CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

[Ethylenebis[nitrilobis(methylene)]]tetrakisphosphonic acid, sodium salt

EC Number: **244-742-5**

CAS Number: **22036-77-7**

Index Number: **-**

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ABBREVIATIONS

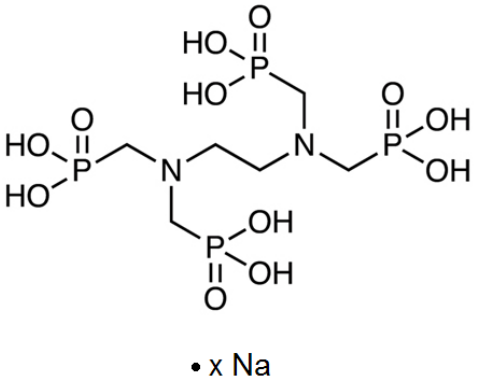
AD	average dose
ALP	alkaline phosphatase
bw	body weight
CHO	Chinese hamster ovary
COM	European Commission
CSR	Chemical Safety Report
d	day
DMSO	dimethyl sulfoxide
DS	dossier submitter
DSC	differential scanning calorimetry
GLP	Good Laboratory Practice
HPLC	high performance liquid chromatography
Hprt	hypoxanthine phosphoribosyltransferase
i.v.	intravenous
LOAEL	lowest observed adverse effect level
LSC	liquid scintillation counting
MA	metabolic activation
MLA	mouse lymphoma assay
MTD	maximum tolerated dose
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OECD TG	OECD testing guideline
pKA	acid dissociation constant
QSAR	quantitative structure-activity relationship
QMRF	QSAR model reporting format
QPRF	QSAR prediction reporting format
RAC	Committee for Risk Assessment
SCL	specific concentration limit
SD rats	Sprague-Dawley rats
T25	estimated chronic dose at which 25 % increase in the incidence of a specified tumour type is expected
TK	toxicokinetics

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	[ethylenebis[nitrilobis(methylene)]]tetrakisphosphonic acid, sodium salt
Other names (usual name, trade name, abbreviation)	Phosphonic acid, P,P',P",P"'-[1,2-ethanediylbis[nitril obis(methylene)]]tetrakis-, sodium salt (1:?) [CAS]; Phosphonic acid, [1,2-ethanediylbis[nitrilobis-(methylene)]]tetrakis-, sodium salt; Phosphonic acid, [ethylenebis(nitrilodimethylene)]-tetra-, sodium salt; Ethylenediaminetetrakis(methylenephosphonic acid) sodium salt; Sodium (ethylenediamine)tetramethylenephosphonate; Sodium EDTMPA; Phosphonic acid, [[(phosphonomethyl)imino]bis[2,1-ethanediylnitrilobis(methylene)]]tetrakis-, x sodium salt; EDTMP-Na
EC number (if available and appropriate)	244-742-5
EC name (if available and appropriate)	[ethylenebis[nitrilobis(methylene)]]tetrakisphosphonic acid, sodium salt
CAS number (if available)	22036-77-7
Other identity code (if available)	-
Molecular formula	C6H20-xN2O12P4.xNa
Structural formula	
Molecular weight or molecular weight range	458 - 612 g/mol
Degree of purity (%) (if relevant for the entry in Annex VI)	<=100

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
EDTMP-Na (CAS No. 22036-77-7; EC No. 244-742-5)	<=100*		

**The compositional information reported in the REACH registrations submitted for this substance show concentration levels of "[ethylenebis[nitrilobis(methylene)]]tetrakisphosphonic acid, sodium salt" <80 %. Therefore, the identification of these compositions deviates from the Guidance for identification and naming of substances under REACH and CLP.*

The current CLH proposal also covers these compositions.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No entry										
Dossier submitters proposal	tba	[ethylenebis[nitrilobis(methylene)]]tetrakisphosphonic acid, sodium salt	244-742-5	22036-77-7	Carc. 1B	H350	GHS08 Dgr	H350		Carc. 1B; H350: C ≥ 1 %	
Resulting Annex VI entry if agreed by RAC and COM											

Table 4: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)		
Oxidising gases		
Gases under pressure		
Flammable liquids		
Flammable solids		
Self-reactive substances		
Pyrophoric liquids		
Pyrophoric solids		
Self-heating substances		
Substances which in contact with water emit flammable gases		
Oxidising liquids		
Oxidising solids		
Organic peroxides		
Corrosive to metals		
Acute toxicity via oral route		
Acute toxicity via dermal route		
Acute toxicity via inhalation route		
Skin corrosion/irritation		
Serious eye damage/eye irritation		
Respiratory sensitisation		
Skin sensitisation		
Germ cell mutagenicity	**Data lacking**	**Yes**
Carcinogenicity	**Carc. 1B, H350**	**Yes**
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure		
Specific target organ toxicity-repeated exposure	**Data conclusive but not sufficient for classification**	**Yes**
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment		
Hazardous to the ozone layer		

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no current harmonised classification for the substance **EDTMP-xNa**[1] ([ethylenebis-[nitrilobis(methylene)]]tetrakisphosphonic acid, sodium salt; EC no. 244-742-5; CAS no. 22036-77-7).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level for CMR properties.

[B.] Justification that action is needed at Community level for the hazard class STOT RE:

The reason for assessing STOT RE in this dossier are differences in the available self-classifications. Some notifiers have classified the substance as STOT RE (H373- bone, blood) and most have not. Including assessment of this hazard class in the present dossier gives clarifications on correct classification and labelling of the substance.

5 IDENTIFIED USES

The substance is widely used as complexing or chelating agent with transition metals and calcium or magnesium, corrosion inhibitors and stabilising agents. It is used in the following products: water softeners, air care products, fillers, putties, plasters, modelling clay, polishes and waxes, washing and cleaning products, and cosmetics and personal care products.

6 DATA SOURCES

In addition to information that is available on the website of ECHA (e.g. ECHA's dissemination site) and in the REACH registration dossier, including study reports for carcinogenicity, an extensive literature search was conducted by the date of September 2021 in several relevant online resources (e.g. PubMed, Embase, Web of Science, Science Direct).

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20 °C and 101,3 kPa	Solid (manufactured and marketed as aqueous solution: straw-coloured liquid)	REACH registration data	Experimental result (visible inspection)
Melting/freezing point	Read-across to EDTMP-H (acid-form): Decomposition with melting from approximately 180 °C (salts would be expected to melt at a higher temperature than the acid, and to decompose)	REACH registration data	Read-across to EDTMP-H [for EDTMP-H: Experimental result (EU Method A.1 (Melting / Freezing Temperature); OECD Guideline no. 102 "Melting Point/Melting Range"; differential scanning calorimetry (DSC))]
Boiling point	Read-across to EDTMP-H (acid-form): thermal decomposition without boiling	REACH registration data	Read-across to EDTMP-H

[1] The "x" in the substance name stands for a different (sometimes unknown) Na content in the substance. If known for the substances in the toxicity studies, the specific content is given in the substance name, such as e.g. EDTMP-5Na (e.g. Study Report 1981b). To simplify data presentation EDTMP-xNa1 is named EDTMP-Na here irrespectively of the real Na^+ ion count.

Property	Value	Reference	Comment (e.g. measured or estimated)
Relative density	Specific gravity: 1.3 - 1.36 g/cm³ at ambient conditions for various commercial solutions; no relative density of solid values available	REACH registration data	Experimental results [secondary sources]
Vapour pressure	Read-across to EDTMP-H (acid-form): Vapour pressure (EDTMP-Na): <2.7E-09 Pa at 25 °C (The vapour pressure of the salt would be expected to be lower than for the acid form.)	REACH registration data	Read-across to EDTMP-H; estimated [(Q)SAR model]
Surface tension	Not applicable (based on structure, surface activity is not expected).	REACH registration data	-
Water solubility	Very soluble (> 10 000 mg/L); water solubility (EDTMP-Na): > 400 g/l at ambient conditions (phosphonate salts are highly water soluble and are supplied as aqueous solutions; free acids are less soluble than the salts)	REACH registration data	Experimental results [secondary sources]
Partition coefficient n-octanol/water	Read-across to EDTMP-H (acid-form): Octanol-water partition coefficient (EDTMP-Na): -4.1 at 23 °C	REACH registration data	Read-across to EDTMP-H [for EDTMP-H: Experimental result (log Kow of the substance was measured by equilibrating aqueous solutions of radiolabelled compounds with n-octanol. The concentration of the substance in each phase was determined by Liquid Scintillation Counting (LSC))]
Granulometry	The substance is marketed and used in a non-solid form (aqueous solution).	REACH registration data	-
Dissociation constant	Read-across to EDTMP-H (acid-form): Dissociation constant (EDTMP-Na): EDTMP acid has eight ionisable phosphonate groups (at 20-25 °C): pKa1 = 1.3 pKa2 = 2.7 pKa3 = 4.2 pKa4 = 5.7 pKa5 = 5.9 pKa6 = 7.3 pKa7 = 8.8 pKa8 = >10	REACH registration data	Read-across to EDTMP-H [for EDTMP-H: Estimated ((Q)SAR model; QMRF/QPRF)]

The information in this table marked with „REACH registration data“ is based on information taken from the REACH registration dossier and ECHA’s public registration information as accessed on 08-07-2020.

8 JUSTIFICATION OF READ-ACROSS

As available data for EDTMP-Na ([ethylenebis-[nitrilobis(methylene)]]tetrakisphosphonic acid, sodium salt; EC no. 244-742-5; CAS no. 22036-77-7) are considered not to be sufficient for assessment of the endpoints toxicokinetics, mutagenicity, carcinogenicity and repeated dose toxicity, the data set was complemented using tests performed with EDTMP-H ([ethane-1,2-diylbis[nitrilobis(methylene)]]tetrakisphosphonic acid; EC no. 215-851-5; CAS no. 1429-50-1).

Justification for this read-across is the expected common hydrolytic behaviour of EDTMP-Na and EDTMP-H. EDTMP-Na is the salt of the parent acid EDTMP-H. The result of hydrolysis of EDTMP-Na is the same $EDTMP^-$ anion that is expected to trigger the toxic effects. From a toxicological point of view, the resulting alkaline counter cations from hydrolysis (Na^+ or hydronium ions) are considered to be of low relevance in case of neutralisation of the test solution. Indeed, neutralisation of EDTMP-H often is performed using sodium hydroxide suggested to result in a similar ion composition of both EDTMP-H and EDTMP-Na, respectively. In addition, because EDTMP-H and EDTMP-Na hydrolyse at neutral or low pH value, exposure to the intact substances in neutral test solutions or after oral application is considered unlikely.

9 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

10 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

As available data for EDTMP-Na ([ethylenebis-[nitrilobis(methylene)]]tetrakisphosphonic acid, sodium salt; EC no. 244-742-5; CAS no. 22036-77-7) are considered not to be sufficient for assessment of the endpoint toxicokinetics, the data set was complemented using tests performed with EDTMP-H ([ethane-1,2-diylbis[nitrilobis(methylene)]]tetrakisphosphonic acid; EC no. 215-851-5; CAS no. 1429-50-1). Justification for this read-across procedure is described in section 8 (Justification of read-across).

Table 6: Summary table of toxicokinetic studies[2]

Method, guideline, deviations if any	Test substance, dose levels duration of exposure	Results	Reference
Basic toxicokinetics *in vivo* Similar to OECD TG 417 GLP: Yes	Substance: ^{14}C-labelled **EDTMP-Na (EC no. 244-742-5)** Purity: Not reported Species: **Rat (Sprague-Dawley) ♂ and ♀** Number of animals per group: Single dose: 20 Multiple doses: 20 Administration route: **Oral (gavage)** Dose levels: **Single dose: 15 mg/kg bw (only ♂), 150 mg/kg bw (♂ and ♀)**	**Supporting study (reliable with restrictions)** **Results: Accumulation in bone (trabecular bone); half-life in bones 15 – 26 d; no dose-dependence or sex-related differences** **Single dose:** - most (no more specific data on dissemination site) of administered dose eliminated in faeces during first 48 h - only 1 % of dose excreted in urine in 24 h - recovery in soft tissue not exceeding 0.1 % - only limited amount detected in blood (max. 0.03 %) - recovery in bone 0.1 - 0.8% in low-dose	(Study Report, 1987) ECHA's dissemination site 004 (EDTMP-H)

[2] Information shown in this table is taken from the REACH registration dossiers and ECHA's dissemination sites of EDTMP-H or EDTMP-Na (last accessed 2021-11-17, weblinks to ECHA dissemination sites can be found under References)

Method, guideline, deviations if any	Test substance, dose levels duration of exposure	Results	Reference
	Multiple doses (10 daily doses): 15 mg/kg bw (only ♂), 150 mg/kg bw (♂and ♀) Treatment time: 4 animals of each group sacrificed at 6 h, 24 h, 96 h, 14 d and 28 d after dosing; blood sampling of rats (96 h group) at 1, 2, 4, 8, 24, 48, 96 h after dosing; excreta (from 14 & 28 d group) sampled at 8, 24, 48, 72, 96 and 168 h after dosing, tissues bones and organs analysed for ^{14}C content	and 0.2 - 0.5 % in high-dose group (♂ and ♀) **-** no dose-dependence or sex-related differences - substance does not appear to be metabolised greatly **Multiple doses:** - primarily elimination via faeces - only 1.2 - 1.4 % via urine in 168 h period; - excretion in ♂ and ♀ similar - <0.1 % in blood and low radioactivity in soft tissues - relatively high levels in bone marrow: 0.6 - 1.2 % in low-dose and 1.3 - 2.8 % in high-dose group) (highest in trabecular bone) - half-life in bone 14 – 27 d - uptake in bones increased by multiple dosing (6 - 8 fold) - no dose-dependence or sex-related differences - substance does not appear to be metabolised greatly	
Basic toxicokinetics *in vivo* Similar to OECD TG 417 GLP: Yes	Substance: ^{14}C-labelled **EDTMP-Na (EC no. 244-742-5)** Purity: Not reported Species: **Mice (B6C3F1)** ♂ Number of animals per group: 20 Administration route: **Oral (gavage)** Dose levels: **Single dose: 15, 150 mg/kg bw**	**Supporting study (reliable with restrictions)** **Results: Accumulation in bone** **Single dose: -** most (no more specific data on dissemination site) of dose administered eliminated via faeces within 24 h - only 1.5 – 2 % via urine within 24 h and additionally 0.4 – 1 % within remaining 168 h - tissues and blood < 0.05 % - in bone 0.2 % (low-dose) and 0.2 - 0.5 % (high-dose) recovered	(Study Report, 1987) ECHA's dissemination site 004 (EDTMP-H)
Basic toxicokinetics *in vivo* Similar to OECD TG 417 GLP: Yes	Substance: ^{14}C-labelled **EDTMP-Na (EC no. 244-742-5)** Purity: 99 % Species: **Rats, Sprague-Dawley,** ♂ Number of animals per group: 20 ♂ Administration route: **Oral (diet)** Dose levels: **15, 150 mg/kg bw/d**, no control animals Treatment time: 10 d	**Supporting study (reliable with restrictions)** **Results: Accumulation in bone** **-** primarily in faeces within 24 h - < 1 % in urine - insignificant amounts in blood - accumulation in bone (0.6 - 1.1 % at low-dose and 0.3 - 0.7 % at higher dose), mainly in femur and tibia; half-life in bone 20 – 43 d (femur, tibia, vertebrae) - less absorption in diet compared to gavage	(Study Report, 1987) ECHA's dissemination site 001 (EDTMP-H)
Basic toxicokinetics *in*	Substance: ^{14}C-labelled **EDTMP-H**	**Supporting study (reliable with**	(Study

Method, guideline, deviations if any	Test substance, dose levels duration of exposure	Results	Reference
vivo Similar to OECD TG 417 GLP: Yes	**(EC no. 215-851-5)** Purity: Not reported Species: **Rat (Sprague-Dawley),** ♂ Number of animals per group: 4 Administration route: **Oral (gavage)** Dose levels: **15, 150 mg/kg bw** Treatment time: Single dose; animals sacrificed at day 1 or 14 after dosing	**restrictions)** **Results: Accumulation in the bone (epiphyseal growth plate)**	Report, 1989) ECHA's dissemination site 003 (EDTMP-H)
Basic toxicokinetics *in vivo* Similar to OECD TG 417 GLP: Yes	Substance: ^{14}C-labelled **EDTMP-H (EC no. 215-851-5)** Purity: Not reported Species: **Mice (B6C3F1),** ♂ Number of animals per group: 4 Administration route: **Oral (gavage)** Dose levels: **15, 150 mg/kg bw** Treatment time: Single dose; animals sacrificed at day 1 or 14 after dosing	**Supporting study (reliable with restrictions)** **Results: Accumulation in the bone (epiphyseal growth plate)**	(Study Report, 1989) ECHA's dissemination site 003 (EDTMP-H)
Basic toxicokinetics *in vivo* Similar to OECD TG 417 GLP: Yes	Substance: Non-labelled or ^{14}C-labelled, **EDTMP-Na (EC no. 244-742-5)** Purity: Not reported (HPLC analysis of excreta indicated the presence of impurities) Species: **Rats (Sprague-Dawley),** ♂ Number of animals per group: 20 ♂; 4 sacrificed from each group at 6 h, 24 h, 96 h, 14 d and 28 d after dosing Administration route: **Intravenous** Dose levels: **15 mg/kg bw** (probably single dose, no specific information on ECHA's dissemination site) Sampling of excreta at 8, 24, 48, 72, 96 and 168 h; analysis of tissues and organs for ^{14}C content	**Supporting study (reliable with restrictions)** **Results: High affinity to bone after i.v. administration; long half-life in bone- 20 d** - most administered radioactivity recovered in bone (55 % at 6 h), higher levels in trabecular bone than cortical bone - disappearance from bone slowly ($t_{1/2}$ = 20 d) - HPLC data of excreta suggests that substance is not metabolised to any great extent (no further specific information on ECHA's dissemination site)	(Study Report, 1987) ECHA's dissemination site 002 (EDTMP-H)
Basic toxicokinetics *in vivo* Similar to OECD TG 417 GLP: Yes	Substance: Non-labelled or ^{14}C-labelled **EDTMP-Na (EC no. 244-742-5)** Purity: Not reported (HPLC analysis of excreta indicated the presence of impurities) Species: **Mice (B6C3F1),** ♂ Number of animals per group: 20 ♂; 4 sacrificed from each group at 6 h,	**Supporting study (reliable with restrictions)** **Results: High affinity to bone after i.v. administration; long half-life in bone -26 d** - 33 % of administered dose recovered in bone (at 6 h) - disappearance from bone slowly ($t_{1/2}$ = 26 d)	(Study Report, 1987) ECHA's dissemination site 002 (EDTMP-H)

Method, guideline, deviations if any	Test substance, dose levels duration of exposure	Results	Reference
	24 h, 96 h, 14 d and 28 d after dosing Administration route: **Intravenous** Dose levels: **15 mg/kg bw** (probably single dose, no specific information on ECHA's dissemination site) Sampling of excreta at 8, 24, 48, 72, 96 and 168 h; analysis of tissues and organs for ^{14}C content	- HPLC data of excreta suggests that substance is not metabolised to any great extent (no further specific information on ECHA's dissemination site)	

10.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

There are several GLP-compliant toxicokinetic studies available performed similar to OECD TG 417 in rats and mice after single or repeated, oral or intravenous EDTMP-H or EDTMP-Na administration.

From the oral studies in rats and mice it can be concluded that EDTMP-H/Na is primarily excreted via faeces and only about 1 % via urine (e.g. after repeated dosing in rats only up to 1.4 % in a 168 h period).

The studies also showed a limited distribution of EDTMP-H/Na in blood and soft tissues (< 0.1 % after single or repeated oral substance administration in rats and mice).

All available oral and intravenous toxicokinetic studies reveal a high affinity of EDTMP-H/Na to the bone with detected bone tissue concentrations of up to 2.8 % of the administered dose mainly in femur and tibia with a long half-life of up to 27 d (repeated dosing in rats).

Absorption was observed to be less in rat oral feeding studies compared to rat oral studies performed using gavage (similar dose levels).

Oral and intravenous studies indicate that EDTMP-H/Na is not metabolised to any great extent. No marked species- or sex-related differences were observed in the oral and intravenous TK studies.

11 EVALUATION OF HEALTH HAZARDS

11.1 Acute toxicity

Not assessed in this dossier.

11.2 Skin corrosion/irritation

Not assessed in this dossier.

11.3 Serious eye damage/eye irritation

Not assessed in this dossier.

11.4 Respiratory sensitisation

Not assessed in this dossier.

11.5 Skin sensitisation

Not assessed in this dossier.

11.6 Germ cell mutagenicity

As available data for EDTMP-Na are considered not to be sufficient for mutagenicity assessment *related to induction of chromosome aberrations*, the data set was complemented using mutagenicity tests performed with EDTMP-H. Justification for this read-across is described in section 8 (Justification of read-across).

Table 7: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	**Test substance**	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Bacterial reverse mutation assay Similar to OECD TG 471 Deviation: - neither a *E.coli* WP2 strain nor the *S.typhimurium* tester strain TA102 has been tested - concentrations not given as µg/plate - no data on cytotoxicity and precipitations GLP: No	**EDTMP-5Na, (CAS no. 7651-99-2)**	**Supporting study** **(reliable with restrictions)** **(together with Study Report (2012): Key information on Ames test available)** Bacterial strains: *S. typhimurium* TA1535, TA 1537, TA98, TA100 Test concentrations (spot test 25 µl): Plate incorporation 0.001, 0.004, 0.01, 0.02, 0.04, 0.1, 0.2, 0.3, 1, 3, 10 µl (stock concentration 500 µl/ml; volume per plate 20 µl) with and without MA MA: Aroclor 1254 induced rat liver S9 Treatment time(s): 48 h incubation at 37 °C Vehicle: Water/ DMSO Negative control: Yes Positive control: Yes	**Results: Negative** (with and without metabolic activation) Cytotoxicity: No data available Precipitations: No data Controls: Valid	(Study Report, 1981a) ECHA's dissemination site: 007 (EDTMP-H)
Bacterial reverse mutation test Similar to OECD TG 471 Deviation: - only one strain tested GLP: Yes	**EDTMP-Na (CAS no. 22036-77-7, EC no. 244-742-5)** Purity: Not reported	**Supporting study** **(reliable with restriction)** **(together with Study Report (1981a): Key information on Ames test available)** Bacterial strains: *S. typhimurium* TA102 Test concentrations: 3, 10, 33, 100, 333, 1 000, 2 500, 5 000 µg/plate with and without MA MA: Phenobarbital/beta-Naphtoflavone induced rat liver S9 Treatment time(s): 1 h preincubation, 72 h incubation Vehicle: Water Negative control: Yes Positive control: Yes	**Results: Negative** (with and without metabolic activation) Cytotoxicity: ≥ 100 µg/plate Precipitations: No data available Controls: Valid	(Study Report, 2012) ECHA's dissemination site: 008 (EDTMP-H)
***In vitro* mammalian cell gene mutation test using the thymidine kinase gene (MLA test)**	**EDTMP-5Na (CAS no. 7651-99-2)** Purity: Not reported	**Key study** **(reliable with restrictions)** Cell culture: Mouse lymphoma L5178Y cells Test concentrations: 1 000, 1 571, 2 143, 2 714, 3 286, 3 857, 4 229, 5 000 µg/ml with and	**Results: Negative** (with and without metabolic activation) Cytotoxicity: No data Precipitation: No data	(Study Report, 1981b) ECHA's dissemination site:

Method, guideline, deviations if any	Test substance	Test substance, dose levels duration of exposure	Results	Reference
Similar to OECD TG 490 Deviations: - no detailed data on results (tables) GLP: Yes		without MA MA: Aroclor 1254 induced rat liver S9 Treatment time(s): 4 h at 37 °C, Sampling time: 2 d expression time Vehicle: Water Negative control: Yes Positive control: Yes	Controls: Valid	004 (EDTMP-Na)
***In vitro* mammalian cell gene mutation test using the Hprt gene)** Similar to OECD TG 476 **Deviations:** - only 3 concentrations tested - detailed result table not available GLP: Yes	**EDTMP-Na (CAS no. 22036-77-7, EC no. 244-742-5)** Purity: Not reported	**Supporting study (reliable with restrictions)** Cell culture: Chinese hamster ovary (CHO) cells Test concentrations: 1, 2, 5 mg/ml with and without MA MA: Aroclor 1254 induced rat liver S9 Justification for top concentration: Not needed Treatment time(s): 3 h Expression time: 7 - 9 d Vehicle: Sodium hydroxide solution (for neutralisation) Negative control: Yes Positive control: Yes, ethylmethanesulfonate, benz(a)pyrene	**Results: Negative** (with and without metabolic activation) Cytotoxicity: > 5 mg/ml Precipitation: No data reported Controls: Valid	(Study Report, 1986a) ECHA's dissemination site: 004 (EDTMP-H)
***In vitro* mammalian chromosome aberration test** Similar to OECD TG 473 Deviation: - harvesting time was too early for short term exposure (already at 3 or 6 h after beginning of treatment) - only 100 instead of 300 cells per culture screened - no justification for top concentration for short term exposure (MI not greatly reduced in lot B, only for	**EDTMP-H (CAS no. 1429-50-1; EC no. 215-851-5)** Purity: Not reported	**Disregarded study** **(not reliable to conclude on negative outcome)** Cell culture: Chinese hamster ovary (CHO) cells Test concentrations: *test 1*: 0, 30, 40, 50 µg/ml, *test 2*: 0, 100, 200, 500 µg/ml, with and without MA MA: Aroclor 1254 induced rat liver S9 Justification for top concentration: Cytotoxicity Treatment time(s): 3, 6, 12 h with and without MA Sampling time: directly after treatment of 3, 6 and 12 h with and without MA Vehicle: Growth medium Negative control: Yes Positive control: Yes, methylmethanesulfonate (without S9), Lot A positive control gave negative results	**Results: Negative** (with and without metabolic activation) Cytotoxicity: Yes for long-term exposure Precipitation: No data reported Controls: Valid	(Study Report, 1986a) ECHA's dissemination site: 005 (EDTMP-H)

Method, guideline, deviations if any	Test substance	Test substance, dose levels duration of exposure	Results	Reference
12 h harvest) GLP: Yes				

Table 8: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test Substance	Dose levels, duration of exposure	Results	Reference
***In vivo* mammalian somatic cell study: cytogenicity/ bone marrow chromosome aberration** Similar to OECD TG 475 Deviations: - only 50 cells per animal evaluated - repeated dosing (which normally is not foreseen for this test) - samples collected only once - no information if bone marrow was reached GLP: Yes	**EDTMP-H (EC no. 215-851-5)** Purity: Not reported	Disregarded study **(not reliable to conclude on negative outcome because of missing information on bone marrow exposure)** Species: Rat, Sprague-Dawley Number of animals per group: 5/sex/group Target organ(s): Bone marrow cells Administration route: Gavage Dose levels: 240, 800, 2 400 mg/kg bw Treatment time(s): Once daily for 5 consecutive days Sampling time(s): 20 h after last treatment colchicine treatment intraperitoneal (1 mg/kg) causing mitotic arrest; bone marrow cells collected 2 – 4 h afterwards Vehicle: Corn oil Negative control: Yes Positive control: Yes, methylmethanesulfonate	**Results: Negative** Animal toxicity and clinical signs: No toxicity observed Controls: Valid	(Study Report, 1981c) ECHA's dissemination site (EDTMP-H)

11.6.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

In vitro data related to induction of gene mutations

Bacterial reverse mutation tests:

For EDTMP-Na, two bacterial reverse mutation tests (Study Report (1981a) and Study Report (2012)) are reported which are considered reliable and, taken together, comprise key information for *in vitro* mutagenicity in bacteria. There were no indications for gene mutations in *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and TA 102 with and without metabolic activation.

In vitro mammalian cell gene mutation tests

There are two *in vitro* mammalian cell gene mutation tests available with EDTMP-Na, one based on the thymidine kinase gene (MLA test, Study Report (1981b)) and one based on the *Hprt* gene (HPRT test, Study

Report (1986a)). The tests were performed according to the respective OECD TGs and were GLP compliant and in spite of some deviations from the guidelines they are considered reliable. Both tests yielded negative results and did not indicate the induction of gene mutations in mammalian cells by EDTMP-Na.

To summarise, results from reliable *in vitro* gene mutation tests do no raise a concern for the induction of gene mutations in bacterial and mammalian cell systems with and without metabolic activation for EDTMP-Na.

In vitro data related to induction of chromosome aberrations

No *in vitro* cytogenicity study was available performed with EDTMP-Na. There is one negative *in vitro* mammalian chromosome aberration test available performed with EDTMP-H (Study Report, 1986a). However, the test has some major methodological deficiencies (e.g. harvesting time was too early for short term exposure - already at 3 or 6 h after beginning of treatment, only 100 instead of 300 cells per culture screened and no justification for top concentration given for short term exposure; see also Table 7). Even if the test result was negative and there was no hint for the induction of chromosomal aberrations in this test it cannot firmly be concluded that EDTMP-H and thus EDTMP-Na do not have the potential to induce chromosomal aberrations *in vitro* in mammalian cells (see deficiencies named for the test in Table 7).

In vivo data

One *in vivo* mammalian bone marrow chromosome aberration test is available performed with EDTMP-H (Study Report, 1981c), similar to OECD TG 475 and GLP compliant; it is the only *in vivo* cytogenicity study available. The test yielded negative results. However, because of several methodological deviations (such as only 50 cells per animal evaluated, repeated dosing - which normally is not foreseen for this test, samples collected only once) and as exposure to bone marrow (target tissue) has not been shown the negative test result is not considered reliable.
Thus, valid data is lacking to allow a firm assessment whether EDTMP-Na has the potential for induction of chromosome aberrations.

11.6.2 Comparison with the CLP criteria

As all available *in vitro* and *in vivo* mutagenicity tests either performed with EDTMP-Na or EDTMP-H yielded negative results, no concern is raised for EDTMP-Na to induce gene mutations or chromosome aberrations. Thus, classification for germ cell mutagenicity based on the available data is not warranted for EDTMP-Na.

However, whereas the available data are considered sufficient to conclude that EDTMP-Na does not induce gene mutations in bacteria and mammalian cell systems, valid data is missing to allow a firm assessment whether EDTMP-H has the potential for induction of chromosome aberrations at the time being. This is important as the observed reduced latency, high malignancy and metastasis of found osteosarcomas could hint to a genotoxic mode of action. Moreover, in a review by Broadhead et al. (2011) it is outlined that a number of chromosomal and genetic abnormalities have been linked to osteosarcoma.

11.6.3 Conclusion on classification and labelling for germ cell mutagenicity

As all available data for the hazard class germ cell mutagenicity are negative, classification for EDTMP-Na for germ cell mutagenicity is not warranted at the time being (data lacking).

11.7 Carcinogenicity

As available data for EDTMP-Na are considered not to be sufficient for carcinogenicity assessment, the data set was complemented using data from EDTMP-H ([ethane-1,2-diylbis[nitrilobis(methylene)]]tetrakisphosphonic acid; EC no. 215-851-5; CAS no. 1429-50-1). Justification for this read-across procedure is described in section 8 (Justification of read-across).

Carcinogenicity studies with EDTMP-H/-Na are available for the oral route only. There are no human data. Studies considered relevant are summarised in Table 9.

Table 9: Summary table of animal studies on carcinogenicity

<table>
<tr><th>Method, guideline, deviations if any</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Combined repeated dose and carcinogenicity

Similar to OECD TG 453

GLP: No information given in the available study report.</td>
<td>EDTMP-Na, EC no. 244-742-5 (EDTMP-H adjusted with sodium hydroxide to pH 7.0 - 7.4)

Purity: 96-97 %

Species: Rat, Sprague-Dawley

Number of animals per group: 60/sex/group

Administration route: Oral (gavage)

Dose levels: 0, 15, 50, 150 mg/kg bw/d (increased to 333 mg/kg bw on day 329 of study because expected increases in alkaline phosphatase had not occurred)

Treatment time: 94 – 107 weeks, daily, dosage was increased to 333 mg/kg bw on day 329 of study

Vehicle: Water

Post exposure period: 1 week

Dose level selected based on effects observed in 28-day dose range-finding study</td>
<td>Key study (reliable without restrictions)

Results: Carcinogenic in ♂ and ♀ Sprague-Dawley rats (increased incidence of osteosarcomas)

<u>Mortality, body weight and food consumption:</u>
- group mean body weights in high-dose ♂ significantly decreased from week 55 until termination
- increased mortality observed: See confidential annex

<u>Neoplastic effects:</u>
- osteosarcomas primarily of the long bones (tibia, femur and/or humerus) in both sexes, in the majority tumours originating from the epiphyseal plate of the long bones

<table>
<tr><th>Osteosarcoma</th><th>No. of animals with lesions / no. of animals in group</th></tr>
<tr><td>♂

HCI[a]: Mean 0.4 % (range 0 - 1.1 %) in 791 control ♂</td><td>0 mg/kg bw/d: 0/60 (0 %)
15 mg/kg bw/d: 0/60 (0 %)
50 mg/kg bw/d: 1/60[#] (1.7 %)
150/333 mg/kg bw/d: 28/60[*] (47 %)</td></tr>
<tr><td>♀

HCI[a]: Mean 0.1 % (0 - 1.1 %) in 790 control ♀</td><td>0 mg/kg bw/d: 0/60 (0 %)
15 mg/kg bw/d: 0/60 (0 %)
50 mg/kg bw/d: 0/60 (0 %)
150/333 mg/kg bw/d: 4/60[#] (7 %)</td></tr>
</table>
[*]statistically significant
[#]considered biologically significant by authors of the study (rare tumour type)
[a] historical control incidence from various laboratories; data provided by supplier of the rats for this study)

- highest incidence of osteosarcoma in tibia
- first palpable bone tumour evident in ♂ after 35 weeks and in ♀ after 43 weeks of treatment (before dosage of high-dose group was increased); most limb masses discovered between week 51 and 89
- metastasis in lungs, liver, regional lymph nodes, adrenals, kidneys and heart
- the authors of the study considered osteosarcomas to be the cause for morbidity or death for 20 of 28 tumour bearing male rats and all four tumour bearing female rats

<u>Non-neoplastic effects:</u>
- significantly increased incidence of metaphyseal osteosclerosis in femur, rib and sternum in high-dose ♂ and mid- and high-dose ♀ (see chapter 11.10)

(alkaline phosphatase level not significantly changed in this study; increases found in pilot study at 350 mg/kg bw/d, data unpublished)

Fluorescent bone labelling: Cortical and trabecular bone mass increased in treated ♂, trabecular bone mass in ♀</td>
<td>(Study Report, 1985)

ECHA’s dissemination site 001 (EDTMP-H)</td>
</tr>
</table>

Method, guideline, deviations if any	Test substance, dose levels duration of exposure	Results	Reference
Subchronic and chronic toxicity study, oral Similar to OECD TG 453 GLP: No	**EDTMP-H, EC no. 215-851-5** Purity: 97 % Species: Rat, Fischer 344 Number of animals per group: 50/sex/group Administration route: Oral (diet) Dose levels: 0, 4, 20, 100 mg/kg bw/d Treatment time: 118 – 122 weeks, daily Dose level selected based on effects observed in 13-week study	**Supporting study (reliable with restrictions)** **Results: Not carcinogenic in rats** ***Mortality, body weight and food consumption:*** - statistically significant increase in mortality in high-dose ♀ from week 119 - no significant changes in food consumption and body weights ***Neoplastic effects:*** - incidence of combined-pancreatic islet-cell adenomas and carcinomas significantly increased in high-dose ♀ (0/50, 2/50. 3/50, 5/50, 10 %) at study termination; the authors of the study considered them to be spontaneous age-related alterations and not to be treatment related. The authors considered the incidence in the controls as unusually low. The (mean) incidence was 4.4 % in 340 female Fischer rats from seven studies of the same laboratory (conducted from 1978 to 1982) ***Non-neoplastic effects:*** - no evidence of an increased incidence of islet-cell hyperplasia accompanying tumours in any treatment group - no adverse effects on calcium homeostasis, bone growth or bone morphology - no further evidence of toxicity	(Calvin et al., 1988)
Carcinogenicity, oral Similar to OECD TG 453 Deviation: - Co-administration of NaF GLP: Yes	**Mixture of EDTMP-Na, EC no. 244-742-5** (EDTMP-H adjusted with sodium hydroxide to pH 7.0 - 7.4) **and sodium fluoride** Purity: 94.4-96.7 % Species: Rat, Sprague-Dawley Number of animals per group: 45/sex in control group; 40/sex in treated groups Administration route: Oral (gavage) Dose levels: **EDTMP-Na**: 0, 15, 75, 150 mg/kg bw/d; **NaF** mixed each: 0, 1.139, 5.695, 11.390 mg/kg bw/d (analytical conc.) Treatment time: 2 years, daily Vehicle: Water	**Supporting study (reliable with restrictions)** **Results: Increased incidence of osteosarcoma in rats** ***Mortality, body weight and food consumption:*** - early death; growth and food intake affected: details see confidential annex - these observed effects are, according to authors of the study, most likely a consequence of the carcinogenic effect ***Neoplastic effects (only related to osteosarcoma)*** - increased incidence of osteosarcoma in mid- and high-dose group - detailed incidences see confidential annex ***Non-neoplastic effects (related to osteodystrophic and osteoproliferative changes)*** - increased bony limb masses in mid- and high-dose groups - enlargement of costochondral junctions and tissue masses of the appendage or bone - osteodystrophic changes of skeletal elements	(Study Report, 1986c) ECHA's dissemination site 003 (EDTMP-H)
Carcinogenicity, oral Similar to OECD	**EDTMP-Na, EC no. 244-742-5 (**EDTMP-H, adjusted with sodium hydroxide to pH 7.0 -	**Supporting study (reliable with restrictions)** **Results: Not carcinogenic in B6C3F1 mice**	(Study Report, 1986c)

Method, guideline, deviations if any	Test substance, dose levels duration of exposure	Results	Reference
TG 451 Deviation: - Limited parameters studied - 2 dose groups only GLP: Yes	7.4) Purity: 96 % Species: Mice, B6C3F1 Number of animals per group: 85/sex/group Administration route: Oral (gavage) Dose levels: 0, 15, 75 mg/kg bw/d Treatment time: 24 months, daily Vehicle: Water	***Mortality, body weight and food consumption:*** - no effect observed ***Neoplastic effects:*** - increased incidences (not statistically significant) of alveologenic adenoma in (see confidential annex for details) - benign tumour with high spontaneous incidences (in mice as high as 28% as stated in Study Report 1986d; in HCD of NTP (B6C3F1 mice, gavage, water) for alveolar adenoma: 19.33 %) ***Non-neoplastic effects:*** - statistically significant increase in fibrous osteodystrophy in ♀ (details: See confidential annex) - statistically significant increase in alkaline phosphatase levels in high-dose ♀ and ♂ at the 6 month interval (see confidential annex for details)	ECHA's dissemination site 002 (EDTMP-H)

In Study Report (1986c), EDTMP-H was co-administered with sodium fluoride (NaF) which itself was suspected to induce carcinogenicity to bone in humans. An oral carcinogenicity study using NaF performed by NTP revealed some equivocal evidence on its carcinogenic potential (NTP, 1990) (Table 10).

Table 10: Summary table of other studies relevant for carcinogenicity – carcinogenic potential of NaF

Method, guideline, deviations if any	Test substance, dose levels duration of exposure	Results	Reference
Carcinogenicity, oral Similar to OECD TG 451 GLP: Yes	**Sodium fluoride** (CAS no. 7681-49-4) Purity: > 99 % Species: **Rats, F344/N** Number of animals per group: 100/sex/group in control and high-dose; 70/sex/group in low- and mid-dose Administration route: Oral (drinking water) Dose levels: 0, 25, 100, 175 ppm (conversion factor for older rats 14; 0, 1.8, 7.1, 12.5 mg/kg bw) Treatment time: 2 years, daily	**Key study (reliable without restrictions)** **Results: Equivocal evidence in ♂ rats based on osteosarcoma, no evidence in ♀ rats** ***Survival, body weight and food consumption:*** Survival: ♂ (increasing dose levels): 42/80 (52.5 %), 25/51 (49 %), 23/50 (46 %), 42/80 (52.5 %) ♀ (increasing dose levels): 59/80 (73.7 %), 31/50 (62 %), 34/50 (68 %), 54/81 (66.7 %) ***Neoplastic effects:*** Osteosarcoma of bone: Malignant, one metastasised to the lung ♂ (increasing dose levels): 0/80 (0 %), 0/51 (0 %), 1/50 (2 %), 3/80 (4 %), ♀: None Historical control incidence: 37/6131 (0.6 %) ***Non-neoplastic lesions:*** ♂: Dentine dysplasia, degeneration of ameloblasts, attrition, deformity and discoloration of teeth ♀: Osteosclerosis, dentine dysplasia, degeneration of ameloblasts, attrition, deformity and discoloration of teeth	(NTP, 1990)
Carcinogenicity, oral Similar to OECD	**Sodium fluoride** (CAS no. 7681-49-4)	**Key study (reliable without restrictions)** **Results: No evidence of carcinogenic activity in ♂ and ♀**	(NTP, 1990)

Method, guideline, deviations if any	Test substance, dose levels duration of exposure	Results	Reference
TG 451 GLP: Yes	Purity: > 99 % Species: **Mice, B6C3F1** Number of animals per group: 100/sex/group in control and high-dose; 70/sex/group in low- and mid-dose Administration route: Oral (drinking water) Dose levels: 0, 25, 100, 175 ppm Treatment time: 2 years, daily	**mice**	
Carcinogenicity, oral Similar to OECD TG 451 GLP: No information	**Sodium fluoride** (CAS no. 7681-49-4) Purity: > 99% Species: **Rats, Sprague-Dawley** Number of animals per group: 70/sex/group Administration route: Oral (diet) Dose levels: 0, 4, 10, 25 mg/kg bw/d Treatment time: 99 weeks, daily	**Key study (reliable without restrictions)** **Results: No evidence of carcinogenic activity in ♂ and ♀ rats**	(Maurer et al., 1990)

11.7.1 Short summary and overall relevance of the provided information on carcinogenicity

There are four carcinogenicity studies available which were performed with either EDTMP-Na or EDTMP-H (Table 9). All four studies are oral life-time (two-years) studies with daily substance administration in rodents, three using rats (Study Report (1985), Calvin et al. (1988), Study Report (1986c)) and one using mice (Study Report, 1986c). Except for the study by (Calvin et al., 1988) test substance administration was performed using gavage. As the pH value in the test solutions of the oral gavage studies was adjusted with sodium hydroxide, the actual substance tested is EDTMP-Na in these studies. However, as discussed above, hydrolysis of EDTMP-H and EDTMP-Na takes place at neutral or physiological pH and exposure to the undissociated substance after oral application is considered to be less likely. Therefore, the test substance name is referred to as EDTMP-H or EDTMP-Na for the gavage studies whereas the general discussion refers to EDTMP-H/-NA.

Study Report (1985) in rats was judged to be the most relevant carcinogenicity study for EDTMP-H/-Na as the study was performed similar to OECD TG 453 covering a wide dose range and is comprehensively documented (available as study report).

In the following, all four available carcinogenicity studies are discussed and compared with the focus on observed neoplastic effects.

Carcinogenicity studies in rats

In Study Report (1985) male and female Sprague-Dawley rats were administered 0, 15, 50, 150 mg/kg bw EDTMP-Na for 94 – 107 weeks. On day 329 (week 47), the highest dose was increased to 333 mg/kg bw as the expected increase in alkaline phosphatase levels was not detected at 150 mg/kg bw/d. Alkaline phosphatase levels are considered to be as an indicator of altered bone metabolism and a tumour marker with high specificity to osteosarcoma in humans and animals (Kim et al., 2017). Interestingly, alkaline phosphatase levels were not significantly changed in the study at any of the dose levels tested. As mentioned by the authors of the study, increases were detected at a higher dose level (350 mg/kg bw/d) in a pilot oral 28-day study (no data available, cited in Study Report (1985)). Nevertheless, osteosarcomas originating from the epiphyseal plate of the long bones (tibia, femur and/or humerus) were observed in male and female animals. In male animals, the occurrence of osteosarcomas was concentration-dependent, at an incidence of 1.7 % (1/60) at the mid-dose and of 46.7 % (28/60) at the high-dose. At this dose level, the increase was statistically significant. In female animals, an incidence of 6.7 % (4/60) of osteosarcomas was observed only at the high-dose level. The first bone tumours were already noted after week 35 in males and after week 43 in females in the high-dose groups before this dosage was increased. The observed rates of osteosarcoma in male and female animals (47 % and 7 %) at the high-dose level clearly exceeded the historical control values[3] of 0.4 % in males (791 control animals) and 0.1 % in females (790 control animals). As osteosarcomas are a rare tumour type in SD rats, the one tumour in the mid-dose males and the four tumours in the high-dose females are considered biologically relevant and treatment related by the authors of the study even if not being statistically significant. This is supported by the DS. The location with the highest incidence of osteosarcomas was the tibia. Importantly, tumours metastasised to the lungs, liver, regional lymph nodes, adrenals, kidneys and heart. No other types of tumours have been observed. Corresponding with the reported occurrences of osteosarcoma, statistically significant increases of metaphyseal osteosclerosis in femur, rib and sternum were observed in high-dosed males and in females in the mid- and high- doses groups. Trabecular bone mass was found to be increased in treated males and females; in addition, increased cortical bone mass was observed in males. The authors of the study considered osteosarcoma as the cause for morbidity and death of female and male animals. As described above, the study was assessed to be of good quality and high relevance as performed similar to OECD TG 453 with the necessary number of female and male animals and covering a wider dose range (from 15 to 333 mg/kg bw/d). This study is considered a key study and the results are regarded as reliable without restrictions. Based on the observed neoplastic and non-neoplastic effects the increase of the high dose from 150 to 333 mg/kg bw/d over the course of the study is not considered to have interfered with the reliability of the study results. Nevertheless, this increase could have influenced the overall tumour incidences.

Another life-time toxicity study (Calvin et al., 1988) is available as a published journal article in which male and female F344 rats were orally dosed in diet with EDTMP-H at dose levels of 0, 4, 20 and 100 mg/kg bw/d. The study set-up was similar to OECD TG 451. Osteosarcomas were not reported in this study in either male or female animals. Instead, a statistically significant increased incidence (10 %) of combined-pancreatic islet-cell adenoma and carcinoma was observed in high-dose females, which was not considered to be treatment-related by the authors of the study. As the tumour's incidence in the controls was considered to be unusually low compared to the expected control values (5.4 % and 4.4 %, Calvin et al., 1988), and because of the identification of the tumours only upon study termination, along with the absence of an increased incidence of islet-cell hyperplasia in treated females, the authors considered the observed neoplasms to be spontaneous age-related alterations. This is supported by the DS mainly because the incidence of islet-cell hyperplasia in control females was high (14 %) (higher than high-dose females (4 %)). The study is considered to be reliable but of lower relevance compared to Study Report (1985). The chosen dose levels were relatively low (highest dose level was 100 mg/kg bw/d compared to 333 mg/kg bw/d in Study Report (1985), which could be an explanation why osteosarcomas were not observed. High incidences of osteosarcomas were observed only at the high-dose level of 150/333 mg/kg bw/d in Study Report (1985); at the lower dose of 50 mg/kg bw/d the incidence was low at 1.7 % in male animals only. Both studies were

[3] Data were provided by supplier of the SD rats to the authors of the study.

performed using different rat strains; Sprague-Dawley rats in Study Report (1985) and F344 rats in the study by Calvin et al. (1988). This could entail different sensitivities and be a reason why no increased incidences were observed at 100 mg/kg bw/d in the study by Calvin et al. (1988). Moreover, intestinal absorption from EDTMP-H in food is suggested to be lower because of the chelating ability of EDTMP. This is supported by toxicokinetic data, which indicate a lower absorption after feeding compared to gavage studies. Thus, actual (internal) dose levels could have been lower compared to the nominal dose levels. Therefore, both studies, Study Report (1985) and Calvin et al. (1988), are not judged to be contradictory.

There is another life-time carcinogenicity study available in which male and female Sprague-Dawley rats were orally administered to a mixture of EDTMP-Na and sodium fluoride (NaF) (Study Report, 1986c) Selected dose levels were 0, 15, 75, 150 mg/kg bw/d for EDTMP-Na and 0, 1.139, 5.695, 11.390 mg/kg bw/d (analytical conc.) NaF. Although being a well-conducted guideline study compliant to GLP, the study is judged to be of **low relevance** for the hazard assessment of EDTMP-H because of the administration of a **substance mixture**. Increased incidences of osteosarcomas were also observed in males and females (24 % and 11 % at the high-dose level). Moreover, non-neoplastic effects related to osteodystrophic and osteoproliferative changes such as increased bony limb masses were reported. NTP (1990) conducted an oral carcinogenicity study in which F344/N rats (Table 10) were administered low doses of solely NaF (0, 1.8, 7.1, 12.5 mg/kg bw/d), comparable to dose levels used in the co-administration study (Study Report, 1986c). From the study results, NTP (1990) concluded for NaF an equivocal evidence of osteosarcoma in male rats and no evidence in female rats. Nevertheless, in the 'NaF only' study osteosarcomas occurred in male animals in a dose-dependent manner, in the mid and high-dose groups (incidences of 2 % and 4 %, respectively), above the levels of historical control incidences. For female animals, no neoplastic effects were observed but osteosclerosis was found. Collectively, the bone tissue appears to be a target tissue for both EDTMP-Na and NaF, with similarities of the non-neoplastic osteodystrophic/proliferative effects and the tumour types observed in the two studies, suggesting similar mode of action between NaF and EDTMP-H related to altered bone metabolisms and the potential to induce osteosarcomas. In two further oral studies in rats (Maurer et al., 1990) and mice (NTP, 1990) with NaF (Table 10) no evidence of carcinogenic activity was found. Noting that the dose levels were low in the NaF studies no comprehensive assessment of the carcinogenic potential of NaF is therefore possible. Nevertheless, the results of the co-administration study (Study Report, 1986c) are considered to support the carcinogenic potential of EDTMP-H/-Na because of the high osteosarcoma incidences observed at EDTMP-Na dose levels of 75 and 100 mg/kg bw/d and the concurrent low NaF (5.7 and 11.4 mg/kg bw/d) dose levels.

Carcinogenicity studies in mice

There is one life-time oral carcinogenicity study in mice available for EDTMP-Na. Groups of 85 male and female B6C3F1 mice were administered 0, 15 and 75 mg/kg bw/d EDTMP-Na supplied daily for 24 months by gavage (Study Report, 1986c). As performed similar to OECD TG 451 the study is considered reliable but because only **two (low) dose levels** were employed, the relevance for prediction of the carcinogenic potential of the substance is considered limited. Increased incidences of alveologenic adenomas were observed in males (19 % and 16 %) in the 15 and 75 mg/kg bw/d dose groups, respectively, compared to 8 % in the controls. But as this increase was not statistically significant, nor related to the dose level, and since alveologenic adenomas are known to be a benign tumour with high spontaneous incidences in mice (as high as 28 %; (Study Report, 1986c)), the occurrence of these adenomas is not considered treatment-related. Other neoplastic effects including osteosarcomas were not observed. An increase in alkaline phosphatase levels, considered as an indicator of altered bone metabolism, was found in dosed females and high-dose males and a statistically significant increase of fibrous osteodystrophy in females (27 %, 46 %, 41 %, at 0, 15, 75 mg/kg bw/d, respectively) was identified. These findings indicate that the bone is a target tissue in mice (Study Report, 1986c).

Overall, under the experimental conditions of one life-time oral carcinogenicity study (Study Report, 1985) considered relevant and reliable the oral administration of EDTMP-Na led to increased incidences of osteosarcomas in male and female Sprague-Dawley rats. This is supported by a second oral study (Study Report, 1986c) in which EDTMP-Na co-administered with low doses of NaF also showed an increased incidence of osteosarcomas in male and female Sprague-Dawley rats. There was no evidence of

carcinogenicity in male and female B6C3F1 mice in an oral life-time study EDTMP-Na. All available carcinogenicity data are not judged to be contradictory to each other.

11.7.2 Comparison with the CLP criteria

According to the CLP Regulation for the purpose of classification as carcinogen, substances are allocated to one of three categories (Category 1A, Category 1B or Category 2) based on available data, strength of evidence and additional considerations.

According to Table 3.6.1 of the CLP regulation: *"a substance is classified in **Category 1** for carcinogenicity on the basis of epidemiological and/or animal data.*

Hereby, classification criteria for **Category 1A** (known or presumed human carcinogens) are as follows (Table 3.6.1): "*A substance …, known to have carcinogenic potential for humans, classification is largely based on human evidence,…".*

Substances are classified into **Category 1B** if there are animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (Table 3.6.1): "*A substance… presumed to have carcinogenic potential for humans, classification is largely based on animal evidence".*

Following Annex I (3.6.2.2.3, CLP Regulation) **sufficient evidence** of carcinogenicity in experimental animals is defined as: "*A causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.*"

If the strength of evidence in experimental animals can be evaluated as only limited, the placing of the substance in **Category 2** is foreseen.

Limited evidence of carcinogenicity in experimental animals is considered if (Annex I, 3.6.2.2.3, CLP Regulation): "*The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.*"

Human studies investigating the epidemiological evidence related to the carcinogenic potential of EDTMP-Na/-H are not available. **Hence, classification in Category 1A is not appropriate.**

An increased incidence of tumours (osteosarcoma) in both sexes of a single species (rats) has been observed in a well-conducted carcinogenicity study similar to OECD TG 451 (Study Report, 1985). In this study, a causal relationship has been established between EDTMP-Na and an increased incidence of malignant neoplasms (osteosarcomas). Osteosarcomas are rare spontaneous neoplasms in rats and in this study occurred at an incidences of 47 % in males and 7 % in females at a dose level of 150/333 mg/kg bw/d/ that. The findings of this study are supported by the results of a second study in the same species showing increased incidences of osteosarcomas at 75 and 150 mg/kg bw/d, in both sexes. The latter study, however, is judged to be of lower relevance because of co-administration with another substance (NaF).

There is no information available to the DS if the study (Study Report, 1985) was conducted under GLP. GLP is, however, no prerequisite (only ideally) to provide sufficient evidence.

Thus, the following criteria which are named for **sufficient evidence in animals are considered to be fulfilled**: "*An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.*"

The criteria for Category 2 were not fulfilled.

According to Annex I (3.6.2.2.3, CLP Regulation)

a) evidence of carcinogenicity is not restricted to a single experiment (Study Report, 1985; Study Report, 1986c),

b) there are no unresolved questions regarding the adequacy of the design, conduct or interpretation of the key study,

c) there are no uncertainties considering the neoplastic potential and

d) the study is not considered to demonstrate only promoting activity in a narrow range of tissues, as osteosarcomas were observed in many different types of bones and usually do not occur in control animals.

Consequently, available animal data are considered to provide sufficient evidence of carcinogenicity of EDTMP-Na in rats which warrants a Category 1B classification.

However, following Annex 3.6.2.2.4. (CLP Regulation) *beyond the determination of the strength of evidence for carcinogenicity from* animal studies*, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans.*

Thus, in the following, factors as described in section 3.6.2.2.6 of the CLP Regulation, are reflected to enable a conclusion on the overall likelihood whether EDTMP-**Na** also poses a carcinogenic hazard in humans and for the final decision of classification of the substance in **Category 1B or 2.**

a) Tumour type and background

In the oral life-time study in rats, osteosarcomas originating from the epiphyseal plate in long bones mainly tibia, femur and humerus were observed (Study Report, 1985). Osteosarcomas are a rare but known malignant tumour type in humans and are considered to be highly relevant to humans. Osteosarcomas are the most common bone tumour type in humans with an reported age standardised incidence per million persons per year of about 2.97 (95 % CI 2.59 - 3.35) (Eyre et al., 2010). Eyre and colleagues (Eyre et al., 2010) observed incidence peaks in the 15 - 29 age group which was considered by the authors to be consistent with other studies also reporting incidence of osteosarcoma after the onset of puberty, when young people are undergoing a growth spurt and bones experience rapid growth. A second incidence peak in the elderly has also been reported (Eyre et al., 2010; Savage and Mirabello, 2011).

Osteosarcomas are a rare tumour type in control rats, as historical control data showed incidences of only 0.4 % in control males and 0.1 % in control females (Study Report, 1985). These historical control data were directly provided by the supplier of the rats for this study and are considered highly relevant. In this life-time carcinogenicity study in rats EDTMP-Na (Study Report, 1985), exposure caused an increased incidence in osteosarcoma in both male and female animals. In males osteosarcomas occurred dose-dependently and reached statistical significance at the highest dose level (150/333 mg/kg bw; incidence: 28/60, 47 %). Lower incidences in mid-dose males (1/60; 1.7 %) and high-dose females (4/60; 6.7 %) are also considered biologically relevant because of increased incidence of the same tumour type at higher doses and as no other neoplasms (in different tissues) were observed.

Therefore, based on the observed type of tumour and low background incidences the carcinogenic evidence is considered to be biologically relevant for humans and the available information is not considered sufficient to downgrade a classification from Category 1B to Category 2.

b) Multi-site responses

The only observed treatment-related tumour type following EDTMP-Na administration is osteosarcoma. Osteosarcomas were detected at different sites of long bones including tibia, femur and humerus. In addition, metastasis to the lungs, liver, regional lymph nodes, adrenals, kidneys and heart was observed (Study Report, 1985). In line with the postulated (non-mutagenic) carcinogenic mode of action (as described in section j "mode of action and its relevance for humans") related to bone metabolism, induction of other types of tumours is not expected for EDTMP-Na. Thus, available information regarding 'multi-site responses' is not considered an issue to downgrade a classification from Category 1B to Category 2.

c) Progression of lesions to malignancy

The observed osteosarcoma is a malignant tumours. Malignant tumours usually constitute sufficient evidence of carcinogenicity supporting Category 1B (rather than Category 2). Moreover, metastasis in different tissues such as lungs, liver, regional lymph nodes, adrenals, kidneys and heart were found underlining the malignant potential of the sarcoma observed.

d) Reduced tumour latency

The reported latency for tumour development was quite short. Bone tumours were first evident after 35 weeks in males and 43 weeks in females. This adds to the weight of evidence for the carcinogenic potential of the substance and supports a Category 1B classification.

e) Whether responses are in single or both sexes

Even if female animals seem to be less sensitive, increased tumour incidences compared to controls were found in both sexes. In male animals, osteosarcomas occurred in a concentration-dependent manner, at an incidence of 1.7 % (1/60) in the mid-dose group and a higher incidence of 47 % (28/60) in the high-dose group. At the highest dose level, the increase was statistically significant. In female animals, osteosarcomas were observed only at the highest dose level and at a lower incidence of 7 % (4/60). As osteosarcomas are a very rare tumour type in SD rats, the one tumour in the mid-dose group of males and the four tumours in the high-dose group of females were considered biologically relevant and treatment-related by the authors of the study even if not statistically significant. This is supported by the DS. The observed sex-related difference could be explained by higher bone turnover rates and faster growth in male compared to female animals as osteosarcomas mainly occurred in the distal femur and proximal tibia associated with intensive growth rates.

The observed osteosarcomas in both sexes support that data provide sufficient evidence for animal carcinogenicity (Category 1B).

f) Whether responses are in a single species or several species

Next to the several carcinogenicity studies in rats, there exists one carcinogenicity study performed with mice (Study Report, 1986d). At dose levels of up to 75 mg/kg bw/d EDTMP-Na no carcinogenic activity was observed. Information on carcinogenic effects in mice are lacking for higher dose levels up to the MTD level. However, the evidence of non-neoplastic effects in bones in mice comparable to those seen in rats indicates that species other than rats may also sensitive to bone tumour development. There are no further carcinogenicity studies available with EDTMP-H/-Na in other species than rats and mice.

As already described above, a single study in one species might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour and age at onset, which is considered to be fulfilled by the carcinogenicity study in rats (Study Report, 1985).

Thus, the fact that sufficiently reliable carcinogenicity data are only available for one species (rat) is not considered to be sufficient to downgrade a classification from Category 1B to Category 2.

g) Routes of exposure

Increased tumour incidences compared to controls in rats were observed after oral substance administration generally considered a relevant route of exposure. Dermal and inhalation carcinogenicity studies are not available for EDTMP-Na. Hence, from the available set of data it is not conclusively proven that no other than the oral route could cause the hazard.

h) Comparison of absorption, distribution, metabolism and excretion between test animals and humans

No data on absorption, distribution, metabolism and excretion of EDTMP-Na are available for humans. Thus, no conclusion can be drawn whether a direct comparison would modify the carcinogenic concern for humans. By default and in the absence of appropriate data, toxicokinetic behaviour is assumed to be similar in animals and humans at least from a qualitative perspective. From the TK data available for rats, no reasons can be identified why this should be different for EDTMP-Na. Thus, available information is not sufficient to downgrade a classification from Category 1B to Category 2.

i) The possibility of a confounding effect of excessive toxicity at test doses

At the highest tested dose-level in the carcinogenicity study in rats (Study Report, 1985) group mean body weights significantly decreased from week 55 until termination in male animals. Moreover, a significant increase in mortality in high-dose males from week 64 onwards was reported. These effects were not observed in high-dose female animals. Bone tumours were first evident after 35 weeks where a reduction in body weight or increased mortality rate were not observed. Thus, the observed osteosarcomas are interpreted as the cause and not a consequence of the decrease in body weights and higher mortality rate. The authors of Study Report (1985) also considered osteosarcoma the cause of morbidity or death for 20 of 28 tumour bearing male rats and all four tumour bearing female rats. Moreover, osteosarcomas are a rare tumour type and do not belong to common, spontaneously occurring tumours (only 0.4 % in control males and 0.1 % in control females). Thus, observed (systemic/non-specific) toxicity is not considered a confounding effect and there is no reason to downgrade the classification from Category 1B to Category 2.

j) Mode of action and its relevance for humans

Based on the available standard *in vitro* and *in vivo* genotoxicity data for EDTMP-H/Na a genotoxic mechanism for the induction of osteosarcoma has not been demonstrated (see section 11.611.6 for details). However, because of missing valid data related to the potential for induction of chromosome aberrations uncertainty remains. In a review by Broadhead et al. (2011), it is outlined that a number of chromosomal and genetic abnormalities have been linked to osteosarcomas. In addition, the observed reduced latency, high malignancy and metastasis of osteosarcomas could point towards a possible contribution of genotoxic events/activity.

From *in vivo* toxicokinetic studies in mice and rats, a high affinity to bone and long half-life times in bone were observed for EDTMP-H after oral and i.v. substance administration showing a “bone seeking” property of EDTMP-H. For this property, EDTMP-H radionuclide derivates such as ^{153}Sm-EDTMP and ^{177}Lu-EDTMP are used as bone-seeking radiopharmaceuticals in bone pain palliation therapy.

Bisphosphonates, a chemical group sharing the bone-seeking properties of EDTMP, are known to influence the balance of bone homeostasis. It is assumed that they lead to an inhibitory effect on bone resorption. This knowledge is based on bisphosphonate pharmaceutical drugs (such as etidronic acid) used to treat osteoporosis in humans (Lewiecki, 2011).

The shift of bone homeostasis towards accelerated bone generation could be a postulated mechanism for EDTMP-Na to induce osteosarcomas in rats which is highly relevant also for humans. This mode of action is supported by the fact that no other treatment-related tumour types were found and by the non-neoplastic findings observed after long-term administration of EDTMP-H/-Na in rats and mice related to increased bone masses. A significant increased incidence of metaphyseal osteosclerosis was observed in male and female rats after repeated dose application (Study Report, 1985). In female mice, a statistically significant fibrous osteodystrophy was found after repeated exposure. Moreover, an increase in alkaline phosphatase (ALP) levels was detected in exposed female and male mice (Study Report, 1986c). ALP is considered a marker of bone formation and was found to be a valuable tumour marker with high specificity to

osteosarcoma in patients. Even if ALP levels were not significantly changed in Study Report (1985) in rats at all the tested dose levels, increases were detected at higher dose levels (350 mg/kg bw/d) in a pilot oral 28-day study (mentioned by authors in Study Report (1985), data not available).

According to Savage and Mirabello (2011), the contribution of environmental exposures in the induction of osteosarcoma in children and young adults is not known because of the heterogeneity and relative rarity of these cancers. However, the authors concluded that it is likely that a combination of environmental exposure and genetic risk factors might contribute to cancer risk. Broadhead et al. (2011) also mention chemical agents as being linked to osteosarcoma formation.

Overall, no specific mode of action has been demonstrated and because a rat-specific one cannot be verified it is concluded that the bone tumours observed in rats are also relevant for humans.

A compilation of all those factors taken into consideration and discussed above for the carcinogenicity assessment of EDTMP-H/-Na is shown in Table 11.

Table 11: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	**Tumour type and background incidence**	**Multi-site responses**	**Progression of lesions to malignancy**	**Reduced tumour latency**	**Responses in single or both sexes**	**Confounding effect by excessive toxicity?**	**Route of exposure**	**MoA and relevance to humans**
Rats, Sprague-Dawley, ♂ and ♀	Treatment related increased incidence of osteosarcoma; incidence high above historical control incidence	No; osteosarcoma as the only treatment related tumour type observed	Osteosarcoma = malignant tumours; metastases identified in several tissues	Yes; first tumours evident after 35 weeks (♂)	Osteosarcoma identified in ♂ **and** ♀**,** with higher incidences in ♂	No	Studies performed with oral (**gavage**) application	Mode of action: unknown, possible contributions by inhibitory effects on bone resorption Target tissue and mechanistic elements relevant to humans

In summary, the available data for EDTMP-H/Na are sufficient to allow a substantiated evaluation of the carcinogenic potential of that EDTMP-Na. The criteria mentioned in section 3.6.2. (CLP Regulation) are fulfilled to conclude a sufficient evidence of carcinogenicity for EDTMP-Na in animals. The carcinogenic potential of EDTMP for the oral route of exposure was demonstrated in male and female Sprague-Dawley rats, in a well conducted life-time carcinogenicity study. Osteosarcomas occurred to an unusual high degree with regard to incidence, site, type of tumour, and at an early time of onset.

Several factors were considered to assess the overall concern of EDTMP-Na to induce osteosarcoma in humans. In conclusion, based on these considerations, weight was added to the likelihood that EDTMP-Na also poses a carcinogenic hazard in humans and that available information is not considered sufficient to downgrade classification from Category 1B to Category 2. The fact that osteosarcomas are malignant tumours that occur in humans, that no confounding effects of excessive toxicity were identified and that the underlying mechanistic elements were not identified was also considered. In conclusion, these tumours are considered relevant for humans.

11.7.3 Conclusion on classification and labelling for carcinogenicity

Based on all information available, and considering the criteria indicative of evidence according to Annex I (3.6.2.2.3, CLP Regulation)

a) evidence of carcinogenicity was observed in two rat studies (Study Report, 1985; Study Report, 1986d),

b) the studies were performed similarly to relevant OECD TG and in some cases were GLP compliant,

c) osteosarcoma is a rare tumour and in one study the incidence reached statistical significance at the high dose,

d) osteosarcomas were observed at several sites of bones and usually do not occur in control animals (very low incidence in historical controls),

a classification of EDTMP-Na as **Carc. 1B (H350)** is warranted.

Specific concentration limits for Category 1 carcinogens:

To decide on the setting of a specific concentration limit for EDTMP-Na, a T25 value was determined according to EC (1999) as a measure for the intrinsic carcinogenic potency of EDTMP-Na. The T25 value estimates the dose level in chronic studies at which particular neoplastic lesions occur in 25 % of the animals of a dose group. For the calculation of the T25 value, a linear relationship between potency and administered dose is assumed. The T25 value was calculated for the statistically significant treatment-related incidences of osteosarcomas in male rats based on Study Report (1985). As the initial daily dose of 150 mg/kg bw/d was increased to 333 mg/kg bw/d at day 329 in the study, an average dose (AD) level was estimated (Table 12) for the T25 calculation.

Table 12: Calculation of T25 value for osteosarcomas in male rats (Study Report, 1985)

Lesion	**Osteosarcoma**			
Dose (mg/kg bw/d)	0	15	50	253[a] (AD)
Exposure (days/week)	7	7	7	7
Number of animals	60	60	60	60
Incidences	0	0	1	28
Incidence (%)	0	0	2	47
T25				**135**[*]

[a]**Average dose (AD):** (328*150 mg/kg bw/d + 421* 333 mg/kg bw/d)/ (749 d) = **253 mg/kg bw/d** (increase of the dose level at day 329); duration of study calculated as follows: 107 weeks * 7

[*]**Calculations according to Dybing et al. (1997): T25 (mg/kg bw/day) = (average daily dose) * (25 / Net incidence (%)**

Based on the key study (Study Report, 1985), the estimated **T25** dose descriptor in rats is **135 mg/kg bw/d.** As the T25 value is > 100 mg/kg bw/d EDTMP-H can be considered as a carcinogen of **low potency**.

Based on the numerical T25 value alone, an SCL of 1.0 % would be justified. In the following, additional potency elements are considered to understand whether a change in potency class might be appropriate (see also Table 14).

First of all, there is uncertainty because of the available supporting carcinogenicity study with mixed treatment of EDTMP-Na and NaF (Study Report, 1986c). T25 values calculated based on results of this

study are more than two-fold lower compared to the key study: 50 resp. 62.5 mg/kg bw/d (calculation see Table 13). However, as NaF treatment itself leads to an increased incidence of osteosarcoma in male F344/N rats (NTP, 1990), the contribution of NaF to the observed total tumour incidence in the mixed rat study (Study Report, 1986c) is uncertain.

Table 13: Calculation of a T25 value for observed osteosarcoma in male rats in the co-administration study of EDTMP-Na and NaF (Study Report, 1986c)

Lesion	**Osteosarcoma**			
Dose (mg/kg bw/d)	0	15	75	150
Exposure (days/week)	7	7	7	7
Number of animals	45	40	40	40
Incidences	0	0	15	24
Incidence (%)	0	0	37.5	60
T25			**50**	**62.5***

***Calculations according to Dybing et al. (1997): T25 (mg/kg bw/day) = (average daily dose) * (25 / Net incidence (%)**

Moreover, evidence of high malignancy, metastasis, and a short latency period may be considered as suggestive a possible contribution of genotoxic actions. However, all available *in vitro* and *in vivo* genotoxicity studies are negative and there is no indication of mutagenic action from the available genotoxicity information.

Table 14: Potency elements which affect the classification

T25 in human studies	**T25 in animal studies**	**Dose-response relationships**	**Site/species/ strain/gender activity and degree of malignancy**	**Genotoxicity**	**Mechanistic relevance to humans**	**Toxi co-kinetics**	**Other elements relevant to potency classification**	**Changes in potency class**	**Allocation of potency class**
NA[a]	135 mg/kg bw/d (mixture study EDTMP and NaF: T25 is 50 resp. 62.5 mg/kg bw/d)	Yes no tumours at the lowest tested dose level (15 mg/kg bw/d) and only one tumour at the medium dose level (50 mg/kg bw/d)	SA[b]	No evidence on genotoxic action, (however, high rate of malignancy (100 % of bone tumours), metastases and short latency period could point to genotoxic action)	SA[b]	SA[b]	Unknown MoA,	Yes	**Low (based on the T25 dose level alone)**

[a]NA: Not applicable
[b]SA: Starting assumption

Thus, the uncertainties in the additional potency elements are regarded to be high and are not regarded to give solid reasoning to deviate from the SCL of 1.0 % based on the T25 of **135 mg/kg bw/d**. **For this reason, an SCL of 1.0 % is supported.**

11.8 Reproductive toxicity

Not assessed in this dossier.

11.9 Specific target organ toxicity-single exposure

Not assessed in this dossier.

11.10 Specific target organ toxicity-repeated exposure

As available data for EDTMP-Na are considered not to be sufficient for assessment for specific target organ toxicity (repeated exposure), the data set was complemented using tests performed with EDTMP-H. Justification for this read-across procedure is described in section 8 (Justification of read-across).

Table 15: Summary table of animal studies on STOT RE via oral route

Method, guideline, deviations if any	Test substance, dose levels duration of exposure	Results	Reference
Combined repeated dose and carcinogenicity Similar to OECD TG 453 GLP: No information given in the available study report.	**EDTMP-Na, EC no. 244-742-5** (EDTMP-H adjusted with sodium hydroxide to pH 7.0 - 7.4) Purity: 96-97 % Species: Rat, Sprague-Dawley Number of animals per group: 60/sex/group Administration route: Oral (gavage) Dose levels: 0, 15, 50, 150 mg/kg bw (increased to 333 mg/kg bw on day 329 of study because expected increases in alkaline phosphatase had not occurred) Treatment time: 94 – 107 weeks, daily, dosage was increased to 333 mg/kg bw on day 329 of study Post exposure period: 1 week Dose level selected based on effects observed in a 28-day dose range-finding study	**Key study (reliable without restrictions)** **NOAEL: 15 mg/kg bw/d** **LOAEL: 50 mg/kg bw/d** (based on metaphyseal osteosclerosis) ***Mortality, body weight and food consumption:*** - significant increase in mortality in high-dose ♂ from week 64 onwards and in high-dose ♀ from month 18 - group mean body weights in high-dose ♂ significantly decreased from week 55 until termination ***Neoplastic effects:*** - see chapter 11.7 ***Non-neoplastic effects:*** - organ weight, haematology, clinical chemistry, urine analysis: No effects observed - metaphyseal osteosclerosis significantly increased in the femur, rib and sternum of males at the high-dose level and in the females at the mid- and high-dose levels. (See confidential annex for details.)	(Study Report, 1985) ECHA's dissemination site 001 (EDTMP-H)
Carcinogenicity, oral Similar to OECD TG 451 Deviation: Limited parameters 2 dose groups only GLP: Yes	**EDTMP-Na, EC no. 244-742-5** (EDTMP-H adjusted with sodium hydroxide to pH 7.0 - 7.4) Purity: 96 % **Species: Mice, B6C3F1** Number of animals per group: 85/sex/group Administration route: Oral (gavage) Dose levels: 0, 15, 75 mg/kg bw Treatment time: 24 months, daily	**Supporting study (reliable with restrictions)** **Results:** **LOAEL: 15 mg/kg bw (**based fibrous osteodystrophy in ♀) ***Mortality, body weight and food consumption:*** - no effect observed ***Neoplastic effects:*** - see chapter 11.7 ***Non-neoplastic effects:*** - organ weight, haematology, clinical chemistry, urine analysis: No effect observed	(Study Report, 1986c) ECHA's dissemination site 002 (EDTMP-H)

Method, guideline, deviations if any	Test substance, dose levels duration of exposure	Results	Reference
		- statistically significant increase in fibrous osteodystrophy in ♀ (see confidential annex for details) - increase in alkaline phosphatase in ♀ and in high-dose ♂	
Subchronic and Chronic toxicity study, oral Similar to OECD TG 453 GLP: No	**EDTMP-H, EC no. 215-851-5** Purity: 97 % Species: Rat, Fischer 344 Number of animals per group: 50/sex/group Administration route: Oral (diet) Dose levels: 0, 4, 20, 100 mg/kg bw/d Treatment time: 118 – 122 weeks, daily Dose level selected based on effects observed in 13-week study	**Supporting study (reliable with restrictions)** **NOAEL: 20 mg/kg bw/d** **LOAEL: 100 mg/kg bw/d** (based on statistically significant increase in mortality in high-dose ♀) ***Mortality, body weight and food consumption:*** - statistically significant increase in mortality in high-dose ♀ from week 119 - no significant changes in food consumption and body weights ***Neoplastic effects:*** - incidence of combined-pancreatic islet-cell adenomas and carcinomas increased in high-dose ♀ but not considered to be treatment related ***Non-neoplastic effects:*** - no adverse effects on calcium homeostasis, bone growth or bone morphology - no further evidence of toxicity	(Calvin et al., 1988)
Subchronic non-guideline repeated dose study in dogs Principle of the study: Investigation restricted to rib biopsies, measurement targeted to certain bone parameters; determination of the bone formation rate not possible	EDITEMPA *(N N N N*-ethylenediaminetetra(methylene phosphonic acid)) **co-administration** of tetracycline-HCl **(no CAS no. or EC no. given!)** Purity: No data Species: ♀ beagles Number of animals per group 5/sex/group Administration route: Oral (feed) Dose levels: 0, 2, 50, 100, 200 mg/kg bw/d (in aqueous solution) Treatment time: 26 weeks	**Supporting study (targeted to investigation of some bone parameters)** **NOEL: 2 mg/kg bw/d** **LOEL: 50 mg/kg bw/d** (based on changed bone parameters) - at 100 and 200 mg/kg bw/d statistically significant differences in bone parameters compared to controls such as osseous tissue porosity, osteoid seams per area, percentage osteoid, osteoid seam width, specific osteoid surface - some of these effects also evident at 50 mg/kg bw/d - according to authors of the study the dominant toxic effect noted was accumulation of osteoid in the forming osteons of cortical bone > 50 mg/kg bw/d	(Jee et al., 1988)

11.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Human evidence for specific target organ toxicity caused by repeated exposure of EDTMP-H/-Na is not available.

Pertinent information for the purpose of hazard characterisation of EDTMP-Na can be obtained from studies on EDTMP-H/-Na in animals. The most appropriate information can be derived from two chronic repeated dose oral toxicity studies performed similar to OECD TG 451 or OECD TG 453, respectively. These studies are a two-year repeated dose and carcinogenicity study in rats (Study Report, 1985) and a two-year carcinogenicity GLP-compliant study in mice (Study Report, 1986c), both performed using gavage substance

administration. Furthermore, there are two non-guideline repeated dose feeding studies available, namely a chronic toxicity study in rats (Calvin et al., 1988) and a sub-chronic repeated dose study in female beagles (Jee et al., 1988).

There are no repeated dose studies with other than the oral substance administration route available for EDTMP-H/-Na.

In the **two-year carcinogenicity guideline study in rats,** the bone was considered the primary target organ of EDTMP-Na administration. Statistically significant effects on the bone structure such as metaphyseal osteosclerosis in femur, rib and sternum in both male (≥ 150/333 mg/kg bw/d) and female (≥ 50 mg/kg bw/d animals were observed. At the highest dose level of 150/333 mg/kg bw/d nearly all treated animals were affected. Moreover, fluorescent bone labelling revealed increased trabecular bone mass in males and females and increased cortical bone mass in males. The **LOAEL** of the study was considered to be **50 mg/kg bw/d** based on the observed metaphyseal osteosclerosis.

Also in the guideline conforming **two-year carcinogenicity study in mice,** the bone was found to be the target organ of EDTMP-Na administration. A statistically significant increase in fibrous osteodystrophy compared to control animals was observed in female animals ≥ 15 mg/kg bw/d. Moreover, an increase in alkaline phosphatase in females and in high-dose males was detected, which is a sign for altered bone metabolism. The **LOAEL** of the study was considered to be **15 mg/kg bw/d** based on the observed osteodystrophy.

Whereas in the **chronic non-guideline feeding study in rats** no adverse effects of EDTMP-H on bone growth or bone morphology were found up to 100 mg/kg bw/d **(Calvin et al., 1988)**, in the **sub-chronic non-guideline feeding study in dogs** (Jee et al., 1988) statistically significant differences compared to controls in bone parameters such as osseous tissue porosity, osteoid seams per area and specific osteoid surface were detected at ≥ 100 mg/kg bw/d. Some of these effects were also evident at 50 mg/kg bw/d. According to the authors of the study the dominant toxic effect noted was the accumulation of osteoids in the forming osteons of cortical bone because of impaired or delayed mineralisation of bone. It was speculated whether the observed effects were signs of increased activation of bone remodelling. The findings of this study underline that bone is the target organ after EDTMP-H administration in dogs.

Overall, from the available repeated dose studies it can be concluded that the bone is the target organ after oral EDTMP-H/-Na administration in rats, mice and dogs.

In the following the LOAELs observed in the two-year guideline studies in rats and mice are extrapolated to a 90-day exposure (see Table 16).

Table 16: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study (Category 1)	Classification supported by the study (Category 2)
(Study Report, 1985) (rats)	LOAEL 50 mg/kg bw/d	94 – 107 weeks	Ca. 400 mg/kg bw/d	No classification (> 10[#] mg/kg bw/d)	No classification (> 100[#] mg/kg bw/d)
(Study Report, 1986c) (mice)	LOAEL 15 mg/kg bw	24 months	Ca. 120 mg/kg bw	No classification (> 10[#] mg/kg bw/d)	No classification (> 100[#] mg/kg bw/d)

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study (Category 1)	Classification supported by the study (Category 2)
[#] Guidance values as given in table 3.9.2 and 3.9.3 of the CLP Regulation (1272/2008)					

11.10.2 Comparison with the CLP criteria

"Target organ toxicity (repeated exposure) means specific, target organ toxicity arising from a repeated exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included." (CLP Regulation 1272/2008, 3.9.1.1.)

The following two hazard categories are differentiated:

Category 1 (STOT RE1):
"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. 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. Guidance dose/ concentration values are provided below (see 3.9.2.9), to be used as part of a weight-of- evidence evaluation."

Category 2 (STOT RE 2):
"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. Guidance dose/concentration values are provided below (see 3.9.2.9) in order to help in classification.

The respective guidance dose values related to sub-chronic (90 d) oral exposure for the two categories are as follows:

STOT RE 1: C ≤ 10 mg/kg bw/d

STOT RE 2: 10 ≤ C ≤ 100 mg/kg bw/d

Whereas the observed adverse bone effects in rats and mice are considered relevant for human health, the derived effective dose levels, if extrapolated to a sub-chronic 90-day exposure, are above the guidance dose values relevant for classification as STOT RE 1 and STOT RE 2 (see Table 16).

Therefore, classification as STOT RE is not considered to be warranted for EDTMP-Na.

11.10.3 Conclusion on classification and labelling for STOT RE

Severe effects mainly related to the bone have been observed after chronic oral EDTMP-H/-Na administration in rodents. However, effective dose levels (LOAELs) are above the guidance values that would warrant classification as STOT RE 1 or 2. Therefore, no classification as STOT RE is proposed for EDTMP-Na.

11.11 Aspiration hazard

Not assessed in this dossier.

12 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed in this dossier.

13 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier.

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