

# Diethylenetriaminepentakis- (methylenephosphonic acid) and its sodium salts

<b>MAK value</b>	<b>not established, see Section IIb of the <i>List of MAK and BAT Values</i></b>
<b>Peak limitation</b>	–
<b>Absorption through the skin</b>	–
<b>Sensitization</b>	–
<b>Carcinogenicity</b>	–
<b>Prenatal toxicity</b>	–
<b>Germ cell mutagenicity</b>	–
<b>BAT value</b>	–
<b>Synonyms</b>	Diethylenetriamine-N,N,N',N'',N''-penta- kis(methylenephosphonic acid) Diethyle- netriamine pentakis(methylene phos- phonic acid)  Diethylenetriamine penta(methylene phosphonic acid)  Ethylenetriamine penta(methylene phos- phonic acid)
<b>Chemical name</b>	[[[(Phosphonomethyl)imino]bis[2.1- ethane diyl]nitrilobis(methylene)]]tetraki- sphosphonic acid
<b>CAS number</b>	Diethylenetriamine pentakis 15827-60-8 (methylenephosphonic acid)

## 2 Diethylenetriaminepentakis-(methylenephosphonic acid)

	sodium salts, number of sodium ions
	x <sup>1)</sup> 22042-96-2
	1 94987-76-5
	2 94987-75-4
	3 95015-06-8
	4 94987-77-6
	5 61792-09-4
	6 93841-74-8
	7 68155-78-2
	8 95183-54-3
	9 93841-75-9
	10 93841-76-0
Structural formula	
	$\begin{array}{c} \text{CH}_2\text{-PO}_3\text{H}_2 \\   \\ \text{N}((\text{CH}_2)_2\text{-N}(\text{CH}_2\text{-PO}_3\text{H}_2)_2)_2 \end{array}$
Molecular weight	acid: 573.20 salts: 595.18–793.02
Melting point	acid: > 200 °C, estimated (OECD 2005) salts: not specified .
pH	acid: < 2 disodium salt: 2–3 heptasodium salt: 7–9 each in 1% solution (OECD 2005)
log K <sub>ow</sub> <sup>*</sup>	acid: –3.4 (OECD 2005) salts: not specified
Solubility	500 g/l as acid in water, > 500 g/l at pH 6–9 (OECD 2005)
Stability	not specified

1) Includes all sodium salts.

\* n-octanol/water partition coefficient

Production	acid: not specified salts: from the acid with NaOH
Purity	70–80% (OECD 2005)
Impurities	acid: according to sample and manufacturer: typically up to 16% components with 4 methylene phosphonic acid groups, up to 2% other organophosphonates, up to 7% inorganic acids containing phosphorus, up to 12.8% HCl (ACCPACP 2003 a; OECD 2005); salts: up to 10% HCl (ACCPACP 2003 b), here in neutralized form as chloride, as the pH value of the salts is alkaline
Use	emulsifier, dispersing agent, complexing agent, anticorrosive and hardness regulator in household and industrial cleaners, metal-working fluid, water-based processes and in water treatment, stabilizer for peroxides, and as an additive in oil production (OECD 2005)

This documentation is based on the data file of the OECD on “Phosphonic Acid Compounds Group 3” (OECD 2005); the IUCLID Dataset on diethylene triamine pentakis(methylene phosphonic acid) (DTPMP) (AACPACP 2003 a); the data on the sodium salts of DTPMP, which include data on the octasodium salt (CAS No. 95183-54-3) as well as the heptasodium salt (CAS No. 68155-78-2) and in addition the data on salts with an indefinite number of sodium ions (CAS No. 22042-96-2) (ACCPACP 2003 b).

## 1 Toxic Effects and Mode of Action

When used in an aqueous environment, a complete dissociation of the diethylenetriamine pentakis(methylenephosphonic acid) (DTPMP) salts must be expected. With the exception of pH-dependent endpoints, both the acid and the salts can be assumed to have similar effects. Oral and dermal absorption are very low. After ingestion, DTPMP salts are eliminated almost completely with the faeces. The acute toxicity of DTPMP and its salts is low. After exposure for longer periods, effects on iron metabolism occur, which result in anaemia and changes in the

spleen. Disturbances in magnesium, calcium and phosphate homoeostasis, as well as non-specific findings in the liver appear. The salts and the acid are only slightly irritating to the skin. In the eyes, the acid is a strong irritant but the salts are slightly irritating. Sensitization of the skin is not to be expected. The fertility of rats is not impaired up to the highest DTPMP dose tested of about 300 mg/kg body weight and day. Developmental toxicity in the form of vertebral anomalies is observed in rats at and above maternally toxic DTPMP salt doses of 2000 mg/kg body weight and day.

In a number of bacterial mutagenicity tests, the acid and the salts were not mutagenic and the acid was not clastogenic in the bone marrow of the rat as in a chromosome aberration test. On the other hand, tests in mouse lymphoma cells showed positive results for the neutralized and non-neutralized acid with metabolic activation, not, however, for a neutral salt having an undetermined number of sodium ions. A carcinogenicity study yielded no positive findings.

## 2 Mechanism of Action

DTPMP is a potent complexing agent. The anaemia observed in animal experiments can probably be attributed to the absence of available iron. Disturbances of calcium and magnesium homoeostasis have also been observed at high doses. The  $\log_{10}$  of the stability constant of the complexes of metal ions with DTPMP are: Hg 22.6; Cu 19.5; Zn 19.1; Ni 19; Co 17.3; Cd 9.7; Pb 8.6; Ca 6.7; Mg 6.6 (OECD 2005).

## 3 Toxicokinetics and Metabolism

### 3.1 Absorption, distribution, elimination

#### Acid

No data are available on the free acid. As the  $\log$  is  $K_{ow} -3.4$ , greatly discrepant absorption rates of  $2.3 \times 10^{-7}$  (Guy and Potts 1993) and  $6.3 \times 10^{-4}$  mg/cm<sup>2</sup> and hour (Wilschut et al. 1995) are obtained with the model calculations for dermal penetration. Altogether, this indicates that, the dermal absorption of the acid, similar to that of the salts (see below), is negligibly low.

#### Salts

A study of oral and dermal absorption was carried out using <sup>14</sup>C-DTPMP which had been “neutralized” with sodium. Rats received 10 mg/kg body weight of the substance orally and excreted, up to 72 hours after administration of the substance, 98% via the faeces, 1.3% in the urine and 0.4% with the exhaled air in the form of CO<sub>2</sub>. In a study with dermal application of 0.6 mg/kg body weight to rats lasting

72 hours, 89% of the applied dose was found on the skin, < 2% in urine, < 1.5% in the carcass and < 0.01% in the faeces (no other details) (OECD 2005). This means that DTPMP salts are practically not absorbed either orally or dermally, and almost completely eliminated with the faeces after oral administration.

In a study in rats with the <sup>14</sup>C-labelled sodium salt of aminotrismethylene phosphonic acid, the dermal flux in vivo was 0.0016 mg/cm<sup>2</sup> per 24 hours, i.e. 6.7 × 10–5 mg/cm<sup>2</sup> and hour (HERA 2004). A similarly low dermal penetration rate can be expected for DTPMP salts due to their similar structure and size. DTPMP is accumulated in the bones, a fact used in medicine for bone scintigraphy (Laznick et al. 1996).

### **3.2 Metabolism**

There are no data available.

## **4 Effects in Humans**

There are no data available.

## **5 Animal Experiments and in vitro Studies**

### **5.1 Acute toxicity**

#### **5.1.1 Inhalation**

There are no data available.

#### **5.1.2 Ingestion**

##### **Acid**

The LD<sub>50</sub> of DTPMP after administration of a 58% solution to rats was 4164 mg/kg body weight. In the animals that died, the gastrointestinal tract was inflamed and the liver slightly discoloured (no further details) (OECD 2005).

##### **Salts**

For the heptasodium salt of DTPMP, three studies are available. In the first investigation with the salt, 42% in water, 5838 mg/kg body weight were without lethal effects in rats. In a follow-up single-dose study with the salt after 8757 mg/kg body

weight, 9 out of 10 rats died within 48 hours. Symptoms were decreased respiration rate, diarrhoea, ataxia and convulsions. Accordingly, the LD<sub>50</sub> is here between 5838 and 8757 mg/kg body weight. In a further study with a 33% aqueous solution, no mortality occurred up to 14 days after administration of the maximum administered salt dose of 1650 mg/kg body weight. Therefore, this study does not contribute to the finding of the LD<sub>50</sub> (OECD 2005).

In a test in which a 26% solution of the octasodium salt of DTPMP and other components, together making up 43% “active salt” (10% HCl, 3% H<sub>3</sub>PO<sub>3</sub>, 3% HOCH<sub>2</sub>PO<sub>3</sub>H<sub>2</sub> and 1% H<sub>3</sub>PO<sub>4</sub>, all as sodium salts, 57% water; the pH value of the test substance as 1% solution was 10.9) was administered to Sprague Dawley rats by gavage, no mortality occurred up to the highest dose tested of 3870 mg/kg body weight “active salt”. The duration of the recovery period was not given. Thus, the LD<sub>50</sub> is above 3870 mg/kg body weight; (equivalent to > 2340 mg octasodium salt/kg body weight (ACCPACP 2003 b; OECD 2005).

### 5.1.3 Dermal application

#### Acid

After 24-hour occlusive application to rabbits of doses up to the maximum dose tested of 4605 mg/kg body weight, DTPMP as a 58% solution produced no mortality within 14 days. Therefore, the LD<sub>50</sub> is above 4605 mg/kg body weight (no further details) (OECD 2005).

#### Salts

In a study with rats carried out according to OECD Test Guideline 402, the heptasodium salt of DTPMP produced no mortality and no significant toxic effects up to 5838 mg/kg body weight (10 ml/kg body weight, 42% including impurities, occlusive, 24 hours). The dermal LD<sub>50</sub> of the heptasodium salt is therefore higher than 5838 mg/kg body weight. In another study with occlusive application of a 33% formulation of the heptasodium salt for 24 hours, no mortality occurred at 2145 mg/kg body weight within 14 days (ACCPACP 2003 b; OECD 2005).

A 26% aqueous solution of the octasodium salt of DTPMP (10% HCl, 3% H<sub>3</sub>PO<sub>3</sub>, 3% HOCH<sub>2</sub>PO<sub>3</sub>H<sub>2</sub> and 1% H<sub>3</sub>PO<sub>4</sub>, in each case as sodium salts, 57% water; the pH value of the test substance as a 1% solution was 10.9) was tested in rabbits at a dose of 2000 mg/kg body weight (no further details). The octasodium salt dose applied, i.e. 520 mg/kg body weight, induced erythema at the site of application, but no mortality. The duration of the recovery period was not reported. Therefore, the LD<sub>50</sub> of the octasodium salt is above 520 mg/kg body weight (no other details) (ACCPACP 2003 b; OECD 2005).

## 5.2 Subacute, subchronic and chronic toxicity

### 5.2.1 Inhalation

There are no data available.

### 5.2.2 Ingestion

#### Acid

There are no data available.

#### Salts

In a 90-day study carried out according to OECD Test Guideline 408, 12 male and 12 female Wistar rats were given feed containing a sodium salt of DTPMP (CAS No. 22042-96-2) in aqueous solution at concentrations of 0, 100, 1000 or 10 000 mg/kg diet. Related to the DTPMP salt this was equivalent to 8, 83 and 842 mg/kg body weight and day in males and 9, 92 and 902 mg/kg body weight and day in females. Effects on mortality, body weight or gross morphology were not found. Treatment-related findings occurred in the high dose groups only: in the male animals the absolute liver weight was slightly, but significantly decreased. In both sexes, the erythrocyte count was significantly increased, the mean corpuscular haemoglobin and the volume of the erythrocytes were decreased (haemoglobin not reported). In the spleen, iron complexes and age-related pigmentation were decreased. The serum iron concentration was decreased in the females, the iron binding capacity in serum was increased in the males. The total bone density and that of the trabecula were increased in both sexes. The only effect occurring below the highest dose was a reduced incidence of microlithiasis (microcalculus formation) in the kidneys of the females at all doses. The calcium plasma level remained always unchanged. The authors explain the influence on haemoglobin and bone metabolism with the ability of DTPMP to form chelates with calcium and iron. The no observed adverse effect level (NOAEL) was 83 mg/kg body weight and day (OECD 2005).

In an earlier 90-day study (conforming to OECD Test Guideline 408), Sprague Dawley rats were given a diet containing neutralized DTPMP (50% solution) in salt concentrations of 100, 1000 or 10 000 mg/kg, corresponding to 4, 45 and 511 mg/kg body weight and day in males or 6, 57 and 656 mg/kg body weight and day in females. The body and liver weights in the males were decreased in both upper dose groups (no other details). The only other finding in the middle dose group consisted of reduced haemosiderin in the spleen of both sexes. The anaemia of both sexes in the high dose group was characterized by decreased haemoglobin and haematocrit values, a decreased erythrocyte count and a lower plasma iron concentration. In addition, both heart weight and haemosiderin in the spleen were decreased.

At 4 mg/kg body weight and day, the liver weight was decreased in the males without noticeable histopathological findings. The authors attributed these effects to chelation of iron in the serum. These effects were reversible after 60 days. The NOAEL from this study is about 50 mg/kg body weight and day. This study was only available as a secondary citation in the OECD (2005) data compilation.

In a one-year feeding study with F344 rats carried out according to OECD Test Guideline 452, a neutralized solution of DTPMP was also used. In this case, the haemosiderin in the spleen was decreased at 100 mg/kg body weight and day and above, as were haemoglobin, haematocrit, and plasma iron and magnesium concentrations after 500 mg/kg body weight and day. In addition, unspecified changes in the histopathology of the liver were found. Liver and spleen weights were decreased. Effects on the bones were not reported. The NOAEL was 100 mg/kg body weight and day (ACCPAC 2003 b; OECD 2005). This study was also only available as a secondary citation in the OECD (2005) data compilation.

A two-year study carried out according to OECD Test Guideline 451 was also only available to the OECD as a secondary citation: Groups of 50 male and 50 female F344 rats received DTPMP salt by gavage at dose levels of 4, 20 or 100 mg/kg body weight and day. At 4 mg/kg body weight and day and above, the spleen weight was decreased in the males and the phosphate content of the bones decreased in the females. At 4 mg/kg body weight and day, the concentrations of iron in the plasma and of magnesium in the bones were decreased in the males, whereas the latter was increased in the females. Changes in the concentration of globulins, plasma phosphate and magnesium, a drop in the iron content and iron binding capacity of the plasma as well as of the haemosiderin in the spleen were found at 20 and 100 mg/kg body weight and day. The expected effects on bone mineralization, bone formation and resorption were absent (ACCPAC 2003 b; OECD 2005). A NOAEL was not given in OECD (2005). Effects occurred which were contrary or were only present in one sex, therefore, these adverse effects are unclear.

### **5.2.3 Dermal application**

There are no data available.

## **5.3 Local effects on skin and mucous membranes**

### **5.3.1 Skin**

#### **Acid**

Rabbit skin was tested occlusively for 4 hours with 0.5 ml of a 50% aqueous solution corresponding to 350 mg of the acid. The test solution contained 12.8% HCl. Only a very mild erythema and oedema occurred. The substance was considered to be



slightly irritating. The study had only minor deviations from OECD Test Guideline 404, these were not considered crucial for the results (OECD 2005).

In an earlier investigation, a formulation with 58% DTPMP (corresponding to 412 mg DTPMP, with maximum 56.8 mg HCl) was also slightly irritating to the rabbit skin after 24-hour occlusive application (ACCPAC 2003 a; OECD 2005).

In a subsequent study, a 50% solution (426 mg DTPMP with 10% HCl) was tested on intact and abraded skin (not stated whether occlusive) for 24 hours. A mild erythema was only found after 24 but not after 4 hours. The test substance was assessed as slightly irritating (ACCPAC 2003 a; OECD 2005).

### **Salts**

Six rabbits were treated occlusively with 0.5 ml of a formulation containing 42% DTPMP heptasodium salt, equivalent to 292 mg salt, for 4 hours. The study was in accordance with the OECD Test Guideline 404 of 1981. At 1, 24, 48 and 72 hours after application, the formulation produced no signs on the skin. This salt is therefore not irritating to the skin. After an extended application lasting 24 hours (and thus not conforming to OECD Test Guideline 404), 0.5 ml of the heptasodium salt in 33% aqueous solution (corresponding to 215 mg salt) was slightly irritating on the intact and abraded rabbit skin in a semi-occlusive test. Findings were examined only at 0 and 48 hours after termination of exposure. At point 0 only, very mild erythema was found, with additional oedema on the abraded skin. The primary skin irritation index was 0.4 on a scale with a maximum value of 8, the value only for intact skin was 0.25 (ACCPAC 2003 b; OECD 2005).

After 24-hour testing of a formulation with 26% octasodium salt (corresponding to 300 mg applied salt, pH value 10.9 as 1% solution) on intact and abraded skin, a very mild erythema occurred on the abraded skin in 4/6 rabbits and in one rabbit on the intact skin. The primary skin irritation index was 0.25 on a scale with a maximum value of 8, the value for intact skin only was 0.08 (ACCPAC 2003 b; OECD 2005). This salt is therefore not irritating to the skin.

### **5.3.2 Eyes**

#### **Acid**

In an eye irritation study, the tested dose of 0.1 ml of a 58% solution contained 82 mg active acid. In the conjunctival sac of rabbits, 24-hour contact resulted in moderate erythema, moderate discharge, mild oedema and complete recovery within 7 days (OECD 2005). The pH value is not given. According to ACCPAC (2003 a), the quantity of HCl contained in the test substance was not clearly apparent from the data presented; it could either have been 8% or insignificant.

In a subsequent study with a similar protocol, again 0.1 ml of an aqueous solution was used, which contained 85.2 mg active acid including 11.4–14.2 mg HCl. Here, the effects were of greater severity with severe initial pain, corneal cloudiness, reduced response to light, necrosis in the conjunctival sac and mild necrosis in the

lower cornea. The findings were similar when the test substance was rinsed after one minute (OECD 2005). The pH value is not given.

Although differences in the HCl content and consequently in pH value could be responsible for the differing results of both studies, DTPMP must nevertheless be considered as an eye irritant (OECD 2005).

### **Salts**

In a study carried out according to OECD Test Guideline 405, 0.1 ml of a 42% formulation with 58 mg heptasodium salt (including impurities) was slightly irritating to the rabbit eye. After one hour the values for irritation were: iris 1 on a scale with maximum 2 in 1/3 animals, conjunctival reddening 2 with maximum 3 in 2/3 and 1 in 1/3, conjunctival chemosis 2 with maximum 4 in 1/3 and 1 in 2/3 animals. The irritation disappeared within 24 hours. In the authors' opinion, the substance was not irritating though slightly irritating according to OECD (ACCPACP 2003 b; OECD 2005).

A further study with 43 mg of the heptasodium salt (including impurities) in 0.1 ml water showed similar effects. Reddening and oedema of the conjunctiva was still present in all six rabbits after 24 hours; on the other hand, the animals were free of signs two days after application (ACCPACP 2003 b; OECD 2005).

The result of a briefly reported study with 0.1 ml of a formulation, including 26% corresponding to 56 mg octasodium salt of DTPMP (impurities were 10% HCl, 3%  $\text{H}_3\text{PO}_3$ , 3%  $\text{HOCH}_2\text{PO}_3\text{H}_2$  and 1%  $\text{H}_3\text{PO}_4$ , in each case as sodium salts, the rest was water) was reported to be "slightly irritating" to the rabbit eye. According to the data sheet, the pH value of the test substance as a 1% solution was 10.9. The findings are only reported as irritation scores and were still present up to 72 hours after application on the conjunctiva of one animal (ACCPACP 2003 b; OECD 2005).

The salts were thus slightly irritating to the eye, while the acid was the stronger irritant. Differences between acid and salt in their irritant effects to the eye are assumedly explained by the different pH values.

## **5.4 Allergenic effects**

Sensitization tests in guinea pigs are available which, however, cannot be assigned to a particular substance of the group, as the study reports are not available in the original. Accordingly, either the acid or one of its salts was not sensitizing in the Buehler test carried out according to OECD Test Guideline 406 (concentration in both phases, induction and challenge, 5%) or in the maximization test with guinea pigs carried out according to OECD Test Guideline 406. In this case, the induction concentrations were 1% intradermally and 10% dermally, and the challenge concentration 2.5% (ACCPACP 2003 a, b; OECD 2005).

## 5.5 Reproductive toxicity

### 5.5.1 Fertility

#### Acid

In a fertility study groups of 20 male and 20 female Long-Evans rats were given DTPMP in concentrations of 0, 300, 1000 or 3000 mg/kg feed. Treatment began at mating of the  $F_0$  parents. The  $F_1$  animals were exposed from weaning up to day 21 after birth of the  $F_{2b}$  generation, so that only the  $F_1$  animals were exposed for a sufficiently long period in order to establish effects on male fertility. In the high dose group, the  $F_0$  dams bore litters with 21% less living foetuses having a weight at birth lower by 3%, both not significant. Contradictory findings were obtained with the  $F_{2a}$  and  $F_{2b}$  generations, i.e. the weights at birth of the offspring in the  $F_{2a}$  generation were significantly reduced by 6.5%, the gestation rate of the dams not significantly reduced by 17%. However, the weight gain of the offspring developed normally after birth. In the  $F_{2b}$  litters by contrast, the gestation rate of the dams was not affected, the birth weights of the foetuses were not significantly reduced (only 2%). As these findings were contradictory, they were considered to be of questionable toxicological importance in OECD (2005). The NOAEL for the impairment of fertility from this study is given as 3000 mg/kg feed, corresponding to 294 mg/kg body weight and day for males and 312 mg/kg body weight and day for females (ACCPACP 2003 a; OECD 2005).

#### Salts

In the oral study lasting 90 days carried out according to OECD Test Guideline 408 (see Section 5.2.2), ovaries, oviducts, cervix, uterus, testes, epididymides and seminal vesicles were also examined. No treatment-related histopathological changes were found in the female Sprague Dawley rats at doses of up to 842 mg/kg body weight and day and none in the males up to 903 mg/kg body weight and day (OECD 2005).

### 5.5.2 Developmental toxicity

#### Acid

A NOAEL of 1000 mg/kg feed (corresponding to 100 mg/kg body weight and day) for postnatal developmental toxicity was obtained from the study described in Section 5.5.1 (OECD 2005). This statement relates to the birth weights of the offspring reduced by 3% ( $F_1$ ), 6% ( $F_{2a}$ ) and 2% ( $F_{2b}$ ) and the slightly reduced number of living offspring ( $F_1$ ) in the high dose group (3000 mg/kg feed, corresponding to about 300 mg/kg body weight and day).

## **Salts**

A neutral DTPMP salt was administered by gavage to groups of 25 Sprague Dawley rats from days 6 to 19 of gestation in doses of 0, 500, 1000 or 2000 mg/kg body weight and day. The animals were examined on day 20 of gestation. After administration of 2000 mg/kg body weight and day, maternal toxicity was found (body weight gain significantly reduced by 32%). The number of foetuses per litter with skeletal anomalies was 0, 5.9, 4.8 and 15.9% for controls and the three exposure groups, and was not significant even in the high dose group according to available data; no historical control data were given. This involved, in the high dose group, three of 109 foetuses, of which two were with vertebral anomalies and one with a fusion of the ribs. At 1000 mg/kg body weight and day, one vertebral anomaly was observed in one of 124 foetuses. The authors of the study considered the skeletal effects to be treatment-related (ACCPACP 2003 b). Foetuses with subcutaneous haematomas were found in all groups, though the incidence was significantly increased only at 500 mg/kg body weight and day. Owing to the lack of a dose-dependency, these findings cannot be used for assessment. The numbers of corpora lutea, resorptions, post-implantation losses and living foetuses were not affected. Due to the slight and not significantly increased incidence, the occurrence of skeletal anomalies was assessed as being of unclear toxicological relevance, especially at 1000 mg/kg body weight and day. According to OECD (2005), the NOAEL is 1000 mg/kg body weight and day for maternal and developmental toxicity (OECD 2005).

## **5.6 Genotoxicity**

### **5.6.1 In vitro**

#### **Bacteria**

##### **Acid**

In a plate incorporation test carried out according to OECD Test Guideline 471 with *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 a 50% aqueous solution of DTPMP was tested up to concentrations higher than the toxicity threshold of 10 µl/plate with S9 mix and 0.3 µl/plate without S9 mix. No mutagenic effect was found (OECD 2005).

A second study with the same test substance and application of concentrations up to 10 µl/plate (toxic at 1 µl/plate and above without S9 mix and in some strains with S9 mix) in the same strains also produced negative results (OECD 2005).

## Salts

In the *Salmonella* mutagenicity test with preincubation carried out according to OECD Test Guideline 471 in *Salmonella typhimurium* strains TA98, TA100, TA1537 and TA1535 and in the *Escherichia coli* strain WP2 uvr, the sodium salts of DTPMP were not mutagenic up to 5000 µg/plate in the presence and absence of S9 mix. Without S9 mix, doses of 1250 µg/plate and above were toxic (ACCPACP 2003 b; OECD 2005).

## Mammalian cells

### Acid

Three TK<sup>+/−</sup> tests in mouse lymphoma cells L5178Y are available. The first was carried out using a 50% aqueous solution of DTPMP following the procedures which were later to become OECD Test Guideline 476 of 1984. Test concentrations were 70–1400 µg/ml active acid without S9 mix and 35–1050 µg/ml active acid with S9 mix. The peak concentrations caused a relative survival below 50%. In the presence and absence of S9 mix, the mutation frequency increased dose-dependently and reproducibly by a factor of more than two. The lowest effective concentration in the presence of S9 mix was 420 µg/ml, corresponding to 0.73 mM (relative survival 75%). To clarify whether the effect was pH value-related, a second test with neutralized test substance was carried out. In the presence of S9 mix, the results of this test were again positive (ACCPACP 2003 a; OECD 2005).

Another study confirmed that the positive findings were not due to the selection of pre-existent mutants. In this experiment in the presence of S9 mix, positive results were obtained in the expression on the second day, but not on the day incubation started and on day 6. Although the number of mutations increased dose-dependently, also in the absence of S9 mix to a greater extent than in the solvent controls, this was not significant (OECD 2005).

The positive result is supported by a further study with neutralized acid, with which the stability of the induced mutants was confirmed (OECD 2005). The aforementioned studies were all carried out before the publication of OECD Test Guideline 476 in 1984. In these studies, the colonies were evaluated automatically and not separately according to size. A differentiation according to colony size did not become obligatory until the revised test guideline was published in 1997.

In the HPRT test with CHO cells, the 19.7% acid induced no gene mutation at the HPRT locus in the presence and absence of S9 mix. The highest concentration of 8000 µg/ml was higher than that required for this test in OECD Test Guideline 476 and was also cytotoxic (relative survival 65% with and 44% without S9 mix). However, it was not clear whether the concentration referred to the test substance or the active acid (OECD 2005).

**Salts**

Neutralized with NaOH, a sodium salt (purity given with 46.9% as acid), CAS No. 22042-96-2, was tested up to 2200 µg/ml in the presence and absence of S9 mix in mouse lymphoma cells L5178Y. This test was carried out according to the OECD Test Guideline 476 of 1997. An increase in mutation frequency in the ethylmethane sulfonate and benzo(a)pyrene positive controls was observed, but not with the test substance. The toxicity threshold was, however, not attained, as the relative survival was around >75%. In this test, large and small colonies were differentiated between as requested by the test guideline (ACCPACP 2003 b; OECD 2005).

This result stands in conflict with the positive findings obtained using the same test system with the acid, especially as the result also remained positive after neutralization of the acid and a falsification of the result through selection of pre-existent mutants could be excluded. There is no plausible explanation for this discrepancy.

**5.6.2 In vivo****Acid**

There are no data available.

**Salts**

DTPMP in the form of an aqueous solution, neutralized to pH 7.0, was the test substance in a chromosome aberration test with Sprague Dawley rats carried out according to OECD Test Guideline 475. Six rats per sex and dose received up to 1970 mg/kg body weight (calculated as acid) by oral gavage and were killed after 6, 12 or 24 hours. The highest dose induced 25% mortality and body weight loss. The metaphases of 4–6 animals per group were evaluated. There was no decrease in the mitosis index. From this, the authors concluded that there was no exposure of the bone marrow to the substance. The frequency of chromosome aberrations was not increased (OECD 2005).

**5.7 Carcinogenicity****Acid**

There are no data available.

**Salts**

The two-year study in rats described in Section 5.2.2 with a sodium salt, CAS No. 22042-96-2 in the form of a neutralized 50% aqueous solution, was designed as a carcinogenicity study in accordance with OECD Test Guideline 451. It was not available in original form for the data compilation of the OECD (2005). After 0, 4,

20 and 100 mg/kg body weight and day, a total of 171 animals died without noticeable histopathological findings. There was thus no treatment-related effect on mortality. There were no differences in the incidences of neoplasms in the treated and untreated groups (no further details on carcinogenicity) (ACCPACP 2003 b; OECD 2005).

## 6 Manifesto (MAK value, classification)

Both bacterial gene mutation tests and one HPRT test in vitro as well as one chromosome aberration test in vivo yielded negative results. The findings from tests with L5178Y mouse lymphoma cells are inconsistent. Whereas positive results were obtained with DTPMP, also neutralized, in three tests in the presence of S9 mix, one sodium salt was not mutagenic. In this case, however, the maximum dose required by the OECD Test Guideline was not applied. Altogether, the contradictory mouse lymphoma test results cannot be explained.

In the only study on carcinogenicity, no increase in tumour incidences occurred. On the basis of the negative carcinogenicity study and the negative in vivo chromosome aberration test, there is no classification in any of the categories for carcinogens or germ cell mutagens.

The systemic NOAEL obtained from a 90-day study in rats with oral administration is about 80 mg/kg body weight and day. On the basis of two tests in rabbits, a strong irritant effect of the acid to the eyes must be assumed, though the salts are less irritating. As, however, neither investigations in humans nor animal studies are available on local effects after inhalation exposure, and a threshold value based exclusively on the systemic NOAEL (80 mg/kg body weight and day at 10 m<sup>3</sup> respiratory volume and 70 kg body weight would be equivalent to about 560 mg/m<sup>3</sup> in humans) is so high that irritation of the airways must be expected, no MAK value can be established.

Neither the salts nor the acid are acutely toxic after epicutaneous application. The dermal penetration in vivo of the structurally related aminotrimethylene phosphonic acid as a sodium salt is very low in rats, and less than 3.5% of the sodium salt of DTPMP is absorbed by rats through the skin after 72 hours (0.04% per hour). No corresponding data are available for the acid. Model calculations indicate a very low skin penetration. For this reason, DTPMP and its salts are not designated with an "H".

Signs of developmental toxicity in the form of skeletal anomalies were found in rats after administration of a neutral DTPMP salt at the maternally toxic dose of 2000 mg/kg body weight and day. The NOAEL for the developmental toxicity of the salt is 1000 mg/kg body weight and day. Indications of possible developmental toxicity (reduced weight at birth) after about 300 mg/kg body weight and day were obtained with the DTPMP acid (NOAEL 100 mg/kg body weight and day). A developmental toxicity study to describe possible skeletal anomalies has not been carried out.

In the Buehler and the maximization test with guinea pigs, DTPMP or one of its salts is not skin sensitizing. The test reports are, however, not available in the original. No reports on airway sensitization exist. Therefore, there is no designation with “Sh” or “Sa”.

## References

- ACCPACP (American Chemistry Council, Phosphonic Acid Compounds Panel) (2003 a) [[[(Phosphonomethyl)imino]bis[(ethylenenitrilo)bis(methylene)]]tetrakisphosphonic acid, sodium salt. IUCLID dataset, 09.10.2003, ACCPACP, USA
- ACCPACP (2003 b) [[[(Phosphonomethyl)imino]bis[(emlyenenitrilo)bis(methylene)]]tetrakisphosphonic acid, sodium salt. IUCLID dataset, 09.10.2003, ACCPACP, USA
- Guy RH, Potts RO (1993) Penetration of industrial chemicals across the skin: a predictive model. *Am J Ind Med* 23: 711–719
- HERA (Human & Environmental Risk Assessment) (2004) Human & environmental risk assessment on ingredients of European household cleaning products: phosphonates (CAS 6419-19-8; 2809-21-4; 15827-60-8), draft,  
[http://www.heraproject.com/files/30-F-04- HERA Phosphonates Full web wd.pdf](http://www.heraproject.com/files/30-F-04-HERA%20Phosphonates%20Full%20web%20wd.pdf)
- Laznick M, Laznickova A, Budsky F (1996) 99Tcm-DTPMP as a skeletal scintigraphy agent: distribution in rats in comparison with 99Tcm-MDP *Nucl Med Commun* 17: 1016–1020
- OECD (Organisation for Economic Co-operation and Development) (2005) Phosphonic acid Compounds group 3, OECD SIDS Initial Assessment Report, SIAM 18publication available, OECD, Paris, FR  
[http://webnet.oecd.org/hpv/ui/SIDS\\_Details.aspx?id=8285ed4e-e644-40b9-8244-5e7fcbadb585](http://webnet.oecd.org/hpv/ui/SIDS_Details.aspx?id=8285ed4e-e644-40b9-8244-5e7fcbadb585)
- Wilschut A, ten Berge WF, Robinson PJ, McKone TE (1995) Estimating skin permeation. The validation of five mathematical skin permeation models. *Chemosphere* 30: 1275–1296

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