

Dow comments on the harmonized classification and labelling proposals for N-carboxymethyl iminobis(ethylenenitrilo)tetra(acetic acid) [DTPA acid] and pentapotassium 2,2',2'',2''',2''''-(ethane-1,2-diyl)nitriilo) pentaacetate [pentapotassium DTPA].

We welcome the opportunity to provide comments during the public consultation period for the above two substances. As dossier submitter for pentasodium DTPA we have valuable information regarding the data used to support the classification as Reprodevelopmental toxicant Category 2, also for DTPA acid (Cas. No. 67-43-6) and pentapotassium DTPA (Cas. No. 7216-95-7).

As dossier submitter for pentasodium DTPA (Cas. No. 140-01-2) we received a number of comments concerning the OECD 414 Prenatal developmental toxicity study with pentasodium DTPA (BASF 1994) in which a number of malformations, retardations and variations were apparent in the offspring of animals dosed at 1000 mg/kg bw/day or 400 mg/kg bw/day. In response to those comments, we submitted historical control data and we would ask that the data be taken into consideration for both the DTPA acid and pentapotassium DTPA.

From the data, it is clear that at 1000 mg/kg bw/day (the limit dose for this study), statistically significant decreases in bodyweight gain (21%), and overall bodyweights (Day 17 and 20) as a result of reduced food consumption (approx. 7% during treatment period GD 6-15) were apparent (tables 1 and 2). At this dose level, a statistically significant reduction in live fetuses/litter, mean fetal bodyweights and increased numbers of malformations, variations and retardations were observed (tables 3 and 4).

At 400 mg/kg bw/day no overall effect on maternal parameters was observed (tables 1 and 2). However an increase in skeletal retardations versus concurrent controls was apparent. Whilst this increase in retardations was statistically significant, the overall number of retardations was still well within historical control values. Actually, when comparing the variations and retardations observed to historical control data, the only finding of significance at this dose level would be a slight increase in fetuses showing incomplete ossification of the skull (table 4).

We have concluded, based on data available on EDTA (see further response to comment 4), these developmental findings are secondary to zinc insufficiency as a result of DTPA chelating zinc both in the diet and the zinc available systemically in the dam, and at high levels, also secondary to induced maternal toxicity.

At 1000 mg/kg bw/day a statistically significant increased number of malformations was observed in conjunction with considerable maternal toxicity manifested as significantly reduced bodyweights, a significant reduction in body weight gain and reduced food consumption. As already mentioned, at 400 mg/kg bw/day, retardations in ossification were observed in the absence of overt maternal toxicity.

It should be considered however, when conducting a standard OECD 414 study, the number of investigations performed is very limited compared to an OECD 407 study (or OECD 408) in which the investigations are more extensive and detailed in the parent animals e.g. full histopathology, haematology, clinical chemistry and others. Indeed for pentasodium DTPA, a 28-day oral (drinking water) repeat dose study in rats was conducted and dose levels of approximately 420 mg/kg bw/day resulted in changes in clinical chemistry parameters. It is therefore likely in the developmental toxicity study at 400 mg/kg bw/day that maternal toxicity was present but was not detected because of the limited number of investigations performed.

It is well known that skeletal ossification is a zinc-dependent process, and is severely impacted in cases of zinc deficiency. It is also worth noting that as a finding, incomplete ossification of the skull is considered a retardation of low to moderate concern (Moore et al 2013, ECETOC 2002) i.e. minor variations to the norm that would not normally justify classification.

The most plausible explanation for the developmental findings is that at 400 mg/kg bw/day, the DTPA administered is chelating sufficient dietary zinc to induce a deficient state in the mother but no outward signs of maternal toxic effects as found at 1000 mg/kg bw. Under conditions of zinc deficiency, the dam maintains liver zinc levels via increased metallothionein expression at the expense of the circulating plasma concentration and a concomitant reduction in foetal zinc levels would occur. Such processes would help maintain sufficient internal zinc levels in the dam such that outward signs of toxicity would not be apparent.

Given the effects observed at 400 mg/kg bw/day were retardations of low concern, were secondary toxicities associated with primary zinc depletion, and were apparent only when following a dosing regimen that is non-representative of potential human exposure (bolus gavage versus continuous, dietary) we conclude that classification of pentasodium DTPA, as well as of DTPA acid and pentapotassium DTPA, as developmentally toxic, GHS category 2 is considered most appropriate.

References

ECETOC. (2002). Guidance on Evaluation of Reproductive Toxicity Data. Monograph No. 31. European Centre for Toxicology and Ecotoxicology of Chemicals, Brussels

N.P. Moore et. al. (2013) Guidance on classification for reproductive toxicity under the globally harmonized system of classification and labelling of chemicals. *Crit. Rev. Toxicol.* 43(10): pp 850-891

Table 1: Maternal in-life findings

Findings	Control	DTPA-100	DTPA-400	DTPA-1000
Fd GD6-8	26.1±2.04	25.3±2.18	26.4±1.91	22.7±2.06
Fd GD8-10	26.0±1.94	25.7±2.46	26.1±1.85	23.4±2.75
BW GD17	352.6±21.32	349.5±25.55	350.5±27.63	332.8±18.25
BW GD20	405.6±26.64	404.6±28.35	402.8±37.95	378.7±26.93
BWG GD6-8	7.9±4.05	6.7±2.81	7.0±2.90	3.6±5.33
BWG GD15-17	22.4±4.11	22.0±5.06	20.5±6.54	17.5±5.11
BWG GD6-15	43.7±8.01	44.5±6.25	43.6±8.75	34.6±10.23
BWG GD15-20	75.4±9.88	77.1±12.04	72.8±17.61	63.5±13.57
BWG GD0-20	148.0±16.88	150.3±19.09	141.4±26.70	125.2±19.41

Fd – Food consumption (g), BW – Body weight (g), BWG – Body weight gain (g), GD – Gestation days

Table 2: Maternal necropsy findings

Findings	Control	DTPA-100	DTPA-400	DTPA-1000
Uterus wt (g)	80.8±10.75	80.1±13.95	76.9±22.87	64.2±20.01
Carcass wt (g)	324.8±19.20	324.6±24.55	325.9±23.11	314.5±13.83
Adjusted wt gain (g)	38.3±6.49	41.4±9.95	39.6±10.00	33.9±9.67

Table 3: Litter findings (in number and g)

Findings	Control	DTPA-100	DTPA-400	DTPA-1000
Live foetuses	14.3±1.96	14.0±2.54	13.5±4.19	11.9±3.78
Foetal wt (all)	3.7±0.21	3.7±0.23	3.7±0.26	3.4±0.29
Foetal wt (♂)	3.8±0.21	3.8±0.25	3.8±0.24	3.5±0.30
Foetal wt (♀)	3.6±0.22	3.7±0.25	3.6±0.29	3.4±0.28

Table 4: Skeletal examination

Values for each endpoint are number of affected litters per group and percentage of affected fetuses per litter.

Findings	Control	DTPA-100	DTPA-400	DTPA-1000	Historical Control
No. Litters	23	22	22	22	819
<i>Malformations</i>					
Total	7	3	8	16	191
	6.7±14.03%	1.9±4.82%	4.7±6.58%	27.7±31.15%	23.3%
Thoracic vertebra absent	0	0	0	6	5
	0.0±0.00%	0.0±0.00%	0.0±0.00%	12.8±29.39%	0.6% (0.0-9.1)%
Lumbar vertebra absent	0	0	0	5	2
	0.0±0.00%	0.0±0.00%	0.0±0.00%	5.9±15.71%	0.2% (0.0-4.0%)
Sternebra(e) bipartite, ossification centres dislocated	1	0	2	6	37
	0.5±2.61%	0.0±0.00%	1.2±4.06%	5.4±10.11%	4.5% (0.0-13.6%)
<i>Variations</i>					
Total	22	21	21	21	763
	49.6±26.19%	48.6±20.09%	46.7±23.60%	78.4±26.74%	93.2%
Shortened 13 th rib	11	10	10	18	286
	13.6±18.37%	12.3±18.91%	13.0±18.25%	47.5±32.46%	34.9% (13.6-57.1%)
Rudimentary cervical rib(s)	2	5	3	11	119
	2.7±10.63%	2.9±5.42%	1.8±4.61%	21.3±31.20%	14.5% (0.0-33.3%)
Absent 13 th rib	0	0	0	12	4
	0.0±0.00%	0.0±0.00%	0.0±0.00%	21.8±30.57%	0.5% (0.0-4.8%)
<i>Retardations</i>					
Total	22	22	20	21	732
	47.4±24.75%	48.4±26.66%	63.8±33.50%	78.0±31.29%	89.4%

Skull incompletely ossified	1	2	6	7	14
	1.0±4.63%	1.2±3.95%	4.6±8.65%	8.5±16.07%	1.7% (0.0-8.3%)
Sternebra(e) not ossified	8	7	11	18	295
	4.8%	6.8%	17.1%	50.7%	36% (11.1-58.3%)

Ranges indicated in brackets