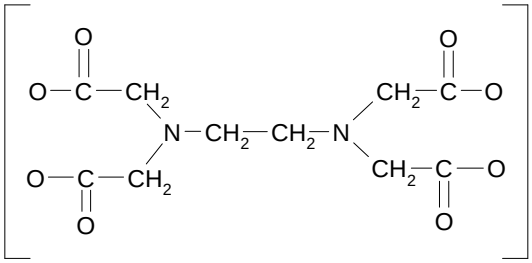
Molecular structure	
Molecular formula	C14 H18 N3 O10 .Na5
Molecular weight	503.3

DTPA-K5

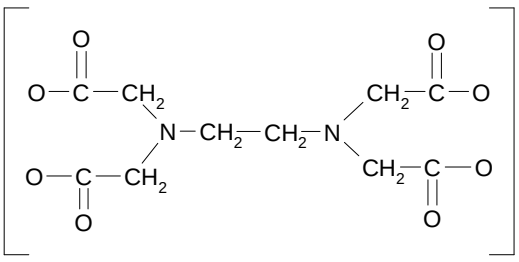
Chemical name	Diethylenetriaminepentaacetic acid, pentapotassium salt
IUPAC name	Pentapotassium 2-[2-[2-(bis(carboxylatomethyl)amino)ethyl-(carboxylatomethyl)amino]ethyl-(carboxylatomethyl)amino]acetate
EC number	404-290-3
CAS Number	7216-95-7
Molecular structure	
Molecular formula	C14 H18 N3 O10 .K5
Molecular weight	583.8

Appendix A2. Details metal chelates (for comparison)

EDTA-CaNa2

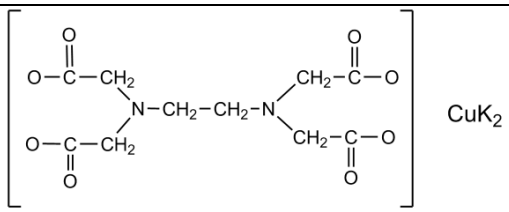
Chemical name	Ethylenediaminetetraacetic acid, calcium disodium complex
IUPAC name	Calcium disodium 2-({2-[bis(carboxylatomethyl)amino]ethyl} (carboxylatomethyl) amino)acetate
EC number	200-529-9
CAS Number	62-33-9 (anhydrous); 23411-34-9 (dihydrate
Molecular structure	 CaNa2
Molecular formula	C10 H12 N2 O8 Ca.Na2
Molecular weight	374.3 (anhydrous); 413.3 (dihydrate)

EDTA-CuNa2

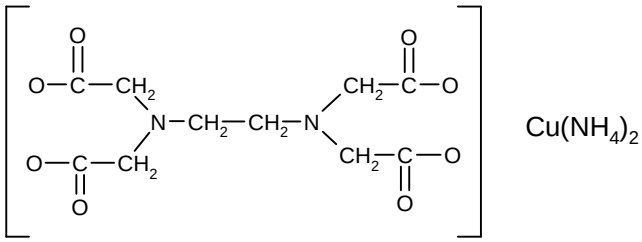
Chemical name	Ethylenediaminetetraacetic acid, copper disodium complex
IUPAC name	Copper(2+) ion disodium 2-({2-[bis(carboxylatomethyl)amino]ethyl} (carboxylatomethyl)amino)acetate
EC number	237-864-5
CAS Number	14025-15-1
Molecular structure	 CuNa2
Molecular formula	C10 H12 N2 O8 Cu.Na2
Molecular weight	397.3

EDTA-CuK2

Chemical name	Ethylenediaminetetraacetic acid, copper dipotassium complex

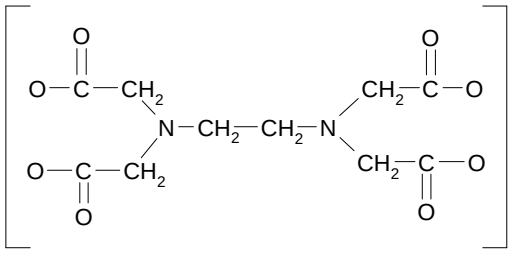
IUPAC name	Copper(2+) ion dipotassium 2-({2-[bis(carboxylatomethyl) amino] ethyl} (carboxylatomethyl)amino)acetate
EC number	277-749-7
CAS Number	74181-84-3
Molecular structure	
Molecular formula	C10 H12 N2 O8 Cu.2K
Molecular weight	430.0

EDTA-Cu(NH4)2

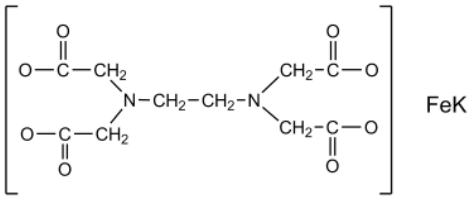
Chemical name	Ethylenediaminetetraacetic acid, copper diammonium complex
IUPAC name	Copper(2+) ion diammonium 2-({2-[bis(carboxyatomethyl)amino] ethyl}(carboxylatomethyl)amino)acetate
EC number	268-018-3
CAS Number	67989-88-2
Molecular structure	
Molecular formula	C10 H12 N2 O8 Cu.(NH4)2
Molecular weight	387.8

EDTA-FeNa

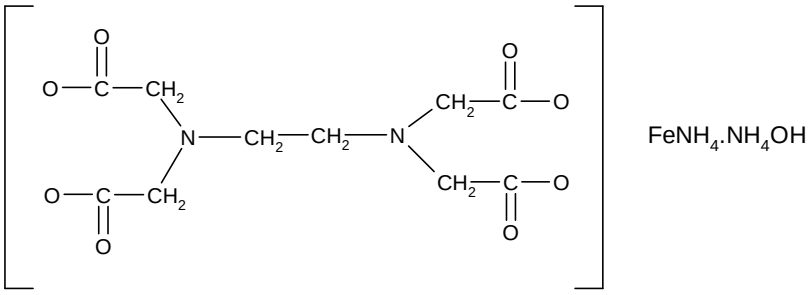
Chemical name	Ethylenediaminetetraacetic acid, ferric-sodium complex
IUPAC name	Sodium; 2-[2-(bis(carboxylatomethyl)amino)ethyl-(carboxylatomethyl)amino]acetate; iron(+3) cation
EC number	239-802-2
CAS Number	15708-41-5 (anhydrous); 18154-32-0 (trihydrate)

Molecular structure	 FeNa
Molecular formula	C10 H12 N2 O8 Fe.Na
Molecular weight	367.1 (anhydrous) 421.1 (trihydrate)

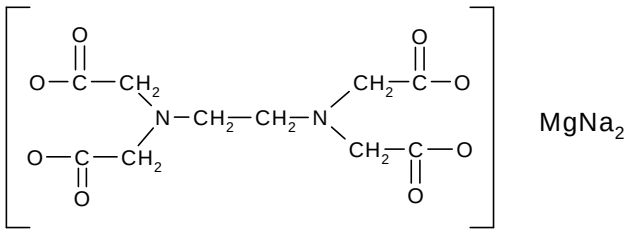
EDTA-FeK

Chemical name	Ethylenediaminetetraacetic acid, ferric-potassium complex
IUPAC name	Potassium; 2-[2-(bis(carboxylatomethyl)amino)ethyl-(carboxylatomethyl)amino]acetate; iron(+3) cation
EC number	259-411-0
CAS Number	277-749-7
Molecular structure	 FeK
Molecular formula	C10 H12 N2 O8 Fe.K
Molecular weight	383.2

EDTA-Fe(NH4)2OH

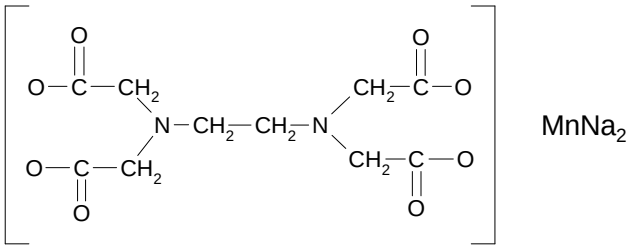
Chemical name	Ethylenediaminetetraacetic acid, ferric-diammonium complex
IUPAC name	Ferrate(2-), [[N,N'-1,2-ethanediylbis[N-[(carboxy-kO)methyl] glycinato-kN,kO]](4-)]hydroxy-, ammonium (1:2), (PB-7-11'-121'3'3)
EC number	270-232-7
CAS Number	68413-60-5
Molecular structure	 $FeNH_4.NH_4OH$
Molecular formula	C10 H21 N4 O9 Fe
Molecular weight	397.2

EDTA-MgNa2

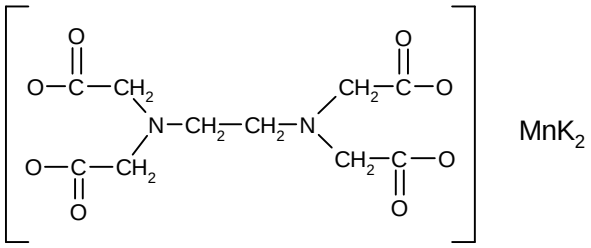
Chemical name	Ethylenediaminetetraacetic acid, magnesium disodium complex
IUPAC name	Magnesium(2+) ion disodium 2-({2-[bis(carboxylatomethyl)amino]ethyl}(carboxylatomethyl)amino)acetate
EC number	238-372-3
CAS Number	14402-88-1
Molecular structure	 $MgNa_2$
Molecular formula	C10 H12 N2 O8 Mg.Na2
Molecular weight	358.5

EDTA-MnNa2

Chemical name	Ethylenediamine-tetraacetic acid, manganese-disodium complex
IUPAC name	Manganese(2+) ion disodium 2-({2-bis(carboxylatomethyl)amino] ethyl} (carboxylatomethyl)amino)acetate

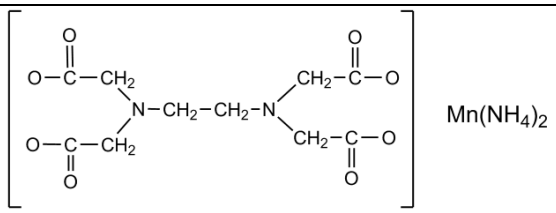
EC number	239-407-5
CAS Number	15375-84-5
Molecular structure	
Molecular formula	C10 H12 N2 O8 Mn.Na2
Molecular weight	389.1

EDTA-MnK2

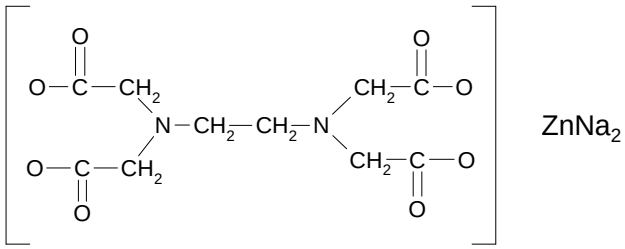
Chemical name	Ethylenediaminetetraacetic acid, manganese dipotassium complex
IUPAC name	Manganese(2+) ion dipotassium 2-({2-[bis(carboxylatomethyl) amino]ethyl}(carboxylatomethyl)amino)acetate
EC number	268-144-9
CAS Number	68015-77-0
Molecular structure	
Molecular formula	C10 H12 N2 O8 Mn.K2
Molecular weight	421.4

EDTA-Mn(NH4)2

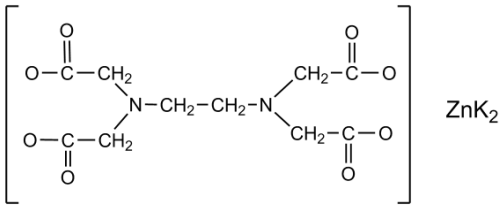
Chemical name	Ethylenediaminetetraacetic acid, manganese diammonium complex
IUPAC name	Manganese(2+) ion diammonium 2-({2-[bis(carboxylatomethyl) amino]ethyl}(carboxylatomethyl)amino)acetate
EC number	304-037-6
CAS Number	94233-07-5

Molecular structure	
Molecular formula	C10 H12 N2 O8 Mn.2H4N
Molecular weight	379.2

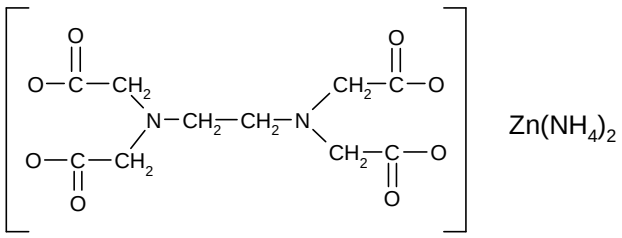
EDTA-ZnNa2

Chemical name	Ethylenediaminetetraacetic acid, zinc-disodium complex
IUPAC name	Zinc (2+) ion disodium 2-({-[bis(carboxylatomethyl)amino]ethyl}carboxylatomethyl)amino)acetate
EC number	237-865-0
CAS Number	14025-21-9
Molecular structure	
Molecular formula	C10H12N2O8Zn.2Na
Molecular weight	399.6

EDTA-ZnK2

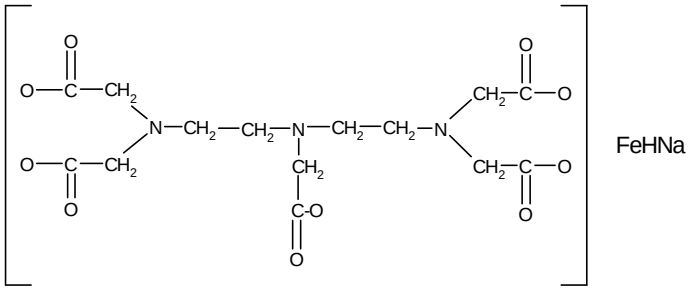
Chemical name	Ethylenediaminetetraacetic acid, zinc-dipotassium complex
IUPAC name	Zinc (2+) ion dipotassium 2-({-[bis(carboxylatomethyl)amino]ethyl} carboxylatomethyl)amino)acetate
EC number	238-729-3
CAS Number	14689-29-3
Molecular structure	
Molecular formula	C10H12N2O8Zn.2K
Molecular weight	431.8

EDTA-Zn(NH4)2

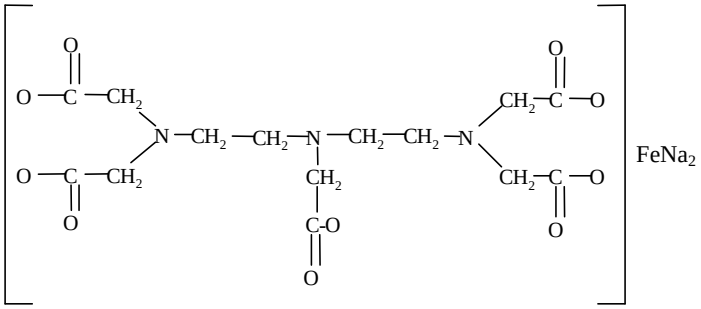
Chemical name	Ethylenediaminetetraacetic acid, zinc-diammonium complex
IUPAC name	Zinc(2+) ion diammonium 2-({2-[bis(carboxylatomethyl)amino]ethyl} (carboxylatomethyl)amino)acetate
EC number	267-400-7
CAS Number	67859-51-2
Molecular structure	
Molecular formula	C10 12 N2 O8 Zn.(NH4)2
Molecular weight	389.7

DTPA-FeHNa

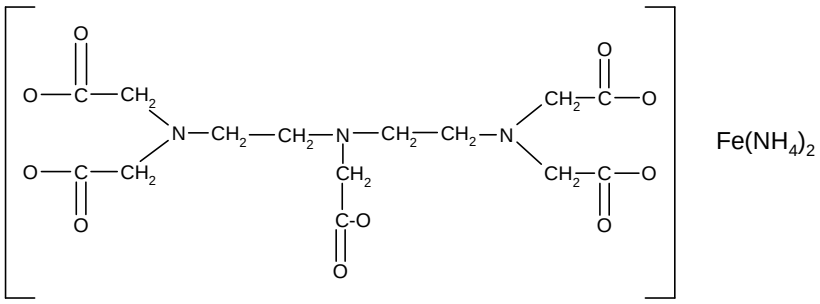
Chemical name	«Chemical_name»
IUPAC name	Iron(3+) ion sodium 5-[bis(carboxylatomethyl)amino]-3-{[bis(carboxylatomethyl)amino]methoxy}pentanoate
EC number	235-627-0
CAS Number	12389-75-2

Molecular structure	 FeHNa
Molecular formula	C14 H18 N3 O10 Fe .H.Na
Molecular weight	468.2

DTPA-FeNa2

Chemical name	«Chemical_name»
IUPAC name	Iron(2+)ion disodium 5-[bis(carboxylatomethyl)amino]-3-{[bis(carboxylato methyl)amino]methoxy}pentanoate
EC number	243-136-8
CAS Number	19529-38-5
Molecular structure	
Molecular formula	C14 H18 N3 O10 Fe.Na2
Molecular weight	490.2

DTPA-Fe(NH4)2

Chemical name	Diethylenetriaminepentaacetic acid, ferric-ammonium complex
IUPAC name	Iron(3+) ion diammonium 5-[bis(carboxylatomethyl)amino]-3-{[bis(carboxylatomethyl)amino]methoxy}pentanoate
EC number	289-064-0
CAS Number	85959-68-8
Molecular structure	
Molecular formula	C14 H18 N3 O10 Fe.(NH4)2
Molecular weight	480.2

Appendix B1. Data matrix for phys-chem endpoints 'empty' chelates (category members)

	Appearance	**Melting point (ºC)**	**Boiling point (ºC)**	**Relative density**	**Particle size <100 micron (%)**	**Particle size <10 micron (%)**	**Vapour pressure (Pa)**
EDTA-H4 CAS: 60-00-4	Solid	Decomposes at 220	Decomposes before boiling	1.46 (g/cm3)	40	2.5	Low
EDTA-Na4 CAS: 64-02-8	Powder	Decomposes at 150	Decomposes before boiling	1.67 (g/cm3)	51	1.7	Low
EDTA-Na2H2 CAS: 139-33-3	Crystalline solid	Decomposes at 252	Decomposes before boiling	1.767 (g/cm3)	29	1.2	Low
DTPA-H5 CAS: 67-43-6	Powder	Decomposes at 206	Decomposes before boiling	1.5	44/50 (v/v)	4/5 (v/v)	Low
DTPA-Na5 CAS: 140-01-2	Powder	Decomposes at 150	Decomposes before boiling	1.67 (g/cm3)	40 (v/v)	4 (v/v)	Low
DTPA-K5 CAS: 7216-95-7	Powder	Decomposes at 158	Decomposes before boiling	1.6422	Only sold as liquid	Only sold as liquid	Low
HEDTA-Na3 CAS: 139-89-9	Powder	288	Decomposes before boiling	1.285 g/cm3	10% < 105 um	-	Low
DTPA-Na5 CAS: 140-01-2	Aqueous solution	-22 (freezing)	105.6	1.31	Not applicable	Not applicable	1600
DTPA-K5 CAS: 7216-95-7	Aqueous solution	-22 (freezing)	100-110	-	Not applicable	Not applicable	1600 (read across with DTPA-Na5)

Appendix B2. Data matrix for phys-chem endpoints metal chelates (for comparison)

	Appearance	**Melting point (ºC)**	**Boiling point (ºC)**	**Relative density**	**Particle size <100 micron (%)**	**Particle size <10 micron (%)**	**Vapour pressure (Pa)**
EDTA-FeNa CAS: 15708-41-5	Crystalline solid	Decomposes at 211	Decomposes before boiling	1.781	38/87 (v/v)	10% < 31 um 10% < 3.2 um	Low
EDTA-FeK CAS: 54959-35-2	Powder	Decomposes at 192	Decomposes before boiling	1.621	39.4 (v/v)	2.0	Low
EDTA-MnNa2 CAS: 15375-84-5	Powder	Decomposes at 252	Decomposes before boiling	1.403	52/56 (v/v)	10% < 23/24 um	Low
EDTA-CaNa2 CAS: 62-33-9	Microgranules	Decomposes at 295	Decomposes before boiling	1.647	57.8 / 62.6 (v/v)	3.0 / 3.4 (v/v)	Low
EDTA-CuNa2 CAS: 14025-15-1	Solid	Decomposes at 219	Decomposes before boiling	1.727	49.2 (v/v)	2.9 (v/v)	Low
EDTA-CuK2 CAS: 74181-84-3	Microgranules	Decomposes at 200	Decomposes before boiling	1.791	36.7 (v/v)	1.9 (v/v)	Low
EDTA-MgNa2 CAS: 14402-88-1	Solid	Decomposes at 240	Decomposes before boiling	1.587	46.5 (v/v)	2.5 (v/v)	Low
EDTA-ZnNa2 CAS: 14025-21-9	Microgranules	Decomposes at 263	Decomposes before boiling	1.720	32.7 (v/v)	1.5 (v/v)	Low
EDTA-ZnK2 CAS: 14689-29-3	Microgranules	Decomposes at 245	Decomposes before boiling	1.786	36.7 (v/v)	1.9 (v/v)	Low

EDTA-MnK2 CAS: 68015-77-0	Solid	Decomposes at 252	Decomposes before boiling	1.34 (g/cm3) (liquid)	Only sold as liquid	Only sold as liquid	Low
DTPA-FeHNa CAS: 12389-75-2	Crystals	Decomposes at 180	Decomposes before boiling	1.697	35.2 (v/v)	3.4 (v/v)	Low
DTPA-FeNa2 CAS: 19529-38-5	Microgranules	Decomposes at 159	Decomposes before boiling	1.599	34.7 (v/v)	1.1 (v/v)	Low
HEDTA-Fe(III)Na CAS: 51181-50-1	Microgranules	Decomposes at 203	Decomposes before boiling	1.362	37.8 (v/v)	1.6 (v/v)	Low
EDTA-Fe(NH4)2OH CAS: 68413-60-5	Aqueous solution	-20 – 0 (freezing)	100-110	1.326 (g/cm3)	Not applicable	Not applicable	6400 (read across with ferric ammonium; 25ºC)
EDTA-Cu(NH4)2 CAS: 67989-88-2	Aqueous solution	-20 – 0 (freezing)	100-110	1.330 (g/cm3)	Not applicable	Not applicable	2030
EDTA-Zn(NH4)2 CAS: 67859-51-2	Aqueous solution	-20 – 0 (freezing)	100-110	1.320 (g/cm3)	Not applicable	Not applicable	5100 (read across with DTPA-Fe(NH4)2
EDTA-Mn(NH4)2 CAS: 94233-07-5	Aqueous solution	-20 – 0 (freezing)	100-110	1.206 (g/cm3)	Not applicable	Not applicable	2030 (read across with EDTA-Cu(NH4)2
DTPA-Fe(NH4)2 CAS: 85959-68-8	Aqueous solution	-20 – 0 (freezing)	100-110	1.280 (g/cm3)	Not applicable	Not applicable	5100

Appendix B1 (cont.). Data matrix for phys-chem endpoints 'empty' chelates (category members)

	Log Kow	**Water solubility**	**Surface tension**	**Flash-point**	**Self-ignition temperature (°C)**	**Flammability**	**Explosive properties**
EDTA-H4 CAS: 60-00-4	-3.86	400 mg/L	Not applicable	Not applicable	>400	No	No
EDTA-Na4 CAS: 64-02-8	-	500 g/L	Not applicable	Not applicable	>200	No	No
EDTA-Na2H2 CAS: 139-33-3	-4.3	108 g/L	Not applicable	Not applicable	>400	No	No
DTPA-H5 CAS: 67-43-6	-4.91	3.5 g/L	Not applicable	Not applicable	392	No	No
DTPA-Na5 CAS: 140-01-2	<-2	At least 65% w/w	Not applicable	Not applicable	386	No	No
DTPA-K5 CAS: 7216-95-7	-2.46	1140 g/L	Not applicable	Not applicable	306	No	No
DTPA-Na5 CAS: 140-01-2	<-2	At least 65% w/w	76.7	No flash detected	See at solid	No	No
DTPA-K5 CAS: 7216-95-7	-2.46	1140 g/L	76.6	Not measured, aqueous solution	See at solid	No	No

Appendix B2 (cont.). Data matrix for phys-chem endpoints metal chelates (for comparison)

	Log Kow	Water solubility	Surface tension	Flash-point	Self-ignition temperature (°C)	Flammability	Explosive properties
EDTA-FeNa CAS: 15708-41-5	-8.841	72 g/L	Not applicable	Not applicable	207	No	No
EDTA-FeK CAS: 54959-35-2	-8.91	310 g/L	Not applicable	Not applicable	200	No	No
EDTA-MnNa2 CAS: 15375-84-5	-8.12	412 g/L	Not applicable	Not applicable	264	No	No
EDTA-CaNa2 CAS: 62-33-9	-10.42	At least 28% w/w	Not applicable	Not applicable	-	No	No
EDTA-CuNa2 CAS: 14025-15-1	-10.4	680 g/L	Not applicable	Not applicable	280	No	No
EDTA-CuK2 CAS: 74181-84-3	-8.91	736 g/L	Not applicable	Not applicable	>400	No	No
EDTA-MgNa2 CAS: 14402-88-1	-10.42	At least 370 g/L	Not applicable	Not applicable	311	No	No
EDTA-ZnNa2 CAS: 14025-21-9	-10.32	At least 543 g/L	Not applicable	Not applicable	315	No	No
EDTA-ZnK2 CAS: 14689-29-3	-8.82	746 g/L	Not applicable	Not applicable	277	No	No
EDTA-MnK2 CAS: 68015-77-0	-8.12	At least 48% w/w	Not applicable	Not applicable	Not applicable (only sold as liquid)	No	No

DTPA-FeHNa CAS: 12389-75-2	-11.9	At least 11.3% w/w	Not applicable	Not applicable	332	No	No
DTPA-FeNa2 CAS: 19529-38-5	-11.9	At least 27% w/w	Not applicable	Not applicable	327	No	No
EDTA-Fe(NH4)2OH CAS: 68413-60-5	-4.57	540 g/L	Not measured (no hydrophobic tail)	Not measured, aqueous solution	Not applicable	No	No
EDTA-Cu(NH4)2 CAS: 67989-88-2	-11.91	At least 55% w/w	Not measured (no hydrophobic tail)	Not measured, aqueous solution	Not applicable	No	No
EDTA-Mn(NH4)2 CAS: 94233-07-5	-5.55	713 g/L	Not measured (no hydrophobic tail)	Not measured, aqueous solution	Not applicable	No	No
EDTA-Zn(NH4)2 CAS: 67859-51-2	-7.5963	At least 54% w/w	Not measured (no hydrophobic tail)	Not measured, aqueous solution	Not applicable	No	No
DTPA-Fe(NH4)2 CAS: 85959-68-8	-13.88	At least 53% w/w	Not measured (no hydrophobic tail)	Not measured, aqueous solution	Not applicable	No	No

Appendix B1 (cont.). Data matrix for phys-chem endpoints 'empty' chelates (category members)

	Oxidising properties	**Self-reactivity**	**Self- heating**	**Viscosity (mPa.s)**
EDTA-H4 CAS: 60-00-4	No	No	No	Not applicable
EDTA-Na4 CAS: 64-02-8	No	No	No	Not applicable
EDTA-Na2H2 CAS: 139-33-3	No	No	No	Not applicable
DTPA-H5 CAS: 67-43-6	No	No	No	Not applicable
DTPA-Na5 CAS: 140-01-2	No	No	No	Not applicable
DTPA-K5 CAS: 7216-95-7	No	No	No	Not applicable
DTPA-Na5 CAS: 140-01-2	No	No	No	32.4
DTPA-K5 CAS: 7216-95-7	No	No	No	Ca. 32.4

Appendix B2 (cont.). Data matrix for phys-chem endpoints metal chelates (for comparison)

	Oxidising properties	**Self-reactivity**	**Self- heating**	**Viscosity (mPa.s)**
EDTA-FeNa CAS: 15708-41-5	No	No	No	Not applicable
EDTA-FeK CAS: 54959-35-2	No	No	No	Not applicable
EDTA-MnNa2 CAS: 15375-84-5	No	No	No	Not applicable
EDTA-CaNa2 CAS: 62-33-9	No	No	No	Not applicable
EDTA-CuNa2 CAS: 14025-15-1	No	No	No	Not applicable
EDTA-CuK2 CAS: 74181-84-3	No	No	No	Not applicable
EDTA-MgNa2 CAS: 14402-88-1	No	No	No	Not applicable
EDTA-ZnNa2 CAS: 14025-21-9	No	No	No	Not applicable
EDTA-ZnK2 CAS: 14689-29-3	No	No	No	Not applicable
EDTA-MnK2	No	No	No	Not applicable

CAS: 68015-77-0				
DTPA-FeHNa CAS: 12389-75-2	No	No	No	Not applicable
DTPA-FeNa2 CAS: 19529-38-5	No	No	No	Not applicable
EDTA-Fe(NH4)2OH CAS: 68413-60-5	No	No	No	12.2
EDTA-Cu(NH4)2 CAS: 67989-88-2	No	No	No	11.8
EDTA-Mn(NH4)2 CAS: 94233-07-5	No	No	No	4.6
EDTA-Zn(NH4)2 CAS: 67859-51-2	No	No	No	14.2
DTPA-Fe(NH4)2 CAS: 85959-68-8	No	No	No	11.8

Appendix C1. Data matrix for toxicity endpoints for 'empty' chelates (category members) - Acute toxicity data, eye irritation, and skin irritation and sensitization

	Acute oral **LD50 (mg/kg bw)**	**Acute dermal** **LD50 (mg/kg bw)**	**Acute inhalation** **4-h LC50 (mg/m3)**	**Skin irritation**	**Eye irritation**	**Skin sensitisation**
EDTA-H4 CAS: 60-00-4	4500 (BASF, 1973)			No (BASF, 1973)	Slight (BASF, 1973)	
EDTA-Na4 CAS: 64-02-8	1780 (BASF, 1983)			No (BASF, 1982)	Yes (BASF, 1978)	
EDTA-Na2H2 CAS: 139-33-3	2800 (BASF, 1973)		30% mortality at 1000 mg/m3 (6-h) (BASF, 2010)	No (BASF, 1973)	No (BASF, 1973)	No (CIT, 2000)
DTPA-H5 CAS: 67-43-6	>2000 (Dow, 1958)			No (Dow, 1958)	Irritating (Dow, 1958)	No (Dow, 2002)
DTPA-Na5 CAS: 140-01-2	Ca. 4550 (BASF, 1968)			No (Sterner, 1983a)	No (Sterner, 1983b)	No (BASF, 1993)
DTPA-K5 CAS: 7216-95-7	>5000 (Kynoch, 1984)	>2000 (Gardner, 1987)		No (Liggett, 1987a)	Irritating (Liggett, 1987b)	No (Kynoch, 1987)

Appendix C2. Data matrix for toxicity endpoints for metal chelates (for comparison) - Acute toxicity data, eye irritation, and skin irritation and sensitization

	Acute oral **LD50 (mg/kg bw)**	**Acute dermal** **LD50 (mg/kg bw)**	**Acute inhalation** **4-h LC50 (mg/m3)**	**Skin irritation**	**Eye irritation**	**Skin sensitisation**
EDTA-FeNa CAS: 15708-41-5	>2000 (Haferkorn, 2007)	>2000 (Haferkorn, 2007b)	>2750 (Haferkorn, 2008)	No (Leuschner, 2007a)	No (Leuschner, 2007b)	No (Haferkorn, 2007c)
EDTA-FeK CAS: 54959-35-2	>2000 (Latour, 2014a)			No (Verbaan, 2014a)	No (Verspeek, 2014a)	
EDTA-MnNa2 CAS: 15375-84-5	>2000 (Beerens, 2010a)		>5160 (Muijser, 2010)	No (Verbaan, 2010a)	No (Verspeek, 2010a)	
EDTA-MnK2 CAS: 68015-77-0	>2000 (Otterdijk, 2013d)			No (Verbaan, 2013f)	No (Verspeek, 2012e)	
EDTA-Mn(NH4)2 CAS: 94233-07-5	>2000 (Latour, 2014b)			No (Verbaan, 2014b)	No (Verspeek, 2014b)	
EDTA-Fe(NH4)2OH CAS: 68413-60-5	>2000 (Beerens, 2010b)	>2000 (Beerens, 2010c)		No (Verbaan, 2010b)	No (Verspeek, 2010b)	
EDTA-CaNa2 CAS: 62-33-9	Ca. 10000 (Dow, 1957)		>1130 (7-h, nominal) (Dow, 1976)	No (Dow, 1978)	No (Dow, 1956)	
EDTA-CuNa2 CAS: 14025-15-1	890 (BASF, 1985)		>5320 (Jonker, 2012a)	No (BASF, 1975)	Yes (borderline) (BASF, 1985)	No (Otterdijk, 2013i)
EDTA-CuK2 CAS: 74181-84-3	>300 and < 2000 (Latour, 2014c)			Yes (Verbaan, 2014c)	Yes (borderline) (Verspeek, 2014c)	

EDTA-Cu(NH4)2 CAS: 67989-88-2	>300 and < 2000 (Otterdijk, 2013a)			Yes (Verbaan, 2013ab)	No (Verspeek, 2012a)	
EDTA-MgNa2 CAS: 14402-88-1	>2000 (Otterdijk, 2013b)			No (Verbaan, 2013c)	No (Verspeek, 2012b)	
EDTA-ZnNa2 CAS: 14025-21-9	>2000 (Stitzinger, 2010)			No (Verbaan, 2013d)	No (Verspeek, 2012c)	
EDTA-ZnK2 CAS: 14689-29-3	>2000 (Latour, 2014d)			No (Verbaan, 2014d)	No (Verspeek, 2014d)	
EDTA-Zn(NH4)2 CAS: 67859-51-2	>2000 (Otterdijk, 2013c)			No (Verbaan, 2013e)	No (Verspeek, 2012d)	
EDTA-MnK2 CAS: 68015-77-0	>2000 (Otterdijk, 2013d)			No (Verbaan, 2013f)	No (Verspeek, 2012e)	
DTPA-CaNa3 CAS: 12111-24-9			>1150 (2-h) (Smith, 1980)			
DTPA-FeHNa CAS: 12389-75-2	>2000 (Otterdijk, 2013e)		>5080 (Jonker, 2012b)	No (Verbaan, 2013g)	No (Verspeek, 2012f)	No (Otterdijk, 2013j)
DTPA-FeNa2 CAS: 19529-38-5	>2000 (Otterdijk, 2013f)			No (Verbaan, 2013h)	No (Verspeek, 2012g)	
DTPA-Fe(NH4)2 CAS: 85959-68-8	>2000 (Otterdijk, 2013g)			No (Verbaan, 2013i)	No (Verspeek, 2012h)	

Appendix C1 (cont.). Data matrix for toxicity endpoints 'empty' chelates (category members) - In vitro and in vivo genotoxicity data

	Ames test	MN / CAT in vitro	MLA in vitro	MN/CAT in vivo	Genotoxicity in germ cells in vivo
EDTA-H4 CAS: 60-00-4					
EDTA-Na4 CAS: 64-02-8					
EDTA-Na2H2 CAS: 139-33-3			Negative (Whittaker, 2001)	Negative (MN; mouse) (BASF, 2000) Negative (MN; mouse) (Russo, 1992) Negative (SCE; mouse) (Zordan, 1990) Negative (Drosophila larvae) (Zordan, 1990)	Negative (hyperhaploidy; mouse germ cells) (Zordan, 1990) Negative (CAT, mouse germ cells) (Russo, 1992) Positive (MN; mouse germ cells) (Russo, 1992) Positive (aneuploidy; Drosophila) (Zordan, 1990)
EDTA-Na3H CAS: 150-38-9	Negative (Dunkel, 1985)	Negative (NTP, 1984)	Negative (NTP, 1984)		
DTPA-H5 CAS: 67-43-6					
DTPA-Na5 CAS: 140-01-2	Negative (BASF, 1989)				
DTPA-K5 CAS: 7216-95-7	Negative (May, 1990)	Negative (Allen, 1987)			

* The overall findings indicate that EDTA-metal complexes lack significant genotoxic potential under conditions that do not deplete essential trace elements required for normal cell function."

Appendix C2 (cont.). Data matrix for toxicity endpoints metal chelates (for comparison) - In vitro and in vivo genotoxicity data

	Ames test	**MN / CAT in vitro**	**MLA in vitro**	**MN/CAT in vivo**	**Genotoxicity in germ cells in vivo**
EDTA-FeNa CAS: 15708-41-5	Negative (Dunkel, 1999)	Aneugenic but no clastogenic effects (de Vogel, 2010a)	Negative (Dunkel, 1999)		
EDTA-FeK CAS: 54959-35-2					
EDTA-Fe(NH4)2OH CAS: 68413-60-5					
EDTA-MnNa2	Negative (vd Wijngaard, 2009)	Negative (de Vogel, 2010b)			
EDTA-MnK2 CAS: 68015-77-0					
EDTA-Mn(NH4)2 CAS: 94233-07-5					
EDTA-CaNa2 CAS: 62-33-9	Negative (Fujita, 1988)				
EDTA-CuNa2 CAS: 14025-15-1	Negative (BASF, 1992)	Aneugenic but no clastogenic effects (Usta, 2013a)			
EDTA-CuK2 CAS: 74181-84-3					
EDTA-Cu(NH4)2					

CAS: 67989-88-2					
EDTA-MgNa2 CAS: 14402-88-1					
EDTA-ZnNa2 CAS: 14025-21-9					
EDTA-ZnK2 CAS: 14689-29-3					
EDTA-Zn(NH4)2 CAS: 67859-51-2					
DTPA-CaN3 CAS: 12111-24-9					
DTPA-FeHNa CAS: 12389-75-2	Negative (Verspeek, 2013c)	Aneugenic but no clastogenic effects (Usta, 2013b)			
DTPA-FeNa2 CAS: 19529-38-5					
DTPA-Fe(NH4)2 CAS: 85959-68-8					

* The overall findings indicate that EDTA-metal complexes lack significant genotoxic potential under conditions that do not deplete essential trace elements required for normal cell function."

Appendix C1 (cont.). Data matrix for toxicity endpoints 'empty' chelates (category members) - Repeated dose toxicity, reproduction and developmental toxicity, and neurotoxicity

	Repeated study (subacute) (mg/kg bw/day or mg/m3)	**Repeated study (subchronic) (mg/kg bw/day or mg/m3)**	**Repeated study (chronic) (mg/kg bw/day)**	**Repro toxicity (mg/kg bw/day)**	**Developmental toxicity (mg/kg bw/day)**	**Neurotoxicity (mg/kg bw/day)**
EDTA-H4 CAS: 60-00-4					NOAEL ≥ 967 No developmental tox (Schardein, 1981)	
EDTA-Na4 CAS: 64-02-8					NOAEL ≥ 1374 No developmental tox (Schardein, 1981)	
EDTA-Na2H2 CAS: 139-33-3	NOAEL: 1125 mg/kg (1-month) (Kawamata, 1980) LOAEC: 30 mg/m3 (6 h/day, 5 days) (BASF, 2010)	NOAEL ≥500 mg/kg (Wynn, 1970) LOAEC: 15 mg/m3, NOAEC: 3 mg/m3 (6 h/day, 5 d/wk) (BASF, 2014)		LOAEL ca. 1000 (Swenerton, 1971)	NOAEL ≥ 1243 No developmental tox (Schardein, 1981) LOAEL ca. 1000 (Swenerton, 1971)	
EDTA-Na3H CAS: 150-38-9	NOAEL: 453 mg/kg (7-wk) (NTIS, 1977)		NOAEL ≥ 500 Not carcinogenic (NTIS, 1977)		NOAEL ≥ 1245 No developmental tox (Schardein, 1981)	
DTPA-H5 CAS: 67-43-6						

DTPA-Na5 CAS: 140-01-2	NOAEL: 75 mg/kg (4-week) (BASF, 2002)				NOAEL: 100 Developmental tox at 400 mg/kg bw (BASF, 1994)	
DTPA-K5 CAS: 7216-95-7	NOAEL: 83 mg/kg (28-day) (Elliott, 1987)					

Appendix C2 (cont.). Data matrix for toxicity endpoints metal chelates (for comparison) - Repeated dose toxicity, reproduction and developmental toxicity, and neurotoxicity

	Repeated study (subacute) (mg/kg bw/day or mg/m3)	**Repeated study (subchronic) (mg/kg bw/day or mg/m3)**	**Repeated study (chronic) (mg/kg bw/day)**	**Repro toxicity (mg/kg bw/day)**	**Developmental toxicity (mg/kg bw/day)**	**Neurotoxicity (mg/kg bw/day)**
EDTA-FeNa* CAS: 15708-41-5		≥ 84 (61-day) (Appel, 2001)				
EDTA-FeK CAS: 54959-35-2						
EDTA-Fe(NH4)2OH CAS: 68413-60-5						
EDTA-MnNa2		NOAEL: 500		NOAEL: 500	NOAEL: 500 Developmental tox at	NOAEL ≥1500

		(Wolterbeek, 2010)		Sperm effects at 1500 (Wolterbeek, 2010)	1500 (Wolterbeek, 2010)	(Wolterbeek, 2010)
EDTA-MnK2 CAS: 68015-77-0						
EDTA-Mn(NH4)2 CAS: 94233-07-5						
EDTA-CaNa2 CAS: 62-33-9	NOAEL ≥ 3636 mg/kg (31-day) (Dow, 1955) LOAEL: 2750 mg/kg (1-month) (Kawamata, 1980)		NOAEL rat ≥ 250 Not carcinogenic (Oser, 1963) NOAEL dog ≥ 338 12-month (Oser, 1963)	NOAEL ≥ 250 (multigeneration) Not reprotoxic (Oser, 1963)	NOAEL ≥ 1340 No developmental tox (Schardein, 1981)	
EDTA-CuNa2 CAS: 14025-15-1		NOAEL: ca. 150 (Lina, 2013)		NOAEL: 500 Mortality at 1500/1050 (Lina, 2013)	NOAEL: 500 Mortality at 1500/1050 (Lina, 2013)	NOAEL: 500 Mortality at 1500/1050 (Lina, 2013)
EDTA-CuK2 CAS: 74181-84-3						
EDTA-Cu(NH4)2 CAS: 67989-88-2						
EDTA-MgNa2 CAS: 14402-88-1						
EDTA-ZnNa2 CAS: 14025-21-9						

EDTA-ZnK2 CAS: 14689-29-3						
EDTA-Zn(NH4)2 CAS: 67859-51-2						
DTPA-CaNa3 CAS: 12111-24-9	NOAEC: 420 mg/m3 (2 h/day, 12 days) (Smith, 1980)				NOAEL: 90 (sc) Developmental tox at 179 mg/kg bw (Fukuda, 1983) LOAEL: 700 (sc) (Brummet, 1977)	
DTPA-ZnNa3 CAS: 11082-38-5					NOAEL ≥ 565 (sc) No developmental tox (Fukuda, 1983) LOAEL: 3000 Brummet, 1977)	
DTPA-FeHNa CAS: 12389-75-2		NOAEL: 500 (Wolterbeek, 2011)		NOAEL: 500 Sperm effects at 1500 (Wolterbeek, 2011)	NOAEL ≥ 1500 No developmental tox (Wolterbeek, 2011)	NOAEL ≥1500 (Wolterbeek, 2011)
DTPA-FeNa2 CAS: 19529-38-5						
DTPA-Fe(NH4)2 CAS: 85959-68-8						

REFERENCES

Allen JA, Brooker PC, Birt DM (1987) DTPA-K5 - Analysis of metaphase chromosomes obtained from CHO cells cultured in vitro and treated with glycine N-N bis [2-[bis(carboxymethyl)amino]ethyl] – pentapotassium salt. Huntingdon Research Centre Ltd., UK, Report no. AKZ 232/87980

Appel MJ, Kuper CF and Woutersen RA. (2001) Disposition, accumulation and toxicity of iron fed as iron (II) sulfate or as sodium iron EDTA in rats. Food Chem Toxicol 39:261-269

Ballou JE (1978) The influence of DTPA on the biological availability of transuranics. Health Phys 34 (5), 445-450

BASF (1968) DTPA-Na5 - Ergebnis der gewerbetoxikologischen Vorprüfung. BASF AG Report no. XVIII/26

BASF (1973) EDTA-H4 Results of the Industrial Hygiene Orientating Investigation, BASF AG Report no. XXIII/2

BASF (1973) EDTA-Na2H2 - Industrial Hygiene Orientating Investigation, BASF AG Report no. XXIII/1

BASF (1975) EDTA-CuNa2 - Results of toxicity tests with Cutrilon. BASF AG Report no. XXIV 36

BASF (1978) EDTA-Na4 - Industrial Hygiene Orientating Investigation, BASF AG Report no. XXVI/290

BASF (1983) EDTA-Na4 - Testing of the Acute Oral Toxicity of "Trilon B Powder" in the Rat, BASF AG Report no. 82/108

BASF (1982) EDTA-Na4 - Acute Skin Irritation According to OECD 404, BASF AG Report no. 82/108

BASF (1985) EDTA-CuNa2 - Report on the study of acute oral toxicity. BASF AG Report no. 84 / 263

BASF (1985) EDTA-CuNa2 - Report on the acute irritation to the eye of the white rabbit based on OECD 405. BASF AG Report no. 84 / 263

BASF (1989) DTPA-Na5 - Report on the Study of Trilon C flüssig in the Ames Test (Standard Plate Test and Preincubation Test with Salmonella typhimurium). BASF AG Report no. 40M1021/884392

BASF (1992) Report on the study of Trilon B-CU 1550 in the Ames test (Salmonella/Mammalian-Microsome Mutagenicity Test - Standard Plate Test and Preincubation Test), BASF AG report no. 40M0401/914239

BASF (1993) DTPA-Na5 - Report on the maximization test for the sensitizing potential of Trilon C Flussig in guinea pigs. BASF AG Report no. 30H812/892379

BASF (1994) DTPA-Na5 - Department of Toxicology. Study of the prenatal toxicity of Trilon C liquid in Wistar rats after oral administration (gavage). BASF AG Report no. 30R0812/89117

BASF (2000) EDTA-Na2H2 - Cytogenetic study in vivo with Trilon BD in the mouse micronucleus

test after two oral administrations. Experimental Toxicology and Ecology Laboratory, BASF AG Report no. 26M0490/984167

BASF (2002) DTPA-Na5 - Trilon C flussig-Repeated dose oral toxicity study in Wistar rats; administration in the drinking water for 4 weeks. Experimental Toxicology and Ecology. BASF AG Report No. 32S0812/89116

BASF (2010) Sub Acute 5-day range finding inhalation study with recovery period in male Wistar rats, dust aerosol exposure. Experimental Toxicology and Ecology, BASF AG Report No 3310628/09053

BASF (2014). Trilon BD - 90-day inhalation study in Wistar rats, dust exposure. Experimental Toxicology and Ecology, BASF SE, Report No 50I0628/09I127

Beerens C (2010a) Assessment of acute oral toxicity with EDTA-MnNa2 in the rat (Acute Toxic Class Method). Notox BV, Den Bosch, the Netherlands. Report no. 494026

Beerens CGM (2010b) Assessment of acute oral toxicity with EDTA-Fe(NH4)NH4OH in the rat (Acute Toxic Class Method). Notox BV, Den Bosch, the Netherlands. Report no. 494022

Beerens CGM (2010c) Assessment of acute dermal toxicity with EDTA-Fe(NH4)NH4OH in the rat. Notox BV, Den Bosch, the Netherlands. Report no. 494023

Bohne F, Nigrovic V, Harmuth-Hoene AE (1976) Metabolism and toxicity of therapeutic chelating agents. II. Effect of DTPA on the intestinal absorption of zinc. Strahlentherapie 134 (2), 293-295

Brummett, ES and Mays CW (1977) Teratological studies of Zn-DTPA in mice. Health Phys 33, 624-626

Cantilena LR and Klassen CD (1982) The effect of chelating agents on the excretion of endogenous metals. Toxicol Appl Pharmacol 63, 344-350

CIT (2000) EDTA-Na2H2 - Skin maximization test in guinea pigs, CIT Centre International de Toxicologie, France, Report no. 30H0490/989166

Cohen and Guilmette (1976) Biological effects of the enhanced excretion of Zinc after calcium diethylenetriaminepentaacetate chelation therapy. Bioinorg Chem 5 (3), 203-210

Dahlback M (1990) A new aerosol delivery system to dogs. J Aerosol Sci 21 (Suppl. 1), S467-S470

Davidsson L, Kastenmayer P, Hurrell RF (1994) Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant: the effect on the absorption and retention of zinc and calcium in women. Am J Clin Nutr 60, 231-237

Davidsson L, Almgren A, Hurrell RF (1998) Sodium Iron EDTA [NaFe(III)EDTA] as a Food Fortificant Does Not Influence Absorption and Urinary Excretion of Manganese in Healthy Adults. J Nutr 128, 1139-1143

Domingo JL, Llobet JM, Colomina MT and Corbella J (1988) The removal of zinc from the mouse by polyamincarboxylic acids (CDTA and DTPA) following semichronic zinc ingestion. Vet Human Toxicol 30 (6), 524-527

Dow (1955) Sub-acute feeding of calcium disodium Versenate and disodium dihydrogen Versenate in the diet of female rats. Dow Report no. K-011830-002

Dow (1956) Results of range finding toxicological tests on calcium disodium ethylene-nitrilotetraacetate. Dow Report no. K-011830-001

Dow (1957) Toxicological investigations of the calcium disodium salt of ethylenedinitrilotetraccetic acid. Food Research Laboratory INC. Report no. K-011830-003

Dow (1958) Results of range finding toxicological tests on diethylene triamine pentaacetic acid (Versenex Acid). Dow Report no. K-018702-002

Dow (1976) Acute toxicological properties and industrial handling hazards of calcium disodiumethylenediaminetetraacetate dihydrate, Reference #1-234-26 TA102. Dow Report no. K-011830-004

Dow (1978) DOT test for corrosiveness to skin and industrial handling hazards of calcium disodium EDTA. Dow Report no. K-011830-005

DOW (2002) XUS-40881.00 Experimental Chelating Agent (DTPA): A Dermal sensitization Study in Hartley Albino Guinea Pigs Modified Buehler Design. Dow Report no. DR-0380-7532-001

Dudley RE, Muggenburg BA, Cuddihy RG and McClellan RO (1980a) Nasal absorption of diethylenetriaminepentaacetic acid in rats. Health Phys 38 (5), 763-768

Dudley RE, Muggenburg BA, Cuddihy RG, and McClellan RO (1980b) Absorption of diethylenetriaminepentaacetic acid from the respiratory tracts of beagle dogs. Am Ind Hyg Assoc J 41 (1), 5-11

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, Rosenkranz H, Simmon V (1985) Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in Salmonella typhimurium and Excherichia coli. Environ Mutagen 7 (5), 1-248

Dunkel VC, San RHC, Seifried HE, Whittaker P (1999) Genotoxicity of iron compounds in Salmonella typhimurium and L5178Y mouse lymphoma cells. Environ Mol Mutagen 33, 28-41

Elliott PH, Smith C, Crook D, Gibson WA, Hadley JC, Gopinath C (1987) Twenty-eight day oral toxicity study in rats with glycine, N-N-bis[2-[bis(carboxymethyl) amino]ethyl]- pentapotassium salt. Huntingdon Research Centre UK, Report no. AKZ 231/871020-3

Foreman H, Vier M and Magee M (1953) The Metabolism of C^{14}-Labeled Ethylenediaminetetraacetic Acid in the Rat. *J Biol Chem* 203:1045-1053.

Foreman H and Trujillo TT (1954) The metabolism of ^{14}C labeled ethylenediaminetetraacetic acid in human beings. *J Lab Clin Med* 43:566.

Fujita H, Nakano M, Sasaki M (1988) Mutagenicity test of food additives with Salmonella typhimurium TA97 and TA102 (III). Ann Rep Tokyo Metr Res Lab PJI, 39, 343-350

Fukuda S, Iida H (1983) Toxicological Study on the Safety of DTPA as a drug (I) Teratological Study in the rat. Hoken Butsuri 18, 37-42

Gardner JR (1987) DTPA-K5 - Acute dermal toxicity to rats of glycine N,N-bis[2-[bis-(carboxymethyl)amine]ethyl]-penta potassium salt. Huntington Research Centre Ltd., UK, Report No. 8713OD/AKZ227/AC

Haferkorn J (2007a) Acute oral toxicity of FeNaEDTA in rats. LPT Laboratory of Pharmacology

and Toxicology GmbH & Co. KG, Hamburg, Germany. Report no. 21617

Haferkorn J (2007b) Acute dermal toxicity of FeNaEDTA in rats. LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG, Hamburg, Germany. Report no. 21618

Haferkorn J (2007c) FeNaEDTA skin sensitization: Local Lymph Node Assay in mice. LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG, Hamburg, Germany. Report no. 21622

Haferkorn J (2008) Acute inhalation toxicity of FeNaEDTA in rats. LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG, Hamburg, Germany. Report no. 21619

Havlicek F (1967) Metabolism and toxicity of therapeutic chelating agents. 3. Effect of EDTA and DTPA on the excretion of endogenous zinc. J Strahlentherapie, 134 (2), 296-305

Heimbach J, Rieth S, Mohamedshah F, Slesinski R, Samuel-Fernando P, Sheenan T, Dickmann R, Borzelleca J (2000) Safety Assessment of Iron EDTA [Sodium Iron {Fe+3} Ethylenediaminetetraacetic Acid]: Summary of Toxicological, Fortification and Exposure Data. Food Chem Toxicol 38: 99-111

Hurley LS, Gowan J, Swenerton H (1971) Teratogenic effects of short-term and transitory zinc-deficiency in rats. Teratol 4, 199-204

Hurrell RF, Ribas S, Davidsson L (1994) Na3Fe + EDTA as a food fortificant: influence on zinc, calcium and copper metabolism in the rat. Br J Nutr 71, 85-93

Jolly L jr, McClearen HA, Poda GA, Walke WP (1972) Treatment and evaluation of a plutonium-238 nitrate contaminated puncture wound - a two-year case history. Health Physics Pergamon Press, 23 (Sept.) 333-341

Jonker D, Triel van JJ (2012a) Acute (4-hour) inhalation toxicity study in rats with EDTA-CuNa2. TNO Triskelion, Zeist, the Netherlands, Report no. 20201/05

Jonker D, Triel van JJ (2012b) Acute (4-hour) inhalation toxicity study in rats with DTPA-FeNaH. TNO Triskelion, Zeist, the Netherlands, Report no. 20201/04

Kalkwarf DR, Thomas VW Jr, Nielson KK, Mauch ML (1983) 1976 Hanford Americanum exposure incident: urinary excretion of trace metals during DTPA treatment. Health Phys 45 (4), 937-947

Kawamata K, Yoshimoto H, Momma J et al. (1980) Comparative toxicity studies of Na2EDTA and CaNa2EDTA in rats. Jpn. J. Pharmacol., 30 (Suppl) 234p

Kimmel CA (1977) Effect of route of administration on the toxicity and teratogenicity of EDTA in the rat. *Toxicol Appl Pharmacol* 40:299-306.

Kynoch SR (1984) DTPA-K5 - Acute oral toxicity to rats of glycine, N-N-bis[2-[bis(carboxymethyl) amino]ethyl]-pentapotassium salt. Huntingdon Research Centre Report no. 84753D/AKZ 172/AC

Kynoch SR and Parcell BI (1987) DTPA-K5 - Delayed contact hypersensitivity in the guinea pig with glycine, N-N-bis[2-[bis(carboxymethyl) amino]ethyl]-pentapotassium salt. Huntingdon Research Centre Report no. 87833D/AKZ 230/SS-3

Latour JEHM (2014) Assessment of acute oral toxicity with EDTA-FeK in the rat (Acute Toxic Class Method). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 505733

Latour JEHM (2014) Assessment of acute oral toxicity with EDTA-Mn(NH4)2 in the rat (Acute Toxic Class Method). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 505736

Latour JEHM (2014) Assessment of acute oral toxicity with EDTA-CuK2 in the rat (Acute Toxic Class Method). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 505735

Latour JEHM (2014) Assessment of acute oral toxicity with EDTA-ZnK2 in the rat (Acute Toxic Class Method). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 505734

Leuschner J (2007a) Acute dermal irritation/corrosion test (patch test) of FeNaEDTA in rabbits. LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG, Hamburg, Germany. Report no. 21620

Leuschner J (2007b) Acute eye irritation/corrosion test of FeNaEDTA in rabbits. LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG, Hamburg, Germany. Report no. 21621

Liggett MP and Smith PA (1987a) DTPA-K5 - Irritant effects on rabbit skin of glycine, N-N-bis[2-[bis(carboxymethyl) amino]ethyl]-pentapotassium salt. Huntingdon Research Centre Report no. 87123D/AKZ 228/SE

Liggett MP and Smith PA (1987b) DTPA-K5 - Irritant effects on the rabbit eye of glycine, N-N-bis[2-[bis(carboxymethyl) amino]ethyl]-pentapotassium salt. Huntingdon Research Centre Report no. 87160D/AKZ 229/SE-3

Lina BAR (2013) Combined oral repeated dose toxicity study with reproduction/developmental toxicity screening test with EDTA-CuNa2 in rats. TNO Triskelion, Zeist, the Netherlands. Report no. V20114

May K (1990) Armogard S462: Assessment of mutagenic potential in histidine auxotrophs of Salmonella Typhimurium (the Ames test). Life Sciences Research Ltd., UK, Report no. 90/AKL084/0441

Muijser H (2010) An acute (4-hour) inhalation toxicity study with EDTA-MnNa2 in rats. TNO Quality of Life, Zeist, the Netherlands. Report no. V8801/03

NTIS (1977) Bioassay of trisodium ethylenediaminetetraacetate trihydrate (EDTA) for possible carcinogenicity. National Cancer Institute Carcinogenesis Technical Report Series No. 11

NTP (1984) EDTA-Na3H - Chromosome Aberration. NTP-Webpage http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm

NTP (1984) EDTA-Na3H – Mouse Lymphoma Assay. NTP-Webpage http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm

Oser BL, Oser M, Spencer HC (1963) Safety evaluation studies of calcium EDTA. Toxicol Appl Pharmacol 5, 142-162

Otterdijk van FM (2013a) Assessment of acute oral toxicity with EDTA-Cu(NH4)2 in the rat (Acute Toxic Class Method). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500352

Otterdijk van FM (2013b) Assessment of acute oral toxicity with EDTA-MgNa2 in the rat (Acute Toxic Class Method). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500360

Otterdijk van FM (2013c) Assessment of acute oral toxicity with EDTA-Zn(NH4)2 in the rat (Acute Toxic Class Method). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500357

Otterdijk van FM (2013d) Assessment of acute oral toxicity with EDTA-MnK2 in the rat (Acute Toxic Class Method). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500363

Otterdijk van FM (2013e) Assessment of acute oral toxicity with DTPA-FeHNa in the rat (Acute Toxic Class Method). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500337

Otterdijk van FM (2013f) Assessment of acute oral toxicity with DTPA-FeNa2 in the rat (Acute Toxic Class Method). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500342

Otterdijk van FM (2013g) Assessment of acute oral toxicity with DTPA-Fe(NH4)2 in the rat (Acute Toxic Class Method). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500345

Otterdijk van FM (2013h) Assessment of acute oral toxicity with HEDTA-Fe(III)Na in the rat (Acute Toxic Class Method). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500348

Otterdijk van FM (2013i) Assessment of contact hypersensitivity to EDTA-CuNa2 in the mouse (Local Lymph Node Assay). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500351

Otterdijk van FM (2013j) Assessment of contact hypersensitivity to DTPA-FeHNa in the mouse (Local Lymph Node Assay). WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500340

Resnick RH, Royal H, Marshall W, Barron R, and Werth T (1990) Intestinal Permeability in Gastrointestinal Disorders. Use of Oral [99mTc] DTPA. Digestive Diseases and Sciences, 35 (2), 205-211

Rubin M and Princiotto JV (1960) Synthetic amino acid chelating agents and iron metabolism, Ann NY Acad Sci 88, 450-459

Russo A and Levis AG (1992) Further evidence for the aneuploidogenic properties of chelating agents: induction of micronuclei in mouse male germ cells by EDTA. Environ Mol Mutagen 19, 125-131

Schardein JL, Sakowski R, Petrere J, Humphrey RR (1981) Teratogenesis studies with EDTA and its salts in rats. Toxicol Appl Pharmacol 61, 423-428

Sato I, Matsusaka N, Suzuki T, Kobayashi H, Sera K, Futatsugawa S (1997) Essential metal contents in the liver of DTPA-treated pregnant mice and their fetuses. Int J PIXE, 7 (1 & 2), 25-29

Smith RM and Martell AE (1974) Critical Stability Constants, 3rd Ed. Plenum Press, New York

Smith VH, Dagle GE, Gelman RA, Ragan HA (1980) Early effects of inhaled Ca-DTPA on the rat lung. Toxicol Lett 7, 9-16

Sterner W and Chibanguza G (1983a) DTPA-Na5 - Test for primary skin irritation of Dissolvine D40. IBR Forschungs-GmbH, Walsrode, Germany, Report no. 1-3-403-83

Sterner W and Chibanguza G (1983b) DTPA-Na5 - Draize animal mucous membrane irritation test of Dissolvine D40. IBR Forschungs-GmbH, Walsrode, Germany, Report no. 1-3-409-83

Stevens E, Rosoff B, Weiner M, Spencer H (1962) Metabolism of chelating agent diethylenetriamine pentaacetic acid (C14 DTPA) in man. Proc Soc Exp Biol Med 111, 235-238

Stitzinger M (2010) Assessment of acute oral toxicity with Zinc-disodium EDTA in the rat (Acute Toxic Class method). Notox BV, Den Bosch, the Netherlands. Report no. 493958

Swenerton H, Hurley LS. (1971). Teratogenic effects of a chelating agent and their prevention by zinc. Science 173, 62-63

Tandon SK (1982) Chelation in metal intoxication: 10. Influence of different polyaminocarboxylic acids and thiol chelators in the excretion and tissue distribution of manganese-54 in rat. Res Commun Chem Pathol Pharmacol, 337-340

Usta B (2013a) In vitro micronucleus test with EDTA-CuNa2 in cultured human lymphocytes. TNO Triskelion, Zeist, the Netherlands, Report no. V20217/03

Usta B (2013b) In vitro micronucleus test with DTPA-FeNaH in cultured human lymphocytes. TNO Triskelion, Zeist, the Netherlands, Report no. V20217/04

Verbaan IAJ (2010a) In vitro skin irritation test with EDTA-MnNa2 using a human skin model. Notox BV, Den Bosch, the Netherlands. Report no. 494025

Verbaan IAJ (2010b) In vitro skin irritation test with EDTA-Fe(NH4)NH4OH using a human skin model. Notox BV, Den Bosch, the Netherlands. Report no. 494021

Verbaan IAJ (2013a) In vitro skin irritation test with EDTA-Cu(NH4)2 using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500353

Verbaan IAJ (2013b) In vitro skin corrosion test with EDTA-Cu(NH4)2 using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 501989

Verbaan IAJ (2013c) In vitro skin irritation test with EDTA-MgNa2 using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500361

Verbaan IAJ (2013d) In vitro skin irritation test with EDTA-ZnNa2 using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500355

Verbaan IAJ (2013e) In vitro skin irritation test with EDTA-Zn(NH4)2 using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500358

Verbaan IAJ (2013f) In vitro skin irritation test with EDTA-MnK2 using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500364

Verbaan IAJ (2013g) In vitro skin irritation test with DTPA-FeHNa using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500338

Verbaan IAJ (2013h) In vitro skin irritation test with DTPA-FeNa2 using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500343

Verbaan IAJ (2013i) In vitro skin irritation test with DTPA-Fe(NH4)2 using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500346

Verbaan IAJ (2013j) In vitro skin irritation test with HEDTA-Fe(III)Na using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 500349

Verbaan IAJ (2014a) In vitro skin irritation test with EDTA-FeK using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 505737

Verbaan IAJ (2014b) In vitro skin irritation test with EDTA-MN(NH4)2 using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 505740

Verbaan IAJ (2014c) In vitro skin irritation test with EDTA-CuK2 using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 505739

Verbaan IAJ (2014d) In vitro skin irritation test with EDTA-ZnK2 using a human skin model. WIL Research Europe BV, Den Bosch, the Netherlands. Report no. 505738

Verspeek CM (2010a) Screening for the eye irritation potential of EDTA-MnNa2 using the bovine

corneal opacity and permeability test (BCOP test). Notox BV, Den Bosch, the Netherlands. Report no. 494024

Verspeek CM (2010b) Screening for the eye irritation potential of EDTA-Fe(NH4)NH4OH using the bovine corneal opacity and permeability test (BCOP test). Notox BV, Den Bosch, the Netherlands. Report no. 494020

Verspeek-Rip CM (2012a) Screening for the eye irritancy potential of EDTA-Cu(NH4)2 using the Bovine Corneal Opacity and Permeability test (BCOP test). WIL Research BV, Europe, Den Bosch, NL. Report no. 500354

Verspeek-Rip CM (2012b) Screening for the eye irritancy potential of EDTA-MgNa2 using the Bovine Corneal Opacity and Permeability test (BCOP test). WIL Research BV, Europe, Den Bosch, NL. Report no. 500362

Verspeek-Rip CM (2012c) Screening for the eye irritancy potential of EDTA-ZnNa2 using the Bovine Corneal Opacity and Permeability test (BCOP test). WIL Research BV, Europe, Den Bosch, NL. Report no. 500356

Verspeek-Rip CM (2012d) Screening for the eye irritancy potential of EDTA-Zn(NH4)2 using the Bovine Corneal Opacity and Permeability test (BCOP test). WIL Research BV, Europe, Den Bosch, NL. Report no. 500359

Verspeek-Rip CM (2012e) Screening for the eye irritancy potential of EDTA-MnK2 using the Bovine Corneal Opacity and Permeability test (BCOP test). WIL Research BV, Europe, Den Bosch, NL. Report no. 500365

Verspeek-Rip CM (2012f) Screening for the eye irritancy potential of DTPA-FeHNa using the Bovine Corneal Opacity and Permeability test (BCOP test). WIL Research BV, Europe, Den Bosch, NL. Report no. 500339

Verspeek-Rip CM (2012g) Screening for the eye irritancy potential of DTPA-FeNa2 using the Bovine Corneal Opacity and Permeability test (BCOP test). WIL Research BV, Europe, Den Bosch, NL. Report no. 500344

Verspeek-Rip CM (2012h) Screening for the eye irritancy potential of DTPA-Fe(NH4)2 using the Bovine Corneal Opacity and Permeability test (BCOP test). WIL Research BV, Europe, Den Bosch, NL. Report no. 500347

Verspeek-Rip CM (2012i) Screening for the eye irritancy potential of HEDTA-Fe(III)Na using the Bovine Corneal Opacity and Permeability test (BCOP test). WIL Research BV, Europe, Den Bosch, NL. Report no. 500350

Verspeek-Rip CM (2013a) Evaluation of the mutagenic activity of DTPA-FeHNa in the Salmonella Typhimurium Reverse Mutation Assay and the Eschericha Coli reverse mutation assay (with independent repeat). WIL Research BV, Europe, Den Bosch, NL. Report no. 500341

Verspeek-Rip CM (2014a) Screening for the eye irritancy potential of EDTA-FeK using the Bovine Corneal Opacity and Permeability test (BCOP test). WIL Research BV, Europe, Den Bosch, NL. Report no. 505745

Verspeek-Rip CM (2014b) Screening for the eye irritancy potential of EDTA-Mn(NH4)2 using the Bovine Corneal Opacity and Permeability test (BCOP test). WIL Research BV, Europe, Den Bosch, NL. Report no. 505748

Verspeek-Rip CM (2014c) Screening for the eye irritancy potential of EDTA-CuK2 using the Bovine Corneal Opacity and Permeability test (BCOP test). WIL Research BV, Europe, Den Bosch, NL. Report no. 505747

Verspeek-Rip CM (2014d) Screening for the eye irritancy potential of EDTA-ZnK2 using the Bovine Corneal Opacity and Permeability test (BCOP test). WIL Research BV, Europe, Den Bosch, NL. Report no. 505746

Vogel de N, Usta B (2010) In vitro micronucleus test with EDTA-FeNa in cultured human lymphocytes, TNO Quality of Life, Zeist, The Netherlands, Report no. V8817/07

Vogel de N (2010) In vitro micronucleus test with EDTA-MnNa2 in cultured human lymphocytes. TNO Quality of Life, Zeist, The Netherlands, Report no. V8417/06

Whittaker P, Seifried HE, San RH, Clarke JJ, Dunckel VC (2001) Genotoxicity of Iron Chelators in L5178Y Mouse Lymphoma Cells. Environ Mol Mut 38 (4) 347-356

Wijngaard van de MJM (2009) Baterial reverse mutation test with EDTA-MnNa2. TNO Quality of Life, Zeist, the Netherlands, Report no. V8405/14

Wittmer E, Groters S, Landsiedel R (2013) HEDTA Acute inhalation toxicity study in Wistar rats – 4-hour liquid aerosol exposure (head-nose only) – Experimental Toxicology and Ecology, BASF AG Report no. 13/0444/10/158

Wolterbeek APM (2010) Combined oral repeated dose toxicity study with the reproduction/developemental toxicity screening test with EDTA-MnNa2 in rats. TNO Quality of Life, Zeist, the Netherlands, Report no. V8650

Wolterbeek APM, Otto M (2011) Combined oral repeated dose toxicity study with the reproduction/developemntal toxicity screening test with DTPA-FeNaH in rats. TNO Triskelion, Zeist, the Netherlands, Report no. V8917

Wynn JE, Riet van't B, Borzelleca JF (1970) The toxicity and pharmacodynamics of EGTA: oral administration to rats and comparisons with EDTA. Toxicol Appl Pharmacol 16 (3), 807-817

Yang SS and Chan SC (1964) Summaries of toxicological data. Fd CosmetToxicol 2, 763-767

Yeung CK, Zhu L, Glahn RP, Miller DD (2005) Tissue Iron Distribution and Adaptation of Iron Absorption in Rats Exposed to a High Dietary Level of NaFeEDTA. J Agric Food Chem, 53, 8087-8091

Zordan M, Russo A, Costa R, Bianco N, Beltrame C, Levis AG (1990) A concerted approach to the study of the aneuploidogenic properties of two chelating agents (EDTA and NTA) in the germ and somatic cell lines of Drosophila and the mouse. Environ Molec Mutagen 15 (4), 205-213

Zylicz E (1975) Effects of DTPA on the deposition of 65Zn, 60Co and 144Ce in pregnant rat and in foetoplacental unit. Int J Radiation Biol & related studies in physics, chemistry & medicine, 125-136