



Should DTPA, an Aminocarboxylic acid (ethylenediamine-based) chelating agent, be considered a developmental toxicant?

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ABSTRACT

Aminocarboxylic acid (ethylenediamine-based) chelating agents, such as DTPA and EDTA, are widely used in a variety of products and processes. Recently the European RAC proposed to classify DTPA as a developmental toxicant Category 1B according to CLP.

This paper provides unequivocal and significant evidence that developmental effects cannot be considered an intrinsic property of the chelating substances themselves since: (1) animals fed a zinc deficient diet during gestation exhibit developmental toxicity of a similar nature and severity to that observed in studies involving such chelates, (2) sufficient supplementation of zinc in the diet, or administration of zinc bound chelates, completely negates the developmental effects. Moreover, the bioavailability of DTPA is very low with > 95% of oral doses excreted unchanged via the feces within 24 h. If DTPA would possess the intrinsic property to be developmentally toxic, simple zinc supplementation should not be sufficient to negate these effects. Furthermore, the relevance of classification is highly questionable since worker or consumer exposure could not lead to a scenario whereby sufficient zinc deficiency would manifest itself. Therefore classification of DTPA for such effects is not protective of human health; instead it leads to onerous and disproportionate restrictions being placed on this substance.

1. Introduction

Diethylene Triamine Penta Acetic acid (DTPA) is a metal complexing (chelating) compound part of the Aminocarboxylic acid (ethylenediamine-based) group. Other members of this group are Ethylene Diamine Tetra Acetic acid (EDTA) and Hydroxy Ethylene Diamine Tetra Acetic acid (HEDTA). The diethylenetriamine structure contains five acetic acid groups, the ethylenediamine structure has four of such groups whereas the hydroxyethylenediamine structure has three acetic acid groups due to the fact that one acetic acid group is substituted with a 2-hydroxyethyl group. These chelating agents are used to bind multivalent ions and have been used in a wide variety of industrial and consumer applications for many years. Pulp and paper, household and industrial cleaning, chemical processing, agriculture and water treatment constitute 80% of their consumption. Textile industry, metal working, and photography are other areas of application of these chelants. Some of these chelating agents are authorized as food additives as enhancers for oral uptake of essential metal ions (e.g. iron), in medicine as carriers for contrast media (e.g. gadolinium or technetium-99) or as

pharmaceutical ingredients for treatment of heavy-metal intoxication.

Recently the European Risk Assessment Committee (RAC) proposed to classify DTPA as a developmental toxicant Category 1B according to the Classification, Labelling and Packaging (CLP) regulation. Essentially, RAC considered that three DTPA substances (H5-DTPA, Na5-DTPA and K5-DTPA (CAS nos 67-43-6, 140-01-2, and 7216-95-7, respectively) should be classified as toxic for development category 1B “based on serious malformations and other developmental effects (retardation) observed also in lower doses, above historical controls and without severe maternal toxicity in a developmental toxicity study in rats with DTPA-Na5 (key study), supported by effects in two additional studies in rats and mice showing a consistent developmental toxicity”. In particular, RAC stated in their opinion that “there are adequate data to support the hypothesis that the teratogenicity resulting from dosing of rats with DTPA salts is a result of an induced deficiency of zinc and presumably also other divalent cations in the mother which subsequently impacts the fetus”. Thus, while RAC acknowledged that developmental effects were the **subsequent** result of the zinc deficiency in the pregnant mothers, it did not consider those effects to be secondary to maternal toxicity or to other

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Abbreviations

CLP	Classification, Labelling and Packaging
DTPA	Diethylene Triamine Penta Acetic acid
EDTA	Ethylene Diamine Tetra Acetic acid
GHS	Global Harmonized System (of Classification and Labelling of Chemicals)
GIT	Gastro Intestinal Tract

HEDTA	Hydroxy Ethylene Diamine Tetra Acetic acid
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
OECD SIDS	Organisation for Economic Co-operation and Development – Screening Information Data Set
RAC	Risk Assessment Committee
RDA	Recommended Daily Allowance

non-specific toxic effects.

The aim of this review is to explore whether the RAC proposal for classification as Category 1B is appropriate scientifically as well as proportionally as article 1 of the CLP Regulation (1272/2008) states that the purpose of this regulation is to ensure a high level of protection of human health and the environment. With that objective in mind it is important to consider how this classification is defined in the CLP regulation and its guidance.

In the following sections a brief overview will be given on chelating constants of the Aminocarboxylic acid (ethylenediamine based) chelating agents DTPA and EDTA to indicate ‘potency’ for metal binding, bioavailability, animal and human toxicity data, and possible exposure to DTPA. As there is limited toxicity data available for HEDTA, this chelating agent is not further included. The commentary will conclude with a discussion on the approach available for the classification of chemicals that have the ability to cause developmental toxicity via an indirect, secondary and non-specific mechanism.

2. Metal binding capacity by chelating agents

DTPA and EDTA predominantly bind di- and trivalent metal ions including zinc, iron, manganese, and calcium (Tables 1 and 2). DTPA and EDTA bind more strongly to zinc compared to e.g. calcium; they have very low affinity for monovalent ions including potassium and sodium. Therefore, in biological matrices where zinc is present, DTPA and EDTA will have a strong tendency to dissociate from metals such as calcium, and even more from sodium or potassium, in favour of binding zinc.

Zinc is one of the most abundant metals in the human body; the average zinc content of a healthy adult is between 1.5 and 2 g (Bedwal et al., 1991). Zinc is present as a cofactor for a large number of enzymes (up to 300), covering almost all classes of enzymes. As such, dietary deficiencies in zinc have produced a wide array of symptoms in test animals, including developmental toxicity such as terata of the skeleton and viscera and many other symptoms of zinc deficiency such as effects on sperm, alopecia, diarrhea, and eye and skin lesions (Stevens et al., 1962; Hurley and Swenerton, 1966; Hurley, 1969, 1971; Hurley et al., 1971; Hurley and Shrader, 1972; Warkany and Petering, 1972; Vojnik and Hurley, 1977; Hickory et al., 1979; Rogers et al., 1985; Record et al., 1985; Ferreira et al., 1989; Jankowski et al., 1995).

To estimate whether chelating agents such as DTPA and EDTA have different potencies to induce a disturbance in zinc homeostasis in a complex physiological system, conditional stability constants towards

ions (such as calcium ions that are present at high concentrations in such environments) have to be taken into account (Tables 1 and 2). The selectivity of chelating agents towards essential ions can be described by subtracting the respective conditional stability constants (e.g. $K_{\text{Zinc-Kcalcium}}$) at different physiological pH values. At physiological pH, DTPA has a 20-fold higher selectivity for zinc compared with EDTA (Table 3). Therefore one might expect any biological activity relating to the chelation of zinc to be more pronounced with DTPA than EDTA. However the ultimate capacity of chelates to bind zinc in the body will be affected by the presence of other metals and the availability of zinc. Concurrence with calcium ions is of high importance, because calcium levels ($100 \text{ mg/L} = 2.5 \text{ mM}$) are approximately 150-fold higher in rat serum than zinc levels ($1 \text{ mg/L} = 15 \text{ }\mu\text{M}$). Correspondingly, the recommended daily allowances for calcium (RDA 1500 mg/day) in the normal diet are approximately 100-fold higher compared to zinc (RDA 15 mg/day). Although DTPA may have a ‘preference’ for zinc over other counter ions such as calcium, the sheer abundance of calcium in the gut will reduce the likelihood of zinc/chelant complexes forming.

3. Bioavailability

Having established that DTPA has a high affinity for zinc, the effect of DTPA administration to experimental animals on zinc status should be considered. There are several studies examining the effects of administration to animals of the calcium salt of DTPA (Ca-DTPA) on the urinary and fecal excretion of zinc (Planas-Bohne and Ebel, 1975; Planas-Bohne and Olinger, 1976; Cohen and Guilmette, 1976; Cantilena and Klassen, 1982; Tandon et al., 1984; Domingo et al., 1988a). When administered via non-parenteral routes (intraperitoneal, intravenous or subcutaneous), either as a single dose, multiple doses in one day, multiple daily doses, or continuous infusion, Ca-DTPA caused an increased excretion of zinc relative to control. Organ concentrations of iron, copper and zinc were also examined after i. p. administration of Ca-DTPA; only zinc was significantly higher in the kidney and lower in the liver after Ca-DTPA administration (Sarić et al., 2004), presumably related to increased zinc excretion (Planas-Bohne and Ebel, 1975; Planas-Bohne and Olinger, 1976; Llobet et al., 1985). In contrast, similar work conducted using the zinc salt of DTPA has demonstrated that administration of Zn-DTPA did not cause an increase in the excretion of zinc after taking into account the amount of zinc in the test substance (Planas-Bohne and Ebel, 1975).

In studies with rats, dogs and humans, the oral absorption of DTPA and DTPA salts is very low with an average intestinal absorption across

Table 1
Conditional complex building constants for DTPA at various pH conditions (Ringbom and Wänninen, 1979).

Metal ion	Conditional stability constants depending on pH value								
	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11
Cu2 +	7.6	9.7	11.5	13.3	15.2	17.0	18.0	18.1	17.7
Fe3 +	12.8	14.0	14.5	14.5	14.5	14.5	14.1	13.5	12.8
Zn2 +	5.7	7.8	9.4	10.9	12.8	14.7	16.1	14.9	12.5
Mn2 +	2.1	4.3	6.3	8.3	10.2	12.2	13.8	14.7	14.9
Ca2 +	–	1.4	2.9	4.1	5.7	7.5	9.1	10.1	10.7
Mg2 +	–	0.4	1.9	3.0	4.3	6.0	7.6	8.6	9.1

Table 2

Conditional complex building constants for EDTA at various pH conditions (Ringbom and Wänninen, 1979).

Metal ion	Conditional stability constants depending on pH value								
	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11
Cu2+	8.3	10.2	12.2	14	15.4	16.3	16.6	16.6	16.1
Fe3+	13.9	14.7	14.8	14.6	14.1	13.7	13.6	14.0	14.3
Zn2+	6.0	7.9	9.9	11.7	13.1	14.2	14.9	13.6	11.0
Mn2+	3.6	5.5	7.4	9.2	10.6	11.7	12.6	13.4	13.4
Ca2+	–	2.2	4.1	5.9	7.3	8.4	9.3	10.2	10.6
Mg2+	–	–	2.2	4.0	5.4	6.5	7.4	8.3	8.6

Table 3

Zinc-selectivity compared to calcium for DTPA or EDTA under physiological pH conditions (Ringbom and Wänninen, 1979).

	Conditional stability constants					
	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
DTPA						
Zn2+	7.8	9.4	10.9	12.8	14.7	16.1
Ca2+	1.4	2.9	4.1	5.7	7.5	9.1
Zn-Ca	6.4	6.3	6.8	7.1	7.2	7.0
EDTA						
Zn2+	7.9	9.9	11.7	13.1	14.2	14.9
Ca2+	2.2	4.1	5.9	7.3	8.4	9.3
Zn-Ca	5.7	5.8	5.8	5.8	5.8	5.6

species of 3–5% (Stevens et al., 1962; Dudley et al., 1980a, 1980b; Resnick et al., 1990). DTPA is expected to be more poorly absorbed through the skin than EDTA after dermal application, as the smaller molecule EDTA is indicated to be absorbed for maximally 0.001% (ECB, 2004). In addition, DTPA substances are unlikely to be absorbed significantly via inhalation due to their large particle size ($> 10 \mu\text{m}$ diameter) when in powdered form and low volatility when in solution. Indeed, in medicinal uses (chelation therapy) chelants are administered intravenously since their bioavailability orally is so low as to render them non-efficacious. Following oral exposure in humans and rats, the limited fraction absorbed is not metabolised and is rapidly excreted with a very short half-life (2 h) via the urine; any systemically absorbed fraction is completely excreted unmetabolized within 24 h (ECB, 2004). Therefore, excretion of these chelates is almost exclusively via the feces. The passage of the DTPA through the gut varies between individuals; however, there is almost complete excretion of the chelating agent within 5 days of administration (Stevens et al., 1962). DTPA is not taken up or concentrated in any particular tissue, and in pregnant rats did not pass into fetal circulation (Zylicz et al., 1975).

4. Animal toxicity data of DTPA

With the exception of being able to complex metal ions, chelating agents including DTPA and EDTA are of low chemical reactivity. OECD SIDS concluded that such chelants possess properties such as skin and eye irritation, repeated-dose toxicity and reproductive/developmental toxicity but that these effects are associated with the chelation of metals. The subsequent toxicological effects related to metal deficiency would therefore only be considered relevant human hazards where there is significant exposure (OECD SIDS, 2012).

4.1. Repeat dose toxicity studies

In an OECD 407 guideline 28-day study in rats (Elliott et al., 1987), gavage administration of the potassium salt of DTPA (CAS no. 7216-95-7) at doses of 0, 83, 333 or 1330 mg/kg bw/day resulted in clear signs of toxicity including mortality at 1330 mg/kg bw/day (4/5 male, 1/5 female). Prior to death, the male animals displayed hunched posture,

abnormal gait, diarrhea, piloerection, yellow-brown staining of fur and decreased respiration rate. High dose animals also consumed less food. Other effects reported included increased serum potassium levels, decreased body weights, and clinical signs. At 83 and 333 mg/kg bw/day there were no mortalities and except for a reduced feed consumption in some mid dose males, there were no clinical signs observed. The NOAEL was 83 mg/kg bw/day. In another OECD 407 guideline 28-day drinking water study with the sodium salt of DTPA (CAS no. 140-01-2), rats received 0, 600, 3000 or 12,000 ppm Na5-DTPA. Body weight reductions and histopathological changes of the urinary tract were observed at 12,000 ppm (ca. 1500 mg/kg bw/day) and 3000 ppm (ca. 375 mg/kg bw/day). The NOAEL was 600 ppm (ca. 75 mg/kg bw/day; BASF, 2002); see Table 4.

Thus, in the two available 28-day studies where DTPA salts were dosed via gavage or the drinking water, there were some clinical signs of a perturbation in nutrition in the high doses consisting of diarrhea, decreased food consumption, and decrease in bodyweight, and in the gavage fed animals there were deaths in the high dose group males. It is very likely that the deaths were associated with diarrhea caused by chelation of metals such as zinc and calcium in the intestinal tract leading to decreased absorption of food and water loss.

4.2. Developmental toxicity studies

In an OECD 414 guideline prenatal developmental toxicity study with Na5-DTPA, (report published on ECHA's homepage; BASF, 1994), rats were administered via oral gavage 0, 100, 400 or 1000 mg/kg bw/day Na5-DTPA on days 6–15 of gestation. DTPA administration resulted in reduced food consumption between gestation days (GD) 6–10, reduced body weight at GD17 and GD20, and reduced body weight gain during GD6–8 and GD15–17 ($p < 0.05$) in high dose group dams. Overall, body weight gain during and after cessation of treatment was lower in this group than in controls (11.5%), although this was not statistically significant (Table 5). Feces were discolored (dark yellow) in this group during treatment, but reverted to normal during the post-treatment phase. There were no differences between the other treatment groups and the control group. Gravid uterus weights in the dams of the high dose group were lower than control (Table 5), as were the live litter sizes and bodyweights of both male and female fetuses (Table 6). At 400 mg/kg bw/day there was a small but statistically significant higher percentage of skeletal retardations (delays in ossification of the skull and sternbrae); the incidences were either within or slightly outside the historical control range (Table 7). At 1000 mg/kg bw/day, in addition to skeletal retardations as seen in animals of the mid dose, there was an increase in the incidence of skeletal malformations (missing thoracic and lumbar vertebrae and bipartite sternbrae) and skeletal variations (Table 7) but no visceral or external malformations were present. The NOAEL for maternal toxicity appeared to be 400 mg/kg bw/day but might have been lower in view of the limited number of parameters required to measure in dams in a developmental toxicity study when compared to e.g. a 28-day study, and the effects seen at similar doses in the 28-day studies (see Table 4). A NOEL for developmental effects was established at 100 mg/kg bw/day

Table 4

NOAELs (parental and developmental) in diet, drinking water and gavage studies with DTPA and EDTA in rats.

	Parental toxicity Diet/drinking water (mg/kg bw/day)			Parental toxicity Gavage (mg/kg bw/day)	Developmental toxicity (mg/kg bw/day)
	subacute	subchronic	chronic		
DTPA-Na5	75 ^b				100 ^c
DTPA-K5				83 ^d	
DTPA-CaNa3					~700 ^{a,c} 90 (sc) ^f
DTPA-ZnNa3					~3000 ^{a,c} ≥565 (sc) ^f
EDTA-Na2H2	1125 ^g 1000 ^j	> 500 ^h		< 1243 ⁱ	≥1243 ⁱ ~1000 ^{a,j}
EDTA-Na3H	453 ^k		≥500 ^k	< 1245 ⁱ	≥1245 ⁱ
EDTA-Na4				< 1374 ⁱ	≥1374 ⁱ
EDTA-H4				< 967 ⁱ	≥967 ⁱ
EDTA-CaNa2	2750 ^{a,g} ≥3636 ^m		≥250 ^l ≥338 ^l	< 1340 ⁱ	≥1340 ⁱ

This table shows that with regard to the DTPA and EDTA chelates (with H, Na, K or Ca as counter ion) that (1) for EDTA, developmental effects are only seen at levels generally higher than 1000 mg/kg bw whereas NOAELs for parental toxicity are similar or lower, (2) for DTPA, developmental effects are seen at lower levels, but at levels at which parental toxicity is also seen. The lower effect levels of DTPA when compared to EDTA are most probably due to the higher preference of DTPA for zinc when compared to EDTA, and (3) if Zn is already bound to DTPA, effect levels are much higher when compared to e.g. Ca as counter ion.

^a LOAEL; sc = subcutaneous.

^b BASF (2002).

^c BASF (1994).

^d Elliott et al. (1987).

^e Brummett and Mays (1977).

^f Fukuda and Iida (1983).

^g Kawamata et al. (1980).

^h Wynn et al. (1970).

ⁱ Schardein et al. (1981).

^j Swenerton and Hurley (1971).

^k NTIS (1977).

^l Oser et al. (1963).

^m Dow (1955).

based on retardations seen at 400 mg/kg bw (BASF, 1994). The increase in the severity of the developmental effects most probably reflected the increase in zinc deficiency.

However, if one were to conduct such a study where metal ions – such as zinc – were supplemented sufficiently it is unlikely that any systemic toxicity, including developmental effects would be observed. This is indeed supported by developmental toxicity studies conducted

Table 5

Litter findings in a rat developmental toxicity study with Na5-DTPA (BASF, 1994).

Findings	0 mg/kg bw/day	100 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day
Live fetuses	14.3 ± 2.0	14.0 ± 2.5	13.5 ± 4.2	11.9 ± 3.8*
Fetal wt (all) (g)	3.7 ± 0.2	3.7 ± 0.2	3.7 ± 0.3	3.4 ± 0.3**
Fetal wt (M) (g)	3.8 ± 0.2	3.8 ± 0.3	3.8 ± 0.2	3.5 ± 0.3**
Fetal wt (F) (g)	3.6 ± 0.2	3.7 ± 0.2	3.6 ± 0.3	3.4 ± 0.3**

Statistics: Dunnett-test (two sided): *p < 0.05; **p < 0.01.

using the zinc salt of DTPA where no developmental (or systemic) toxicity was observed in pregnant mice injected subcutaneously at doses in excess of 1000 mg/kg bw/day. Fisher et al. (1975) administered Zn-DTPA or Ca-DTPA or saline solution to female mice via daily subcutaneous injections. These doses were equivalent to 200 or 1600 mg Zn-DTPA/kg bw and 180 or 1440 mg Ca-DTPA/kg bw. The dosing period started 4 days after the mating period began and continued throughout pregnancy until the pups reached an age of 13 days. In the group of mice dosed with 1440 mg/kg bw Ca-DTPA there were no viable offspring observed. Only one stillborn pup was observed but it appeared grossly normal. In the 180 mg/kg bw Ca-DTPA group there were no adverse effects on reproduction or developmental parameters. Both dose levels of Zn-DTPA, the high dose of which was well above the OECD limit dose via a non-parenteral route, were reported to be completely harmless to the mothers and the pups.

In a subsequent study to further study the effects of Ca-DTPA, Fisher et al. (1976) administered a range of doses of Ca-DTPA to pregnant mice via daily subcutaneous injections. The mice were separated into 3 groups and were dosed during early (days 2–6), mid (days 7–11) or late (days 12–16) gestation. The mice received injections equivalent to 0, 355, 715 and 1430 mg Ca-DTPA/kg bw. The dams were sacrificed on day 18 of gestation and the fetuses examined for morphologic alterations. Ca-DTPA (355 or 715 mg/kg bw) dosed either from day 2–6 or from day 7–11 of gestation resulted in an increase in the percentage of resorptions compared to control. Neither of these doses caused an increase in resorptions when dosed from day 12–16 whereas 1430 mg/kg bw did. Dosing with 715 mg/kg bw produced malformations in fetuses in all dosing period groups. The types of malformation and number of fetuses affected varied however with the dosing schedule. The malformations observed were typical of those associated with zinc deficiency. Dosing with 355 mg/kg bw only produced malformations (3%) when dosed from days 2–6 and these malformations were consistent with those observed with 715 mg/kg bw dosed for the same period.

Brummett and Mays (1977) investigated the developmental toxicity of the zinc salt of DTPA in the mouse using the same mouse strain and a similar protocol to that used by Fisher et al. (1976). Pregnant mice were

Table 5

Maternal findings in a rat developmental toxicity study with Na5-DTPA (BASF, 1994).

Findings	0 mg/kg bw/day	100 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day
Fd GD6-8 (g)	26.1 ± 2.0	25.3 ± 2.2	26.4 ± 1.9	22.7 ± 2.1**
Fd GD8-10 (g)	26.0 ± 1.9	25.7 ± 2.5	26.1 ± 1.9	23.4 ± 2.8**
BW GD17 (g)	352.6 ± 21.3	349.5 ± 25.6	350.5 ± 27.6	332.8 ± 18.3*
BW GD20 (g)	405.6 ± 26.6	404.6 ± 28.4	402.8 ± 38.0	378.7 ± 26.9*
BWG GD6-8 (g)	7.9 ± 4.1	6.7 ± 2.8	7.0 ± 2.9	3.6 ± 5.3**
BWG GD15-17 (g)	22.4 ± 4.1	22.0 ± 5.1	20.5 ± 6.5	17.5 ± 5.1**
BWG GD6-15 (g)	43.7 ± 8.0	44.5 ± 6.3	43.6 ± 8.8	34.6 ± 10.2**
BWG GD15-20 (g)	75.4 ± 9.9	77.1 ± 12.0	72.8 ± 17.6	63.5 ± 13.6*
BWG GD0-20 (g)	148.0 ± 16.9	150.3 ± 19.1	141.4 ± 26.7	125.2 ± 19.4**
Uterus wt (g)	80.8 ± 10.8	80.1 ± 14.0	76.9 ± 22.9	64.2 ± 20.0**

Fd = food consumption, BW = body weight, BWG = body weight gain, GD = gestation days. Statistics: Dunnett-test (two sided): *p < 0.05; **p < 0.01.

Table 7
Skeletal examinations in a rat developmental toxicity study with Na5-DTPA (BASF, 1994).

Findings	0 mg/kg bw/day	100 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day	Historical control [#]
No. fetuses	171	160	152	135	6067
No. litters	23	22	22	22	854
Malformations					
Total malformations					
Fetal incidence, % (N)	7.0 (12)	1.9 (3)	5.3 (8)	28 (38)	3.2 (194) (range, %: 0–10.1)
Litter incidence, % (N)	30 (7)	14 (3)	36 (8)	73 (16)**	18.4 (157) (range, %: 0–43.5)
Affected fetuses/litter, Mean, % \pm SD	6.7 \pm 14.3	1.9 \pm 4.8	4.7 \pm 6.6	27.7 \pm 31.2**	3.3 (range, %: 0–8.7)
Thoracic vertebra absent					
Fetal incidence, % (N)	0 (0)	0 (0)	0 (0)	11 (15)	0.1 (3) (range, %: 0–1.2)
Litter incidence, % (N)	0 (0)	0 (0)	0 (0)	27 (6)**	0.4 (3) (range, %: 0–9.1)
Affected fetuses/litter, Mean-% \pm SD	0 \pm 0	0 \pm 0	0 \pm 0	12.8 \pm 29.4**	0 (range, %: 0–1.1)
Lumbar vertebra absent					
Fetal incidence, % (N)	0 (0)	0 (0)	0 (0)	6.7 (9)	0.1 (6) (range, %: 0–1.3)
Litter incidence, % (N)	0 (0)	0 (0)	0 (0)	23 (5)*	0.6 (5) (range, %: 0–6.5)
Affected fetuses/litter, Mean-% \pm SD	0 \pm 0	0 \pm 0	0 \pm 0	5.9 \pm 15.7**	0.1 (range, %: 0–1.5)
Sternebra(e) bipartite, ossification centres dislocated					
Fetal incidence, % (N)	0.6 (1)	0 (0)	1.3 (2)	5.2 (7)	0.6 (37) (range, %: 0–2.7)
Litter incidence, % (N)	4.3 (1)	0 (0)	9.1 (2)	27 (6)*	4.2 (36) (range, %: 0–20)
Affected fetuses/litter, Mean-% \pm SD	0.5 \pm 2.6	0 \pm 0	1.2 \pm 4.1	5.4 \pm 10.1*	0.6 (range, %: 0–2.7)
Variations					
Total variations					
Fetal incidence, % (N)	50 (86)	50 (80)	48 (73)	81 (110)	47.8 (2899) (range, %: 31–88.4)
Litter incidence, % (N)	96 (22)	95 (21)	95 (21)	95 (21)	94.1 (804) (range, %: 80–100)
Affected fetuses/litter, Mean-% \pm SD	49.6 \pm 26.2	48.6 \pm 20.1	46.7 \pm 23.6	78.4 \pm 26.7**	47.6 (range, %: 29.5–87.8)
Shortened 13th rib					
Fetal incidence, % (N)	13 (23)	13 (20)	14 (22)	51 (69)	10.4 (632) (range, %: 0–25.3)
Litter incidence, % (N)	48 (11)	45 (10)	45 (10)	82 (18)*	37.8 (323) (range, %: 0–75)
Affected fetuses/litter, Mean-% \pm SD	13.6 \pm 18.4	12.3 \pm 18.9	13.0 \pm 18.3	47.5 \pm 32.5**	10.5 (range, %: 0–26.4)
Rudimentary cervical rib(s)					
Fetal incidence, % (N)	2.9 (5)	3.1 (5)	2.0 (3)	19 (26)	1.8 (109) (range, %: 0–6.6)
Litter incidence, % (N)	8.7 (2)	23 (5)	14 (3)	50 (11)**	10.2 (87) (range, %: 0–30.4)
Affected fetuses/litter, Mean-% \pm SD	2.7 \pm 10.6	2.9 \pm 5.4	1.8 \pm 4.6	21.3 \pm 31.2**	1.9 (range, %: 0–5.9)
Absent 13th rib					
Fetal incidence, % (N)	0 (0)	0 (0)	0 (0)	21 (28)	0.03 (4) (range, %: 0–0.6)
Litter incidence, % (N)	0 (0)	0 (0)	0 (0)	55 (12)**	0.2 (2) (range, %: 0–4.2)
Affected fetuses/litter, Mean-% \pm SD	0 \pm 0	0 \pm 0	0 \pm 0	21.8 \pm 30.6**	0 (range, %: 0–0.6)
Retardations					
Total retardations					
Fetal incidence, % (N)	48 (82)	48 (77)	63 (96)	81 (109)	46.5 (2821) (range, %: 0–72)
Litter incidence, % (N)	96 (22)	100 (22)	91 (20)	95 (21)	86.2 (736) (range, %: 0–100)
Affected fetuses/litter, Mean-% \pm SD	47.4 \pm 24.8	48.4 \pm 26.7	63.8 \pm 33.5*	78.0 \pm 31.3**	46.3 (range, %: 0–71.5)
Skull incompletely ossified					
Fetal incidence, % (N)	1.2 (2)	1.3 (2)	3.9 (6)	8.1 (11)	0.8 (46) (range, %: 0–4)
Litter incidence, % (N)	4.3 (1)	9.1 (2)	27 (6)*	32 (7)*	4.6 (39) (range, %: 0–28)
Affected fetuses/litter, Mean-% \pm SD	1.0 \pm 4.6	1.2 \pm 4.0	4.6 \pm 8.7*	8.5 \pm 16.1**	0.8 (range, %: 0–4)
Sternebra(e) not ossified					
Fetal incidence, % (N)	5.3 (9)	6.9 (11)	18 (27)	51 (69)	6.5 (395) (range, %: 0–13)
Litter incidence, % (N)	35 (8)	32 (7)	50 (11)	82 (18)**	31 (265) (range, %: 0–56)
Affected fetuses/litter, Mean-% \pm SD	4.8 \pm 7.1	6.8 \pm 12.9	17.1 \pm 21.6*	50.7 \pm 35.8**	6.4 (range, %: 0–12.1)

Historical control data (study year \pm 4 years, i.e. 1990–1998; BASF); Statistics: Wilcoxon test (one-sided); *p \leq 0.05 or **p \leq 0.01.

subcutaneously dosed with Zn-DTPA daily either from days 2–6 or 7–11 during gestation. The doses of Zn-DTPA used were equivalent to 0, 3160 and 6370 mg/kg bw. Due to the hypertonic nature of the test material an additional group of mice was treated with a solution of NaCl at the same ion concentration, osmolality, pH and volume as the high dose Zn-DTPA treatment. A Ca-DTPA dose group of 715 mg/kg bw dosed daily on days 7–11 was also included in this study. Dosing with Zn-DTPA in this study did not result in any malformations of the fetuses although 6370 mg/kg (days 2–6 and 7–11) and 3160 mg/kg (days 7–11) caused an increase in embryo toxicity relative to controls (aborted litters or resorptions). However, administration of the hypertonic saline solution also caused an increase in aborted litters and resorbed fetuses relative to control. The only malformed fetus observed which had exencephaly was in the Ca-DTPA group. Considering the previous studies on Ca-DTPA the evidence suggests that had it been dosed in this study from days 2–6 then the malformations observed would have been far more extensive. This seems to suggest that it could take several days for DTPA to induce a zinc deficiency and that it might last for a few days once dosing has ceased since organogenesis peaks in

the mouse between days 7 and 11 of gestation; thus the sensitivity to zinc-deficiency induced malformations should be greatest during this time period.

In a follow-up study to that reported by Brummett and Mays (1977), Calder et al. (1979) dosed mice daily via subcutaneous injection 4 days after mating began until birth or until 29 injections had been administered. Two forms of Zn-DTPA were used in this study, one commercial batch with no NaCl present and another made in the lab containing NaCl. No gross malformations were reported in this study at doses up to 6370 mg/kg bw although it appears only an external examination was performed. The authors therefore reported that 6370 mg/kg bw Zn-DTPA (commercial grade) was not toxic to either dams or pups although it did produce a statistically significant drop in pup weight. Doses of 1560 or 3160 mg/kg bw Zn-DTPA (commercial or lab grade) did not significantly alter any of the parameters examined (pup weight, pups/litter, abortion rate) relative to control. The study also followed the progress of the dams and the pups for their remaining lifespan to understand whether there were delayed effects on fertility or viability. There was no evidence of any impairment in the fertility of

the mature pups or the viability of their offspring; however, no additional details are given.

This is supported by developmental toxicity studies conducted in rats using the zinc salt of DTPA where no developmental or systemic toxicity was observed when injected subcutaneously at doses up to 565 mg/kg bw/day, whereas Ca-DTPA caused significant increases in developmental toxicity at 179 mg/kg bw (but not at 90 mg/kg bw; Fukuda and Iida, 1983). As such Ca-DTPA treatment resulted in developmental toxicity at lower levels when compared to Zn-DTPA which supports the notion that - because the affinity of DTPA is much higher for Zn than for Ca (Table 1) – Zn-DTPA did not induce developmental toxicity as DTPA was already bound to zinc.

4.3. Supporting studies

Further evidence of the potential of DTPA and EDTA to cause an increase in zinc excretion and thus zinc loss, comes from work in mice and rats to identify an effective antidote for acute zinc intoxication (Llobet et al., 1985; Domingo et al., 1988b). In this work a number of chelating agents including DTPA and EDTA were compared. In both studies, mice and rats were administered lethal doses of zinc via intraperitoneal injection and then administered doses of chelating agents either immediately or 10 min later also via intraperitoneal injection. The antidotal effectiveness was assessed by comparing the degree of zinc induced mortality. In both studies the calcium salt of DTPA was, compared to EDTA, most protective against acute zinc toxicity. Llobet et al. (1985), in addition, showed an increase in the excretion of zinc in the feces and urine during the study period.

Additional support comes from studies (and treatments) conducted in humans and animals where the zinc salt of DTPA was dosed with no evidence of systemic toxicity at therapeutic doses (Kalkwarf et al., 1983; Sato et al., 1997). Also in an extended OECD 422 rat study (with a 10-week pre-mating treatment period instead of 2 weeks) with Fe-DTPA no developmental toxicity was observed up to and including a dose of 1500 mg/kg bw. In this case, less zinc was bound as DTPA was already bound to iron for which it has a higher affinity (Wolterbeek and Otto, 2011).

Table 8

Estimated combined oral and inhalation exposure to a female worker (60 kg) during manufacture of DTPA or EDTA.

Route of exposure		Total dose remaining in the GIT	Total systemic exposure
Oral (mg/day)	In the absence of data on the potential oral intake of dusty chemicals in the workplace, an exposure level of 25 mg/day is assumed based on a US EPA estimate that the daily adult unintentional soil intake would fall within the range of 0–50 mg/day (USEPA, 1997) which is very likely a gross overestimate of actual worker exposure to DTPA through the dietary route. However, for the purpose of this assessment the midpoint of this range was used. Absorption following oral exposure is approximately 5% (Stevens et al., 1962; Bondesson et al., 2007); as such, the actual systemic dose to DTPA following oral exposure in the workplace would be 1.25 mg/day.	23.75	1.25
Inhalation (mg/day)	Particle size measurements have indicated that the fast majority of the particles is > 10 µm. Particles above 10 µm are only partially inhaled (ICRP, 1994). Using ICRP (1994), 90% of the inspired particles will be deposited in the extra-thoracic airways and eventually swallowed. The remaining 10% are assumed to be deposited in the lung and available for absorption. The Derived-No-Effect-Level (DNEL long-term) of 1.5 mg/m ³ , established under European REACH regulation, was used. This would result in a total exposure of 15 mg DTPA per day. Of this 15 mg amount, due to the large particle size, at least 90% will be swallowed, resulting in an oral dose of 13.5 mg/day of which 5% will be absorbed into the systemic circulation (0.68 mg/day), while maximally 10% enters the deep lung and is assumed to be 100% absorbed (1.5 mg/day). Thus the amount of DTPA entering the systemic circulation would be 2.2 mg/day, and the amount remaining in the GIT is 12.8 mg/day.	12.8	2.2
Dermal (mg/day)	A worst case estimate was used, applying 5 mg/cm ² /day dermal exposure and an exposed area of 840 cm ² (palms and backs of both hands; ECB, 2004) which resulted in 4200 mg/person/day. The systemic dose for a 60 kg (female) person would then be 4200 mg/day x 0.001% = 0.04 mg/day or 0.0007 mg/kg bw/day. As this amount is very low this has not been further included in the exposure assessment.	NA	Negligible
Total (mg/day)		36.6	3.5
Total (mg/kg bw/day)		0.61	0.06

5. Human data (parenteral DTPA exposure)

It has to be noted that the following section comprises only parenteral exposure data, as no human data are available with regard to other exposure routes, most likely due to the very limited bioavailability of DTPA via other (oral, dermal and inhalation) routes.

Data in humans show that intravenous administration of DTPA or EDTA (the calcium salts) is capable of causing an increase in the excretion of zinc, in some cases leading to a deficiency. In one case study (Proksch and Kölmel, 1985), a patient treated with Ca-EDTA and Ca-DTPA for manganese poisoning exhibited zinc deficiency syndrome with acrodermatitis enteropathica-like skin changes. This resolved completely following oral administration of zinc aspartate. In a second study (Heid et al., 1979; Kalkwarf et al., 1983) levels of trace metals were assessed in the urine samples of a worker contaminated in the 1976 Americium incident. This worker had been treated over the course of 3 years with different forms of DTPA (Ca-DTPA or Zn-DTPA; total amount of 560 g of DTPA in the first ca. 2 years, thus on average ca. 1 g/day) in an effort to reduce the radionuclide contamination. Analysis of the worker's urine identified that of all the trace metals assessed, zinc was the only one excreted at much higher levels than 'normal' and the peaks in zinc excretion appeared to correspond to treatments with Ca-DTPA. The treatment schedule also included intermittent zinc supplementation. It was noted that this supplementation or administration of Zn-DTPA compensated for the loss of zinc caused by Ca-DTPA (Heid et al., 1979; Kalkwarf et al., 1983).

6. Exposure assessment for Aminocarboxylic acid (ethylenediamine-based) chelating agents using DTPA as an example

To extrapolate a worst-case exposure scenario, DTPA has been chosen as a candidate of the aminocarboxylic acid (ethylenediamine based) chelants category, due to its 20-fold higher selectivity for zinc in comparison to EDTA. Furthermore, a workplace scenario was used because potential exposure is much higher at the workplace compared to consumer applications where chelants are generally used in very low concentrations.

6.1. Oral exposure

It is generally assumed that oral exposure to industrial chemicals in the workplace can be discounted and DTPA is no exception, making it unlikely that any oral exposure will occur during manufacturing or formulation processes. However, for the purpose of this (worst-case) evaluation the possibility of contamination of food occurring within the workplace is considered as part of the exposure assessment (Table 8).

6.2. Dermal exposure

Data on dermal absorption of DTPA are not available. However, data on EDTA suggest that the rate of absorption is very low. Dermal penetration data for EDTA has been reported by the European Chemicals Bureau (ECB, 2004) as 0.001% absorption. As DTPA has a higher molecular weight than EDTA but a similar log Kow it is assumed that dermal penetration of DTPA will be equivalent to or less than that of EDTA. Given this very low dermal penetration, systemic exposure via the dermal route to DTPA or EDTA is not considered to significantly add to a combined workplace exposure estimate (Table 8).

6.3. Inhalation exposure

DTPA and EDTA are sold either as a solid (a crystalline solid powder) or a ca. 40% aqueous solution. The manufacture and major industrial use of liquid forms of DTPA and EDTA are not anticipated to form aerosols. The main use for DTPA is in the paper and pulp industry and for this use it is supplied as a liquid. Due to the enclosed nature of the production process, the low volatility of liquid DTPA and the use of suitable personal protective equipment by the work force (goggles, gloves and respiratory protection; in case of short-term exposure situations where no technical risk management measures are in place or can be applied), the potential for exposure to liquid DTPA during production and use in the paper and pulp industry is considered to be minimal. A similar conclusion was reached in the EU Risk Assessment report for EDTA (ECB, 2004).

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

Inhalation of DTPA or EDTA powder is likely to be the most significant source of exposure at the workplace. Although DTPA and EDTA powder is manufactured in an enclosed process, there is potential for inhalation exposure when the powder is transferred into containers for transport and from transport containers into formulating vessels (see further Table 8).

6.4. Zinc intake

As indicated previously, DTPA and EDTA are able to bind metals such as zinc, reducing the zinc's bioavailability, in which DTPA shows a ca. 20-times higher activity than EDTA. All evidence supports that the effects observed in animal studies at high oral doses can be ascribed to zinc depletion, a secondary non-specific effect as a consequence of their mode of action. Therefore, to fully evaluate the risk associated with DTPA exposure to workers it is necessary to understand how exposures to DTPA in the workplace could affect zinc status in workers.

The average zinc content of a healthy adult is between 1.5 and 2 g (Bedwal et al., 1991). Past surveys have shown that pregnant women consume an average of 10 mg Zn/day (Swanson and King, 1993) although other data indicate that the zinc intake of women in general (not specific to pregnant women) is approximately 7 mg/day (NDNS,

2005). In 27 reported studies, dietary zinc intake ranged from 5.7 to 22 mg/day in non-vegetarians and from 5 to 12.6 mg/day in vegetarians with a mean of approximately 8 mg/day (King, 2000). The average daily zinc intake through the diet in the USA ranges from 5.2 to 16.2 mg (ATSDR, 2005). The reason for the lower intake in vegetarians is the absence of dietary meat, which is a significant source of zinc. Overall it appears that an average zinc intake for a female vegetarian would be between 5 and 8 mg/day of which approximately 25% would be absorbed, although there are several factors that can influence this (King, 2000). For example, absorption is reduced by the presence of excess calcium, fibre or phytate in the diet, smoking and alcohol abuse (King, 2000). Zinc absorption from the gut is increased in individuals consuming less zinc in the diet or when the person has lower zinc status (Cousins, 1986). Excretion of zinc is also decreased in individuals with a lower intake of zinc (King, 2000).

It should be noted that many estimations of what would be a sufficient intake to maintain zinc homeostasis are based on studies to assess the amount of zinc intake necessary to exactly match the amount of zinc excreted (Hambridge, 2003). However as indicated above, zinc absorption and excretion are not constant, and vary with an individual's zinc status. Therefore assessments of zinc requirement are not necessarily absolute and thus the estimate given above of an 'insufficient' zinc intake is a range and not a single limit.

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

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

At present there is no advice (in Europe and the US) to pregnant women proposing the level of zinc in the diet should be monitored or supplemented. In populations where zinc deficiency is more prevalent due to poor nutrition as a result of poverty, or eating habits that include consumption of significant amounts of clay and cereals, trials to assess the benefits of supplementing the diet with zinc (Shah and Sachdev, 2006) have been inconclusive and in general there appears to be little or no benefit to zinc supplementation during pregnancy in zinc deficient populations.

With respect to the workforce, it is generally accepted the worker population is 'healthier' than the general population. It thus seems defensible to assume that the average worker (pregnant or not), is healthy with a balanced diet containing zinc in the mid-range of 5–8 mg/day, i.e. approximately 6.5 mg zinc/day.

7. Risk Assessment

In order to determine how DTPA would affect an individual's zinc status it is necessary to consider how much zinc is expected to bind to a given dose of DTPA as zinc bound to DTPA is no longer bioavailable.

The Zn-DTPA complex consists of 1 mol of DTPA and 1 mol of zinc which is equivalent to 6 mg of DTPA and 1 mg zinc. A worst-case estimate would therefore be that for every 6 mg of DTPA dosed, 1 mg of zinc will be chelated. There are, however, many factors in a biological system influencing the amount of Zn-DTPA complex formed. DTPA complexes with other metal ions and the amount of each complex is

affected by the presence of other metals, the pH and the concentration of DTPA (increasing the concentration will increase the probability that each metal complex will be formed). In the gut, DTPA will therefore bind to whatever metal ions are present.

To illustrate this, consider a balanced female diet, containing around 2600 mg potassium, 750 mg of calcium, 230 mg magnesium, 10 mg iron, 1 mg copper and 7 mg zinc (NDNS, 2005). Whilst only iron and copper have a greater affinity for DTPA than zinc, the significantly larger amounts of calcium, magnesium and potassium available will mean that DTPA is far more likely to interact with these elements than zinc. Any DTPA that becomes systemically available will face a similar situation, but will also have to compete with the various metal transport proteins in the body, such as metallothionein. Therefore it seems unrealistic to assume that 1 mol of DTPA will bind 1 mol of zinc. Support for this argument comes from the rat developmental toxicity study (BASF, 1994) in which gavage doses of 100, 400 and 1000 mg/kg bw/day DTPA (equivalent to approximately 30, 130 and 330 mg DTPA/day) resulted in no, mild and moderate developmental toxicity respectively. Assuming 1 mol of DTPA will bind 1 mol of zinc, then 30, 130 and 330 mg/day DTPA should be capable of binding approximately 5, 22 and 55 mg/zinc per day. Under such circumstances all three doses would be capable of binding the 1.5 mg/day zinc in the diet (based on 60 mg zinc/kg diet, average food consumption of 25 g diet/day) and thereby produce evidence of a zinc deficiency. The fact only the mid and high dose could have had any significant effect on the daily zinc intake indicates significantly more than 1 mol of DTPA is necessary to prevent the uptake of 1 mol of zinc.

Additional support comes from data of an individual treated with DTPA following an exposure to the isotope 241 Americium (Kalkwarf et al., 1983). Following an intravenous injection of 1 g of the calcium salt of DTPA, zinc excretion in the urine was significantly increased compared to normal with approximately 18 mg of zinc excreted in the urine (other metals were not significantly affected). The number of moles of zinc excreted per mole of DTPA was calculated to be approximately 0.13, i.e. 7 mol (42 mg) of DTPA bind approximately 1 mol (1 mg) of zinc.

As calculated above the total amount of DTPA present in the GIT of a worker would be 36.6 mg/day (Table 8). Based on a ratio of 7 mol (42 mg) of DTPA being required to bind 1 mol zinc, the maximum amount of zinc which could be bound to DTPA present in the GIT is, at most, 1 mg of the 6.5 mg of zinc present in the diet.

In addition, the total estimated worker systemic exposure following a combination of oral and inhalation exposure is 3.5 mg/day. Based on a ratio of 7 mol of DTPA dosed to 1 mol of urinary zinc excretion, it can be estimated that a systemic intake of 3.5 mg of DTPA would cause a loss by urinary excretion of approximately 0.1 mg zinc.

The calculations described above, using extremely conservative oral exposure values, indicate that occupational exposure to DTPA during manufacturing could lead to reductions in the bioavailability of zinc both by reducing the amount of dietary zinc available for absorption from the GIT and by binding zinc already absorbed in the systemic circulation. As described earlier there is on average 6.5 mg of zinc present in the diet of which approximately 25% is absorbed from the GIT into body. Of the 6.5 mg of zinc in the diet the worst-case scenario is that 1 mg might bind to DTPA in the GIT. This however leaves 5.5 mg available for absorption and as only 25% is usually absorbed there is still an excess of zinc. In addition, as any oral exposure is most unlikely to occur during manufacturing or formulating the chemical it is unlikely that worker exposure to DTPA has any impact on the uptake of zinc from the GIT.

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

day, which is still a sufficient dietary intake.

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

Overall, based on the ability of the body to compensate for changes in zinc status and the minimal amount of zinc that could be affected by DTPA, it is highly unlikely that exposure to DTPA in the workplace will adversely affect a (female) worker's zinc status. As exposure via consumer uses is much lower it is even more unlikely that consumer exposure to DTPA will adversely affect an individual's zinc status either. This would also be true for susceptible populations, e.g. children or young adults with lower body weights, because recommended daily zinc allowances are comparable to adults (between 5 and 12 mg zinc per day).

8. Discussion

8.1. Toxicity issues

As previously indicated, with the exception of being able to complex metal ions, DTPA and EDTA are of low chemical reactivity. Therefore, their ability to sequester metal ions is most likely responsible for the observed toxicity.

EDTA has been evaluated by ECB (2004): developmental toxicity of sodium, calcium and zinc EDTA were investigated in studies using rats. After repeated treatment of dams during various periods of gestation using different routes of exposure (diet, gavage, subcutaneous, and intramuscular) impaired embryo/fetal development and the induction of a pattern of gross malformations were observed during these investigations with the exception of one study (Schardein et al., 1981) in which no developmental toxicity was seen at the levels tested (ca. 1000–1200 mg/kg bw; Table 4). These effects were almost exclusively exhibited in studies using maternally toxic dosage levels (e.g. in the presence of moderate to severe diarrhea). From studies with oral application it appeared that developmental effects were more marked when test compounds were provided via the diet than via gavage as there would be more time to interact with dietary zinc (Kimmel, 1977).

Since single dose levels in these studies had been administered mainly, no oral NOAEL for either developmental toxicity or maternal toxicity could be established. However, from the investigations of Swenerton and Hurley (1971), which represent the only feeding study that had applied two different dosages, ECB (2004) concluded that a dose-effect relationship can be derived. In this study, at dietary exposure levels corresponding to 1500 mg/kg bw/day clear developmental toxicity was observed. Effects were also observed at dietary exposure levels corresponding to 1000 mg/kg bw/day. ECB (2004) concluded that a rather steep slope for the dose response for developmental effects can be assumed. Although maternal toxicity was concomitant to the observed developmental effects at both levels of dietary exposure, it appears that the specific outcome of malformations in offspring was not secondary to the substance induced impairment of the dams (as evidenced by reduced food intake, reduced maternal weight (gain) and by diarrhea), but rather resulted from compound specific interference with endogenous Zn homeostasis.

The extent of endogenous zinc depletion during administration of EDTA salts which has to be assumed to be higher compared with the effect of the Ca-EDTA chelate, however, has not been investigated so far. Since it has been demonstrated that zinc deficient diets per se lead to developmental and teratogenic effects in offspring (Hurley and Swenerton, 1966; Hurley et al., 1971), the depletion of zinc in the diet and/or the depletion of endogenous zinc tissue concentrations caused by EDTA treatment appear to be of specific significance for embryo/fetal impairment and the induction of malformations. With sufficient zinc supplementation fetotoxic and teratogenic effects could be

prevented or minimised. The zinc chelate of EDTA obviously lacks a specific teratogenic potential. Hence, ECB did not recommend classifying Na4-EDTA and H4-EDTA as being a reproductive toxicant (ECB, 2004). Since the evaluation by ECB (2004) no new data with regard to developmental toxicity have become available on H4-EDTA, Na4-EDTA, and Na2-EDTA.

According to RIVM (2014), Ca-EDTA is not considered a reproductive toxicant, as long as zinc intake is sufficient. Overall, Ca-EDTA seems to be safe for use as a food additive, as the noted toxic doses are higher than can be achieved via the addition of Ca-EDTA to food (RIVM, 2014).

Whilst the findings mentioned above are a clear indication that DTPA is capable of producing developmental toxicity at high oral doses and EDTA at even higher doses, viz. > 1000 mg/kg bw, in the presence of visible maternal toxicity, the observed effects do not represent an intrinsic property of the chelants and the relevance of these effects to man is questionable. It is considered that the developmental toxicity is occurring secondary to a maternal Zn-deficiency, resulting in a disturbed maternal metal ion homeostasis, which should also be considered maternal toxicity. To reproduce this toxicity in a pregnant woman, these chelating agents would have to be administered in such a way that the zinc status would be negatively impacted throughout pregnancy. This would require significant oral doses of DTPA during a certain period as explained earlier.

The support for the hypothesis that DTPA produces developmental effects by creating a Zn-deficiency comes from an understanding of three key issues:

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

The production of congenital malformations in a mammal by maternal Zn-deficiency was first reported in 1966 in the Sprague-Dawley rat (Hurley and Swenerton, 1966). Since this initial report, there has been considerable work on the teratogenic effects of Zn-deficiency and the mechanisms involved. The types of defects produced by Zn-deficiency are many, and they occur with high frequencies. Fetuses of Zn-deficient rats have been reported to have brain defects, eye defects, cleft palates, and skeletal defects, as well as gross malformations of the cardiovascular, respiratory, and urogenital systems (Hurley and Swenerton, 1966; Hurley, 1969; Mills et al., 1969; Hurley and Shrader, 1972; Warkany and Petering, 1972; Apgar, 1972). In addition to these structural defects, the biochemical development of the lung (Vojnik and Hurley, 1977) and pancreas (Robinson and Hurley, 1981a, 1981b) is also adversely affected by gestational Zn-deficiency in the rat. Subsequently it is very well understood that a nutritional deficiency in zinc in the maternal organism can produce very severe consequences in the developing fetus depending on the extent, duration and timing of Zn-deficiency during pregnancy (Hurley et al., 1971; Hickory et al., 1979; Record et al., 1985). This is important because less extensive effects are associated with a mild or fluctuating Zn-deficiency during pregnancy, and the timing during pregnancy will determine the organ systems/skeletal structures affected (Hurley et al., 1971; Record et al., 1985; Hickory et al., 1979). The effects of short term/transitory Zn-deficiency highlight the fact that in rats the maternal organism does not seem to be capable of compensating for a sudden drop in zinc intake by mobilizing zinc stores (Hurley et al., 1971); in fact it seems that during periods of Zn-deficiency the maternal liver sequesters zinc via induction of the Zn-binding protein, metallothionein, thus restricting the supply of zinc to the embryo/fetus (Rogers et al., 1985). King (2000) noted that in humans and animals, transfer of sufficient zinc to the fetus is dependent on maintenance of normal maternal serum zinc concentrations, therefore interference with the maternal zinc status is the first step in producing a Zn-deficiency in the developing fetus. Importantly, teratogenic effects have resulted from a decrease in the transfer of zinc to the embryo, even in the absence of detectable decreases in absolute zinc levels (Daston et al., 1991; Keen et al., 2003; Leazer et al., 1992; Taubeneck et al.,

1994). Thus it appears possible to induce teratogenicity in the absence of overt visible maternal toxicity. In addition, where a pair fed control group was also included, the restriction in food intake was shown to cause some decrease in maternal body weight gain but it did not result in any developmental toxicity (Hurley et al., 1971; Rogers et al., 1985). These findings implicate Zn-deficiency, and not reduced feed consumption as the causative agent responsible for developmental toxicity.

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

(2) Does DTPA induce Zn-deficiency in the maternal organism?

In the developmental toxicity study with Na5-DTPA (BASF, 1994) there was no assessment of zinc status in either the maternal or fetal organisms. Therefore, from the results of this study alone it is not possible to conclusively state that the zinc status was affected by DTPA administration. However, by considering the physical properties of DTPA and the results of other studies conducted with structurally similar chemicals, there is sufficient evidence available to support the conclusion that DTPA administration is capable of altering zinc status (Swenerton and Hurley, 1971; Fisher et al., 1975, 1976; Fukuda and Iida, 1983; Proksch and Kölmel, 1985; Brownie et al., 1986; Sato et al., 1997). Therefore it is plausible that this occurred in the teratogenicity study in question.

With the exception of the more recent toxicity studies conducted with Na5- or K5- DTPA, all previous experimental investigations of DTPA (calcium or zinc salts) have been conducted using non-oral routes of administration. Therefore there is very little information on the effect of oral administration of DTPA (gavage or dietary) on the general health (including zinc status) of animals and man. However, in the afore-mentioned 28-day study in rats (Elliott et al., 1987), gavage administration of the potassium salt of DTPA at doses up to 1330 mg/kg bw/day resulted in clear signs of toxicity at the highest dose. In the mid and low dose animals (83 and 333 mg/kg) with the exception of a reduced feed consumption in some mid dose males, there were no clinical signs observed. Whilst these data do not directly show that oral dosing of DTPA can cause a Zn-deficiency, it does demonstrate the consistency in toxicity with other chelating compounds such as EDTA which are generally believed to cause a Zn-deficiency (Swenerton and Hurley, 1971; ECB, 2004), albeit at higher doses (> 1000 mg/kg). At physiological pH DTPA has a 20-fold higher selectivity for zinc compared with EDTA as explained earlier. In the available repeated oral dose studies with EDTA, due to a slightly different complexation profile (higher affinity to calcium and lower affinity to zinc in the gut), the diarrhea was more pronounced in treated animals which may have increased the Zn-deficiency even further. The diarrhea might be induced by the higher preference of EDTA to calcium (see Tables 1 and 2) interfering with epithelial membrane stability and water efflux. However, the principle of induced zinc chelation/depletion is comparable between DTPA and EDTA with DTPA being the more potent chelator.

(3) What is the relevance of Zn-deficiency for the effects observed with DTPA in the developmental toxicity study?

The aforementioned studies of Fisher et al. (1975, 1976), Brummett and Mays (1977), and Calder et al. (1979), appear to demonstrate that Ca-DTPA is capable of causing fetotoxicity and malformations consistent with Zn-deficiency and that the frequency and type of these malformations is dependent on the dosage and dosing period during

pregnancy. Conversely, Zn-DTPA dosed at significantly higher dose levels for equivalent dosing periods does not appear to cause malformations. It does result in increased fetotoxicity albeit at extremely high dose levels. The explanation given by the investigators as to why there is a difference in teratogenicity between the calcium and zinc salts of DTPA is that the toxicity is due to the chelation of essential metals such as zinc and manganese (consider the data on increased excretion of zinc following DTPA administration) and that the zinc salt of DTPA cannot chelate any additional zinc. Ca-DTPA on the other hand will release the calcium and bind zinc in the body increasing its excretion and producing a zinc deficient state. It is therefore apparent that the form of DTPA (including DTPA acid, or the sodium and potassium salt of DTPA) which is dosed to pregnant animals is directly related to the potential for chelation of zinc. Thus forms of DTPA that can chelate zinc, i.e. those forms where the DTPA-metal complex has a lower dissociation constant than Zn-DTPA (Table 1), could potentially induce developmental effects; however, only at oral doses that would result in a Zn-deficiency.

Considering the work of Swenerton and Hurley (1971), it is highly plausible that, if the diet used in the developmental toxicity study using Na5-DTPA (BASF, 1994) had been supplemented with zinc as done by Swenerton and Hurley (1971), then the developmental toxicity would have been prevented. The concept of a mode of action involving Zn-deficiency is further supported by the data on other chemicals that can deplete zinc, where zinc supplementation negated the developmental toxicity of a dose known to be toxic to the fetus. This would also be consistent with the difference in the toxicity of Ca-DTPA compared with Zn-DTPA.

Whilst the data above demonstrate that DTPA is capable at high doses of producing malformations when not dosed as the zinc salt, there has been a question about the types of malformations observed in the developmental toxicity study conducted on Na5-DTPA (BASF, 1994). The effects observed in the fetuses in this study were not as severe as those observed with subcutaneous dosing of Ca-DTPA, or when observed in case of a complete Zn-deficiency. Missing vertebrae resulting from a Zn-deficiency have been reported in the past (Swenerton and Hurley, 1971) as have delays in ossification, missing ossification centers, bipartite sternebrae and many other skeletal deformities (Stevens et al., 1962; Hurley et al., 1971; Rogers et al., 1985; Record et al., 1985; Ferreira et al., 1989; Jankowski et al., 1995). As mentioned previously, Zn-deficiency potentially can affect a broad range of developmental processes, with the specific malformations manifested being mainly a function of exposure timing, duration and dose. Assuming that a functional Zn-deficiency requires at least a few days of dosing, the Zn-deficiency likely occurred during a stage of embryogenesis when axial skeleton patterning (i. e. the vertebrae and its derivatives) is being established. Thus, a causal association between DTPA-induced Zn-deficiency and missing vertebrae is entirely plausible. In addition, the duration of dosing and the timing of the drop in zinc availability during pregnancy will determine the degree of teratogenicity as well as of apparent maternal toxicity. Due to the relatively limited spectrum of effects observed in the developmental toxicity study (BASF, 1994) it is probable that the gavage dosing of DTPA did not cause a complete deficiency in zinc throughout pregnancy but instead zinc levels fluctuated, deficient levels coinciding with certain stages in fetal development such as skeletal development and bone ossification. Moreover, in the gavage study the animals were dosed in the morning, whereas they eat at night; so the animals may have (partly) re-compensated their zinc levels from the diet.

8.2. Classification issues

Classification for reproduction toxicity is, in contrast to acute toxicity, skin and eye irritation, skin sensitization, and specific target organ toxicity, **not** based on potency. Rather, classification for CMR (Carcinogenicity, Mutagenicity, Reproduction toxicity) is based on

evidence in humans and/or animals. DTPA appears to be developmentally toxic following administration at high oral doses in rats (retardations at 400 mg/kg bw and retardations/malformations at 1000 mg/kg bw) whereas EDTA exhibits developmental toxic effects at even higher doses i.e. above the limit dose for testing (1000 mg/kg bw). DTPA and EDTA are hardly absorbed from the gut but instead complex with essential metal ions such as zinc in the lumen of the gut preventing these metal ions from becoming bioavailable. At high doses this deprivation of essential nutrients leads to toxic effects in animals such as developmental toxicity due to the importance of zinc in the healthy development of a growing fetus. In determining whether classification would be appropriate the assessment would need to take two parts. First, application of the guidance concerning how to classify for developmental toxicity but also estimation of exposures to the workforce (the group with the highest exposure levels) in conjunction with an assessment of how these exposures may affect the zinc status of the workforce, specifically the susceptible subgroup of pregnant workers.

Chemicals that would need classification as developmental toxicant cat. 1B according to the European Regulation EC 1272/2008 for CLP (CLP, 2008) and the Globally Harmonized System (GHS, 7th revised ed., 2017) are those for which ‘*animal data provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects, the adverse effect on reproduction is considered not to be a secondary nonspecific consequence of other toxic effects*’. In addition, it is indicated in section 3.7.2.2.1 of CLP that ‘*classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction and substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects*’. It is clear that the developmental and other effects of DTPA are only seen in the presence of a nutrient (viz. zinc) deficiency, and should therefore be considered secondary non-specific consequences. In addition, DTPA has no intrinsic, specific property to produce an adverse effect on developing organisms because if DTPA were to be directly toxic to the developing fetus then there should not be any difference in effects following dosing of the Ca-salt of DTPA versus the Zn-salt of DTPA. Moreover, the bioavailability of DTPA is limited, and in pregnant rats did not pass into fetal circulation (Zylicz et al., 1975). Thus there is no relevant systemic exposure; and it will not induce developmental effects in case sufficient zinc is available.

In addition, according to section 3.7.2.3.5. of both CLP and GHS: ‘*If appropriate information is available it is important to try to determine whether developmental toxicity is due to a specific maternally mediated mechanism or to a non-specific secondary mechanism, like maternal stress and the disruption of homeostasis*’. Substantial chelation of zinc in the diet, which occurred at high oral doses in rats, leads to a condition of malnutrition and overall disruption of essential metal ion homeostasis in the dams which in turn leads to a series of non-specific secondary effects in the fetus including abnormal bone formation, a process mediated by zinc containing enzymes. This state is further exacerbated by increased metallothionein production in the dam, leading to increased sequestration and hoarding of systemically available zinc resulting in even less being available to the developing fetus.

In sections 3.7.2.3.2 and 3.7.2.5.5 of CLP/GHS it is also stated that: ‘*If it can be conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals shall not be classified*’. And in section 3.7.2.3.4 of CLP/GHS: ‘*data from animal studies ideally shall provide clear evidence of specific reproductive toxicity in the absence of other systemic toxic effects. However if developmental toxicity occurs together with other toxic effects in the dam, the potential influence of the generalised adverse effects should be assessed to the extent possible*’. Taking conservative assumptions into account, the estimated worker exposures to

Table 9
Summary of conclusions on DTPA.

1.	DTPA as chelating agent binds di- or trivalent metal ions predominantly in the lumen of the gut since it is not absorbed following oral exposure
2.	Toxicity of DTPA is mediated exclusively by zinc deficiency since simple zinc supplementation negates all developmental (and other) toxicity
3.	Reduction in dietary zinc in the dams disturbs zinc homeostasis not only by reducing absorbed zinc but also by increasing sequestration of available zinc by increasing metallothionein production
4.	Direct developmental effects on the fetus are not feasible since following administration to pregnant rats, DTPA is not found in fetal circulation
5.	Since zinc supplementation in the presence of the chelant prevents the developmental effect occurring then it cannot be said that DTPA possesses the intrinsic property to be a developmental toxicant
6.	Appropriate application of Regulation EC 1272/2008 should, when interpreted correctly, result in no classification for DTPA

DTPA during manufacture are low. In addition, consumer exposure levels would even be lower due to the much lower levels of DTPA in formulated products that may be available to the consumer. As indicated earlier, the effect of conservatively estimated worker exposures to DTPA under normal working conditions would not be considered to be detrimental to a worker's (including pregnant women) zinc status. Thus it is highly unlikely that a consumer's zinc status would also be negatively affected, considering the significantly lower level of exposure in the general population. Thus exposure to DTPA would not result in the development of a Zn deficiency; rather, the occurrence of developmental toxicity in humans would require unrealistically high exposure situations and thus is extremely unlikely.

Further, it is stated in section 3.7.2.3.4 of CLP/GHS that: *'The preferred approach is to consider adverse effects in the embryo/fetus first, and then evaluate maternal toxicity, along with any other factors which are likely to have influenced these effects, as part of the weight of evidence. Discounting developmental effects that are observed at maternally toxic doses can only be done on a case-by-case basis when a causal relationship is established or refuted'*. Unfortunately, the extent of maternal toxicity, is not quantified or explained in the regulation or its respective guidance documents. Maternal toxicity was assumed to be present in case of e.g. > 10% death in the dams, or heavily decreased body weight or body weight gain. In the case of DTPA, however, it can be concluded that the dose-related developmental toxicity occurred together with a dose-related Zn deficiency or uncompensable fluctuations in the zinc levels of the dam, and that, as explained before, the fast growing fetuses suffered more from this zinc shortage than their mothers. Although this was not substantiated by measurements of zinc levels (however, maternal plasma zinc levels are of low predictivity for the presence or absence of fetal Zn deficiency (Brownie et al., 1986)), and maternal toxicity was not visible at the 400 mg/kg bw/day level at which a slight increase in retardations but no malformations were observed, classification of DTPA as a developmental toxicant Cat. 2 H361d (oral) would still be overly conservative, especially in view of the lack of the (confirmed) intrinsic property to induce developmental toxicity. However, classification with Cat. 2 would represent a compromise to communicate the potential effects of DTPA on zinc status, and the potential association between a Zn deficiency and developmental effects, while also recognizing the highly unlikely circumstances that could lead to this kind of effect in a pregnant worker.

9. Conclusions

The differences observed in the developmental toxicity of the calcium salts of DTPA and EDTA versus their zinc salts indicate that such

chelates themselves are unlikely to have a direct developmental toxic effect on the developing fetus. If Aminocarboxylic acid (ethylenediamine-based) chelating agents were directly teratogenic, viz. would have an intrinsic developmental toxic property, then the salt form (calcium or zinc) should not significantly influence the teratogenicity particularly following parenteral (intravenous or subcutaneous) dosing.

Based on the data available, it is concluded that oral treatment of pregnant rats with high doses of DTPA and even higher doses of EDTA will lead to a significant amount of zinc ions bound in the gut and excreted in the feces. The potential increase in zinc excretion may then cause a reduction in the zinc available to the mother and the developing fetus. Considering that pregnant rats facing sub-optimal levels of zinc do not appear to be able to mobilize tissue stores of zinc to ensure adequate supply to the fetus, the disturbance in zinc level homeostasis will result in an insufficient supply of zinc to the fetus which in turn produces a. o. malformations and increases fetotoxicity.

There is substantial data to support the hypothesis that the developmental toxicity resulting from treatment of rats with high doses of DTPA is not the result of a specific effect of the absorbed test substance but the result of an induced deficiency of zinc in the mother which subsequently impacts the fetus. Developmental toxicity secondary to a Zn-deficiency should not be considered relevant for classification, as DTPA, when administered in laboratory animals in conjunction with zinc, simply loses the ability to cause developmental effects which should not be possible if it were a truly intrinsic hazardous property of the substance (see summary in Table 9). In addition, as a classification according to CLP criteria should ensure the level of protection, it has been demonstrated that exposure to DTPA in a worst-case assessment is insufficient to produce a deficiency in zinc in the worker or consumer population, including also susceptible populations (e.g. pregnant women).

The current way of classification for reproductive toxicity being only based on available evidence in humans and/or animals, and not on potency, seems to be outdated and should in our view be reconsidered. Moreover, clearer guidance is required both for authorities and industry so that substances can be classified appropriately in the interest of worker safety and public health. It appears as though classification and labelling is meant to effectively identify substances of concern; it should not be a driver for substitution with less efficient chemicals that may be equally or more hazardous, or perhaps hazardous in other ways. Especially so, since proportionality and relevance can be questioned as Article 1 of CLP (1272/2008) states that *'the purpose of this Regulation is to ensure a high level of protection of human health and the environment'*. If DTPA would be classified, lacking as they do a truly intrinsic property to be developmentally toxic, we can only conclude classification under EU rules is superfluous, disproportionate and as currently applied not truly protective of worker safety and public health.

Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.yrtph.2018.06.019>.

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