Human & Environmental Risk Assessment on ingredients of European household cleaning products

Phosphonates

(CAS 6419-19-8; 2809-21-4; 15827-60-8)

DRAFT

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1. CONTENTS

2. EXECUTIVE SUMMARY

Phosphonates are a class of chelating agents and scale inhibitors. Three acids, aminotris(methylene phosphonic acid) (ATMP), 1-hydroxyethylidene diphosphonic acid (HEDP) and diethylenetriamine penta(methylene phosphonic acid (DTPMP), have been assessed. They are used in household cleaning products, personal care products, institutional cleaners and industrial cleaning processes, and as water treatment additives in various applications. Uses in household cleaning products, the scope of HERA, include laundry detergents, hand dishwashing liquids, and various hard surface cleaners.

The total volume of phosphonates used in Europe is not known exactly, but estimated to be in the 10,000 – 50,000 ton/year range on an active acid basis of which 12,000 tonnes/year of the three assessed phosphonates is used in household detergents and cleaning products (HERA, 2003).

An extensive environmental data set is available for phosphonates. On the environmental fate side, this includes biodegradation studies, simulation studies of removal in treatment systems, and a limited amount of field monitoring data. On the environmental effects side, acute as well as chronic single-species data are available.

To determine the Predicted Environmental Concentration (PEC), the removal in wastewater treatment plants was estimated from laboratory simulations and the available field data. Phosphonates do not significantly degrade in wastewater treatment plants, but are removed from the water by adsorption to sludge. The assessment of the fate in surface waters is conservative as known, but poorly understood removal mechanisms such as photodegradation have not been taken into account.

The Predicted No-Effect Concentration (PNEC) was based on chronic ecotoxicity data, although the full three trophic levels have not been studied for all three acids concerned. The data did reveal a discrepancy for HEDP between to two chronic tests on daphnia. As for both tests the validity cannot be properly assessed, an assessment was made with each of the data separately. Retesting for this endpoint is being planned by the ACC Chemstar Panel on Phosphonic Acid Compounds, of which the producer companies are members.

It could be shown that the use of phosphonates in HERA applications (household detergents and cleaning products) typically results in risk characterization ratios less than one, indicating no concern, for all environmental compartments. For HEDP there may be concern for the aquatic environment and for the sediment if the lowest PNEC is being confirmed by the future test results.

An additional exposure scenario was included in this risk assessment, by assuming that 5000 ton of each phosphonate would be used and discharged in Europe for applications outside the scope of HERA. Using the same exposure and effects assessment approach, it is shown that the additional amount of phosphonates does not change the conclusions of the HERA assessment.

Consumers are exposed to the phosphonates ATMP, HEDP and DTPMP through their presence in laundry and cleaning products mainly via the dermal route, but to some extend also via the oral and the inhalatory route.

A substantial amount of toxicological data and information *in vivo* and *in vitro* demonstrates that there is no evidence for ATMP, HEDP and DTPMP being genotoxic, mutagenic or carcinogenic. There is some conflicting data with regard to the mutagenicity of DTPMP, but the overall weight of the evidence suggests it is not mutagenic. There is also no evidence of reproductive toxicity or developmental effects in animals. The long-term toxicity of the acid or salt forms of the phosphonates under review was evaluated in several subacute, subchronic and chronic toxicity studies. In the available chronic and subchronic oral toxicity studies, no adverse effects for ATMP, HEDP and DTPMP were observed at dose level of 500, 24 and 20 mg/kg/day respectively.

To allow comparison of the consumer "systemic" exposure, the NOAELs for all three phosphonic acid compounds were corrected by the lowest systemic availability which was determined to be 2 % for the neutralized form of ATMP acid. The systemic NOAELs for ATMP, HEDP and DTPMT were calculated to be 10, 0.38 and 0.4 mg/kg bw/d, respectively.

The comparison of the aggregate exposure and the systemic NOAEL results in MOEs of 800, 1.200 and >1.000.000 for DTPMP, HEDP and ATMP respectively. These margins of exposure are large enough to account for the inherent uncertainty and variability of the hazard database and inter and intra-species extrapolations, which are usually considered by a factor of 100.

Under normal use conditions with direct skin contact (*e.g.*, in hand laundering or in hand dishwashing) the consumer is exposed to detergent solutions containing 0.001–0.01 % phosphonates. At these concentration levels, phosphonates are not irritating to eyes and do not alter the overall eye irritation profile of the cleaning product. Local dermal effects due to direct or indirect skin contact with phosphonate containing solutions in hand-washed laundry or hand dishwashing are not of concern because phosphonates are not contact sensitizers and are not expected to be irritating to the skin at in-use concentrations.

In summary, the human health risk assessment has demonstrated that the use of ATMP, HEDP and DTPMP in household laundry and cleaning detergents is safe and does not cause concern with regard to consumer use.

3. SUBSTANCE CHARACTERISATION

Phosphonates are a group of chemicals used in laundry detergent products as functional agents, providing beneficial effects such as complexing, anti-redeposition, etc. They are also used in hard surface cleaners as chelating agents.

The phosphonates covered in this assessment are also included in the ICCA HPV programme. The draft documents (SIARs and SIDS packages) were submitted by the Chemstar Phosphonic Acid Compounds Panel (Chemstar PAC) to the UK as the sponsor country in the OECD in 2003 and reviewed at SIAM 18 (April 2004). Data for this HERA assessment have been taken wherever possible from the SIARs.

3.1 CAS No and Grouping information

Phosphonates are multifunctional acids, which structurally have the phosphonic acid group – PO_3H_2 in common. The phosphonate groups are placed on different backbones, often bound through a methylene group to amines (amine methylenephosphonates), or directly onto a carbon atom. Being multifunctional acids, phosphonates will form salts or complexes of different composition, depending on the chemical composition and the pH of the environment.

These substances are used primarily as acids and as sodium salts. Their behaviour in the body or in the environment does not depend on the presence of sodium as the counter ion. For the purpose of the HERA risk assessment, phosphonate salts are therefore being grouped under their respective parent acid (Table 1). A full list of the CAS numbers of the relevant EINECS listed phosphonates is given in the Appendices A and B.

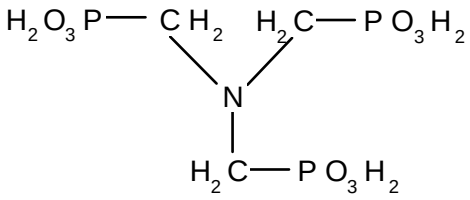
Table 1: Phosphonates most commonly used in the detergent industry

CAS No	Common chemical name	Abbreviation	Applications
6419-19-8	Amino tris(methylene phosphonic acid)	ATMP	Hard surface cleaners
2809-21-4	(1-hydroxyethylidene) diphosphonic acid	HEDP	Hard surface cleaners, laundry detergents, dish wash products
15827-60-8	Diethylenetriamine penta(methylene phosphonic acid)	DTPMP	Laundry detergents, I&I cleaners

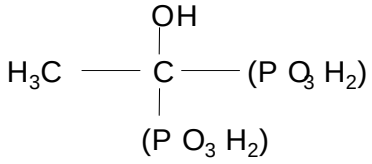
Each of the acids may be commercially supplied as such, or be neutralised to a sodium or other salt as the final product. The products are supplied mostly as aqueous solutions, but some salts are also sold as solids (powder or granules).

3.2 Chemical structure and composition

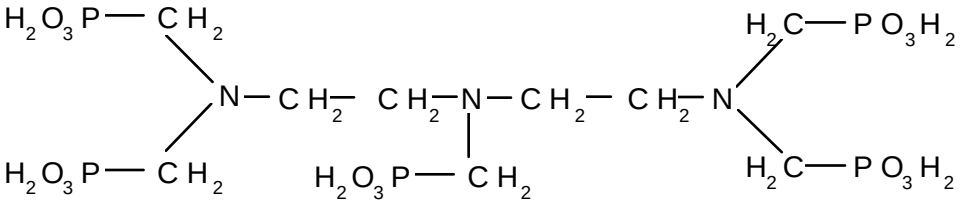
Figure 1 shows the structures of the reviewed phosphonates.



Amino tris(methylenephosphonic acid)
ATMP



(1-Hydroxyethylidene) diphosphonic acid
HEDP



Diethylene triamine penta(methylenephosphonic acid)
DTPMP

3.3 Physico-chemical properties

Table 2 gives a summary of the physico-chemical properties. More extensive discussions have been published (Gledhill and Feijtel, 1992; Landner and Walterson, 1993; W/M, 1997; Jaworska et al., 2002, Chemstar PAC, 2003).

The physico-chemical characteristics determining the health and environmental behaviour of phosphonates are: high water solubility, non-volatility, very low octanol-water partition coefficients, moderate to high sorption coefficients, multi-protic acidity and strong (transition) metal complexation.

Table 2: PHYSICAL-CHEMICAL PROPERTIES[1]

CAS #	Chemical	MW	M.P. °C	Vapour Pressure mmHg	Water Solubility @25 C mg/L	Henry's Constant atm-m^3/mol	Log Kow (2)	State at 25°C
6419-19-8	Phosphonic acid, [nitrilotris(methylene)] tris	299	Ca. 200 (decomp) (3) 225 (decomp) (5)	2×10^{-11} 1.9×10^{-10} Pa (5)	5×10^5 (5)	8.0×10^{-18}	-3.53	solid
2809-21-4	Phosphonic acid, (1-hydroxyethylidene)bis	222	Ca. 195 (decomp)(4) 198-199 (decomp at 228) (5)	1×10^{-10} 1.24×10^{-9} Pa (5)	6.9×10^5 (5)	5×10^{-17}	-3.49	solid
15827-60-8	phosphonic acid, [(phosphonomethyl)imino], bis[1,2 ethanediylnitrilo]bis	573	90 > 200 (estimated) (5)	2×10^{-11} 1×10^{-7} (2) 1.67×10^{-10}Pa (5)	5×10^5 (5)	7.3×10^{-18}	-3.40	solid

1. All unreferenced values from Epiwin: EPIWIN, U.S. Environmental Protection Agency. 2000. EPIWIN. Software for estimating physical/chemical properties.

2. Michael and Kaley, 1980

3. Beilstein and references cited therein: Maier,L., 1973, Helv.Chim.Acta, **56**, 1257; Moedritzer,K.; Irani,R.R., 1966, J.Org.Chemical, 31, 1603

4. Beilstein and references cited therein: Podobaev; Usminskii, 1979, J.Appl.Chem.USSR (Engl.Transl.), **52**, 406 and Zh.Prikl.Khim.(Leningrad), **52**, 442; Henkel, 1977, Patent, US 4060546; Maier,L., Helv.Chim.Acta, 1973, **56**, 1257

5. Chemstar PAC Panel (2003), draft SIARs

Table 2: PHYSICAL-CHEMICAL PROPERTIES (continued)

Stability constants of phosphonic acids (from Owens and Davis, 1980b)

CAS #	Chemical	Ca	Cd	Co	Cu	Hg	Mg	Ni	Pb	Zn
6419-19-8	Phosphonic acid, [nitrilotris(methylene)]tris	7.6	12.7	18.4	17.0	21.7	6.7	15.5	16.4	14.1
2809-21-4	Phosphonic acid, (1-hydroxyethylidene)bis	6.8	15.8	17.3	18.7	16.9	6.2	15.8	Insol	16.7
15827-60-8	phosphonic acid, [(phosphonomethyl)imino], bis[1,2 ethanediylnitrilo]bis	6.7	9.7	17.3	19.5	22.6	6.6	19.0	8.6	19.1

Whereas most of these properties can be reasonably expected from the structure, the phosphonic acid group is responsible for the high adsorption properties. Typical for these chemicals are the high sediment and soil adsorption coefficients (Table 3).

Phosphonates adsorb strongly on sediments and suspended particles, which further reduces the exposure of organisms. Sediment/water adsorption coefficients in the range 250 – 3900 were observed at concentration of 0.05 and 0.1 mg/l (Michael et al, 1980). At these concentrations, the concentration of the phosphonates in the water was reduced by two to three orders of magnitude. Although varying somewhat by the stucture of the molecules, adsorption is consistently much higher than what can be expected for highly water soluble, low Log Kow chemicals. In addition, adsorption is more pronounced at lower concentrations.

Similar results have been obtained by Fischer for HEDP. Freundlich "k" values for clay minerals ranged from 293 to 2378 (Fischer, 1991), for sediments from 407 to 2107 and for sewage sludge from 907 to 2718 (Fischer, 1992) depending on the specific clay, sediment or sewage sludge. For this assessment the mid-value of the reported range was used. The missing K values for DTPMP were assigned based on the similarity with ATMP and HEDP.

In addition to the high sorption coefficients, Michael et al (1980) and Fischer (1993) found that the adsorption of phosphonates is only partially reversible, and that only part of the adsorbed material can be desorbed again.

Table 3: Adsorption coefficients of phosphonates

Phosphonate		$K_{water\text{-}soil*}$	$K_{water\text{-}active\ sludge*}$	$K_{water\text{-}river\ sediment**}$
ATMP	measured	32 – 240	1360 – 3900	1100 – 1300
	used in this assessment	150	2630	1200
HEDP	measured	20 – 190	2600 - 12700	920 - 1300
	used in this assessment	115	8950	1110
DTPMP	measured	n.a.	n.a.	720
	used in this assessment	100	3000	720

* Steber and Wierich (1987, 1986)
** at 0.05 mg/liter (Michael et al, 1980)

Chelation is another important property to understand the behaviour and effects of phosphonates in the environment. Phosphonates form strong complexes with transition metals and with calcium and magnesium. The stability constants were critically reviewed on behalf of IUPAC (International Union of Pure and Applied Chemistry) by Popov, Rönkkömäki and Lajunen (2001). They extensively reviewed the literature, but for reasons of reliability, they did not succeed in putting forward recommended stability constants for many metals of environmental relevance. However, as the precise stability constants are not used in this assessment, the research by Owens and Davis (1980b) can be used as appropriated estimates of the various stability constants. Their results were summarised by Gledhill and Feijtel (1992) which give a useful overview for a range of important divalent metal ions (Table 2). Values reported below are log10 of the overall stability constant.

The work by Owens and Davis (1980b) did not include the measurement of the stability constants of iron (Fe^{2+} and Fe^{3+}). Due to the different experimental conditions and reporting, it is diffucult to compare the stability constants recommended by Popov et al. (2001) with those by Owens and Davis (1980b). Nevertheless, it is clear that Fe^{2+} and Fe^{3+} will form complexes of similar strength as the other transition metals.

These properties, namely complexation (sequestration) and surface effects due to adsorption, are also responsible for the performance characteristics of phosphonates in detergent products. The functional characteristics of phosphonates in laundry detergents are fourfold (Solutia, undated):

1) sequestration, in particular of heavy metals. This improves the stability of peroxide (perborate, percarbonate) and bleach systems. It also improves the removal of specific stains, such as tea, coffee, wine, etc.

2) Threshold effect on phosphate, carbonate and sulphate scales. This prevents the precipitation of insoluble salts and protects the fiber by inhibiting incrustation.

3) Deflocculation. This keeps soil from the laundry in suspension, preventing redeposition on the fabric and helping to retain the colours.

These properties improve the performance of other consumer detergent products in a similar way.

3.4 Manufacturing route and Production/Volume statistics

According to Davenport (2000) in the Chemicals Economics Handbook, organophosphonates are derived from phosphorous acid, which is produced from phosphorus trichloride or as a by-product. The aminomethylene phosphonates ATMP and DTPMP are produced by reaction of phosphorous acid, an amine and formaldehyde. HEDP is made by reacting anhydrous phosphorous acid with acetic anhydride.

Based on the input from the formulator companies (HERA, 2002 and 2003), the consumption of the different phosphonates is shown in Table 4.

Table 4: Consumer detergents phosphonate consumption (Tons of active acid/year)

		Volume		
CAS No	**Substance**	**6 HERA companies**	**Extrapolation to 100% of the market**	**Usage**
6419-19-8	ATMP	44	53	cleaners
2809-21-4	HEDP	7067	8480	all
15827-60-8	DTPMP	3412	4094	all
TOTAL		**10523**	**11627**	

Apart from these most recent figures, there are very limited statistical data on the total use of phosphonates in Europe. Gledhill and Feijtel (1992) provide estimations for the year 1990,

but the HERA data collection indicates that the use pattern may have shifted considerably since. More recent data are limited to an estimation of the total volume of all phosphonates.

Davenport (2000) quotes 14,000 – 15,000 ton of active product. Davenport estimates that 60% of the total volume is used in liquid and powder detergents. This would correspond to 9000 tons and, thus, confirms the order of magnitude of the HERA survey.

Total use volume data have also been reported within the framework of the Existing Substance Regulation (ECB, 2000; ESIS, 2003). The annual volumes, given in Table 5, cover the period 1990-1993 (actually up to March 1994). As these data refer to production, they are not suitable for the estimation of the total marketed quantities in Europe. For these data, ranges have been summed together. The possible use of acids as intermediates for the salt forms, leading to double counting in the reported volumes of the acids, has not been taken into account. Finally, also exports have not been taken into account.

Table 5: Phosphonate volumes reported for Regulation 793/93

CAS No	**Acid**	**Substance**	**Volume reported* (tonnes/year)**
6419-19-8	ATMP	acid	10 000 – 50 000
20592-85-2	ATMP	x Na salt	LPV
2809-21-4	HEDP	acid	10 000 – 50 000
7414-83-7	HEDP	2 Na salt	> 1000 (HPV)
29462-95-1	HEDP	x Na salt	> 1000 (HPV)
15827-60-8	DTPMP	acid	5 000 – 10 000
22042-96-2	DTPMP	x Na salt	10 000 – 50 000

* Production and importation volumes; source: ECB, 2000; except LPV substances and 22036-77-7: ESIS, 2003

Given the difficulty to obtain sufficiently accurate production and "non-HERA use" volume data, it was decided to estimate the contribution of the other uses, influencing the continental and regional background levels, by taking a 5000 ton/year scenario for each of ATMP, HEDP and DTPMP (further called the "5000 ton scenario") into account. The total of such estimated non-detergent uses is 15,000 Tons/year, Combined with the HERA/Consumer detergents volume, it would be about double of the volumes reported by Davenport (2000), but stays below the (unreliable) data from the Existing Substance Regulation (ECB, 2000; ESIS,2003) tonnages.

3.5 Use applications summary

Phosphonates are used in laundry detergents as additives providing a range of properties such as sequestration/complexation, anti-redeposition and dispersion. Phosphonates are also used in laundry detergents as perborate and percarbonate stabilisers, preventing decomposition by transition metals, in automatic dish washing products and in hard surface cleaners.

The major other application of phosphonates is in water treatment of cooling and boiler water as scale inhibitors. Other applications include reverse osmosis water treatment, the photographic industry, the paper and pulp industry and the textile industry. Phosphonates are further used as stabilisers for hydrogen peroxide solutions and formulations.

4. ENVIRONMENTAL ASSESSMENT

In the past a number of hazard and risk assessment have been prepared for phosphonates. The environmental data, including a limited risk characterisation, were reviewed in depth by Gledhill and Feijtel (1992). ATMP was reviewed in the OECD SIDS HPV programme (OECD, 1993). Environmental risk assessments were prepared for Sweden by Landner and Walterson (1993) and the Netherlands by the W/M (1997), recently published by Jaworska et al (2002). Complete hazard assessments were also prepared by the Chemstar PAC Panel (2003) as a contribution to the ICCA/HPV programme for presentation at SIAM 18 (Paris, April 2004).

4.1 Environmental Exposure Assessment

4.1.1 Environmental fate

a) Standard biodegradability screening tests

The classical tests, such as the OECD screening test, BOD20 test or the closed bottle test show only a low degree of ultimate biodegradation. For ATMP and HEDP a DOC (Dissolved Organic Carbon) removal of 23 - 33 % was observed in an inherent biodegradability test (Zahn-Wellens test), but mineralisation was very low even after long-term incubation (Gledhill and Feijtel (1992) and references cited therein; Table 6). However, several studies have shown that phosphonate degrading bacteria can be found in almost any environment whether soil, activated sludge or river water (Schowanek, 1990). At low ortho-phosphate concentration, i.e. if phosphate is the growth-limiting factor, phosphonate degradation occurs with almost complete breakdown of HEDP (94 %). DTPMP showed 60 % degradation under similar conditions (Schowanek, 1990). No quantitative study was done for ATMP. These phosphate-limited conditions are not likely to occur in the European environment.

Inherent biodegradation tests (Zahn-Wellens, SCAS testing, see Table 7) also indicate a low degree of biodegradation under the standard test conditions. For example, biodegradation of radiolabelled ATMP, HEDP and DTPMP resulted in SCAS tests in 0.5 to 10.2 % release of $^{14}CO_2$ over a 210 day period (Saeger, 1978).

As a consequence, for the HERA exposure assessment, no biodegradation is assumed in sewage treatment plants.

TABLE 6: Ready biodegradation data (Gledhill and Feijtel, 1992)

Property	ATMP	HEDP	DTPMP
COD*	0.468	0.44	n.a.
ThOD*	0.481	0.388	0.753
BOD_5*	0	0	n.a.
OECD screening test (% $^{14}CO_2$ evolution after 28 days)	20	1-3	

* expressed in mg O_2/mg of pure acid (not formulated)

TABLE 7: Inherent biodegradation tests

Property	ATMP	HEDP	DTPMP
Zahn-Wellens (% DOC removal after 28 days)	23[1]	33[2]	~50[3]
SCAS at pH 7 (% DOC removal after 26 days)	90 [4]	100 [4]	n.a.
SCAS (% $^{14}CO_2$ evolution over 210 days at 5 mg/l)	0.5-2.0[5]	1.9-6.7[5]	0.9-3.5[5]

[1]Steber and Wierich, 1987

[2]Steber and Wierich, 1986

[3]Cegarra et al., 1994

[4]Grohmann and Horstmann, 1988

[5]Saeger et al., 1978. Some removal observed, only little mineralization; no inhibition of COD or MBAS removal up to 160mg/liter

b) Degradation in river water

Whereas the laboratory tests indicate that biodegradation of phosphonates is poor, it becomes important to study and simulate the biodegradation under real-life conditions and to identify other non-biological removal mechanisms.

Research (Saeger et al., 1978) (Table 8) showed that in non-sterile natural water, the ultimate biodegradation ($^{14}CO_2$ evolution) of phosphonates ranged from 2.0 to 12.3 % in the dark, and 13.6 to 17.2 % in sunlight after 60 days indicating some degradation enhancement by sunlight, at a concentration of 2 mg/l . In sediment/riverwater systems the ultimate biodegradation was 28.6 - 30.7 % for DTPMP at 0.3 – 0.5 mg/l (Saeger et al., 1979).

These data show that the phosphonates are slowly degrading under simulated environmental conditions, and that there are several degradation mechanisms, including biodegradation and photodegradation.

For the HERA exposure assessment, the ultimate biodegradation in water has been estimated as 10% in 60 days for all three phosphonates, based on an average of the data reported by Saeger et al. (1978). The corresponding half-life is 395 days.

TABLE 8: Properties affecting surface waters

Primary degradation (loss of chelation titre, river water, 138 days) (Gledhill and Feijtel, 1992)

% loss	ATMP	HEDP	DTPMP
Dark	55	14	n.a.
Artificial light	50	0	n.a.
Sunlight	96	100	n.a.

Ultimate degradation (14CO2 evolution, River water, 60 days) (Saeger et al, 1978)

% biodegradation	ATMP	HEDP	DTPMP
Sterile			
Dark	0.1	0.2	1.4
Sunlight	6.2	2.7	3
Natural			
Dark	12.3	2	4.8
Sunlight	13.6	17.2	14.3

Ultimate degradation (14CO2 evolution) in surface water/sediment microcosms (Saeger et al, 1979)

% biodegradation	ATMP	HEDP	DTPMP
Lake water[1]			
Sterile	1.6-8.6	0.2-5.2	0.1-9.2
Active	4.7-12.3	4.6-9.6	14.8-16.9
River water[2]			
Sterile	n.a.	n.a.	0.2-1.0
Active	n.a.	n.a.	28.6-30.7

[1] Pristine lake water/sediment, 50 day study, 1 mg/l phosphonate, 16 hrs light/8 hrs dark cycle

[2] Missouri rive water/sediment, 38 day study, 0.3-0.5 mg/l phosphonate, 16 hrs light/8 hrs dark cycle

c) Degradation in river sediments

In the presence of sediment (Saeger et al, 1979) the biodegradation was found to be significantly faster in particular for DTPMP (Table 8). No marked difference is seen for ATMP and HEDP. However, the study also indicated that the phosphonates become tightly adsorbed onto the sediment, for a significant part irreversibly. This leads to the conclusion that the major part of the (bio)degradation may occur in the sediment but not in the water phase. For the HERA exposure assessment of sediments, it is assumed that the degradation occurs fully in the sediment. Half-lives were calculated, assuming an exponential decay, from

the average measured values, i.e. for ATMP 8.8% in 50 days, for HEDP 7.1 % in 50 days and for DTPMP 15.9% in 50 days and 29.6 % in 38 days. The corresponding half-lives are 376 days for ATMP, 471 days for HEDP and 200 days and 75 days for DTPMP. For the latter a half-life of 137.5 days was used in the assessment.

d) Anaerobic degradation

Anaerobic degradation has not been studied extensively. Steber and Wierich (1986, 1987) reported only minor conversion of ATMP and HEDP in model digestors. No inhibitory effect was observed neither for ATMP up to 100 mg/liter and for HEDP up to 5 mg/g dry sludge.

e) Biodegradation in soil

In soils, biodegradation of DTPMP occurs as shown in Table 9 (Saeger et al., 1977, 1978). ATMP and HEDP also show degradation, but slower than DTPMP. When sludges or sediments are disposed of at land, this will ensure mineralisation and removal from the environment.

For the HERA exposure assessment, the degradation half-lifes of ATMP and HEDP were extrapolated from the estimated degree of degradation of resp. 10 % and 20 % at 120 days, assuming an exponential decrease. For DTPMP, the measured half-life was used (45 days).

TABLE 9: Biodegradation in active soil (119 - 148 days) (Saeger V.W. et al., 1977, 1978)

	ATMP	HEDP	DTPMP
% $^{14}CO_2$ evolution	1-15	3-47	63-64
T1/2 (days)	789*	373*	40-50

*extrapolated

f) Hydrolysis

Phosphonates are quite stable in water as evidenced by the dark controls in the photolysis studies (see below). However, Steber and Wierich (1987) found that ATMP would hydrolyse fairly easily at low concentrations (70 ppb) with complete primary degradation in a few days. Saeger (undated) also reported 37 % degradation of HEDP in the presence of Cu ions. Schowanek (1990) studied the hydrolysis of phosphonates in detail, and came to the conclusion that metal ions, aerobic conditions and light were favourable conditions of the hydrolysis/degradation of these substances. Nowack and Stone (2000) identified a possible degradation mechanism for ATMP, catalysed by manganese and oxygen, confirming the earlier findings by Schowanek.

Although hydrolytic degradation mechanisms have been identified, they appear to be strongly dependent on the specific environmental conditions, and in particular on the presences of certain metal ions and light. Schowanek (1990) estimated the hydrolysis halflifes in the range of 50 -200 days at 15 – 25 °C. As average European conditions would be somewhat colder, the half-life for hydrolysis might be of the same order as biodegradation.

For the purpose of this risk assessment, hydrolysis was not taken into account. It is assumed that any hydrolysis is already covered by the reported biodegradation data, although this may underestimate the degradation rate, in particular at low concentrations

g) Photolysis

Photodegradation is another important route of the environmental removal of phosphonates. It is catalysed by transition metal ions and is pH dependent. The data reported by Saeger (undated) are shown in Table 10. It is especially pronounced in the presence of iron ions when 40 to 90 % degradation of the phosphonate-residues to ortho-phosphate occurs in 17 days. Other transition metals also stimulate photodegradation, in particular for HEDP. Further studies on HEDP (Fischer, 1993) confirmed these findings. HEDP was found to be degradable in river waters at neutral pH simulating day-light conditions. The rate of degradation was concentration dependent. At 3 mg/l, 70% was degraded in 8 days, at 10 mg/l, only 12.5 % was degraded. The half life was estimated at ca. 100 hrs at 3 mg/l.

Some limited evidence is available from field studies. Grohmann and Horstmann (1988) found a 50 % degradation of iron complex of PBTC (phosphonobutane tricarboxylic acid) after 5 -7 days and photodegradation was demonstrated down to 2 m depth in surface water. Although this substance contains only one phosphonate group, its behaviour seems quite similar to HEDP.

For the purpose of this risk assessment, photolysis was not taken into account. It is assumed that any photolysis is already covered by the reported biodegradation data in surface waters, as a light/dark cycle was applied. However, this may underestimate the degradation rate, in particular at low concentrations

TABLE 10: Photodegradation (pH 7, 17 days in tap water, % degradation to phosphate, exposure to natural sunlight) (Saeger, undated)

	ATMP	HEDP	DTPMP
Phosphonates only	2	7	14
with ferric ion	44	78	70
Hours of sunlight exposure	87	90	150

4.1.2 Removal in sewage treatment

As it was demonstrated that biodegradation in waste water treatment plants can be neglected, the discussion below focuses on the removal of phosphonates through adsorption onto sludges Many of the waste waters containing phosphonates will be discharged into a sewer and then treated in a factory or municipal waste water treatment plant. The treatment typically consists of a primary physical treatment, in which solids are allowed to settle, and a secondary biological treatment, where organic substances are degraded. Sometimes a tertiary physico-chemimal treatment e.g. for the removal of phosphate, is applied. Both laboratory (see Table 7) and field studies (Table 11) are available.

a) Laboratory tests

Adsorption to sludge (2-3 g DSS/l) from municipal sewage treatment plants was over 90 % for ATMP and HEDP (ca. 1-10 mg/l) after a 24-48 h incubation in a batch test (Steber and Wierich, 1987, 1986). At concentrations of 0.115 mg/l, adsorption was 80-90% for DTPMP (quoted in Gledhill and Feijtel, 1992). As the sludge adsorption coefficient was determined under sludge conditions typical for sewage treatment plants (STP) it is possible to calculate the adsorption-based elimination extent in the activated sludge (biological) stage of a STP. For HEDP an elimination extent of 25-60% was calculated (Steber and Wierich, 1986).

In a modified SCAS test, with the pH buffered at 7, over 90 % removal of DOC was observed for ATMP and HEDP after 26 days (Grohmann and Horstmann, 1988).

These findings suggest that adsorption onto sludge represents a very important removal mechanism for phosphonates in STPs.

b) Removal in mechanical-biological treatment plants

Field studies showed 50 % removal of ATMP and HEDP in the mechanical stage of a 2-stage sewage treatment plant (Müller et al., 1984). These studies were carried out at concentrations of 2 – 2.4 mg/l of phosphonate in the influent. This influent level is higher than what has been found in field studies (Nowack, 1998; Nowack and Saladin, 2000; Nowack, 2002) and what is being estimated for the exposure assessment in this report (highest value: 0.86 mg/l for HEDP; see below). As it is known that the adsorption of phosphonates is dependent on the concentration, and increases with decreasing concentration (refer to section 3.3 for the discussion), the results obtained from field studies with unrealistically high levels of phosphonate may underestimate the real elimination extent. Hence, preference should be given to data from studies obtained at more realistic concentrations, or from monitoring data.

Table 11: Elimination in sewage treatment plants

Property	ATMP	HEDP	DTPMP
Adsorption to sludge (%) (laboratory data)	> 90	> 90	80-90
Primary stage(% removal)	n.a.	50-70	n.a.
Field data (% removal in secondary treatment)		[calc. 60-90] (see 4.1.2.a)	95[1]
Total removal in a mechanical-biological STP incl. in tertiary treatment: field data (% removal)	>= 93[2]	n.a.	>= 85[3] 97[1]

[1] Nowack, 2002
[2] Nowack and Saladin, 2000
[3] Nowack, 1998

Higher removal rates were indeed found in a field study on DTPMP in a Danish WWTP (Nowack, 2002). A removal rate of 95% was observed after the biological step.

Removal is expected to be in a similar order of magnitude taking the comparable sludge adsorption coefficients of the phosphonates into account (cf. 4.1.2 a). This elimination both in the mechanical and biological stage will result in an elimination extent of 60 – 80%

c) Removal in plants with tertiary treatment

Recently a field study was carried out in Switzerland on a number of waste water treatment stations connected to textile plants (Nowack, 1998). All except one station were equipped with a tertiary treatment step (phosphate removal). The study quantified the removal of ATMP and DTPMP. In all but one case, the phosphonate concentration in the effluent was below the detection limit, indicating removal rates of at least 75 - 80 % for ATMP. In the single case where DTPMP was detected in the effluent, the elimination rate was 85 %. A similar study was carried out for ATMP in a German WWTP with tertiary phosphate removal (Nowack and Saladin, 2000) and for DTPMP in a Danish WWTP (Nowack, 2002). With

influent concentration ranging from 0.1 – 1.1 µM (0.03- 0.33 mg/l), no ATMP was found in the effluent (detection limit: 0.05 µM; 0.015 mg/l), leading to a removal rate of at least 93%. The removal of DTPMP in a Danish WWTP (Nowack, 2002) was monitored both after the biological step and after the final precipitation step. The removal rates were not significantly different: 95% after the biological step and 97% after the precipitation step.

The conclusion is that phosphonates at the influent concentrations expected for detergent uses, will be removed efficiently from waste water by adsorption onto the sludges in the treatment plants. The primary stage removal is of the order of 50 %. The removal after passing the secondary biological stage can be up to more than 90 %, with further removal in the tertiary stage.

Based on the discussed information, the following basic conditions were taken into account in the exposure assessment for the removal in waste water treatment (Table 12):

a) % degraded

Degradation in WWTP will be small. For the purpose of this risk assessement, no degradation has been assumed, although in practice a few % would be expected.

b) % to sludge

The main removal mechanism is WWTP is adsorption to sludge. Based on the lab and field testing, removal rates between 60 to 90 % can be expected depending on the type of phosphonate.

c) % to water

Due to the water solubility of phosphonates, the non-adsorbed moiety will stay in the water phase, i.e. 10 – 20 % depending on the type of phosphonate.

d) % to air

Phosphonates are not volatile. The only removal mechanism to air will be through spray. For this risk assessment, release to air is not taken into account.

e) Distribution in WWTP

The calculation in EUSES of the distribution to the various compartments using the measured adsorption coefficients (Table 3) underestimates the elimination of phosphonates from sewage water, as found in various laboratory and field studies e.g. calculation by EUSES for ATMP and DTPMP, using the adsorption coefficients given in Table 3 predicts a 63 % release to water and 37 % adsorbed on sludge. This result does clearly deviate from the observations in the field. Therefore, the EUSES output was corrected with the values shown in Table 12. Values are the same as those used by Jaworska et al. (2002).

Table 12: Overview of distribution in WWTP for each phosphonate

	ATMP	HEDP	DTPMP
Degraded	0	0	0
To sludge	80	80	85
To water	20	20	15
To air	0	0	0

4.1.3 Monitoring Studies

Suitable analytical methods have only quite recently become available for ATMP and DTPMP (Nowack, 1997). Monitoring studies have been limited to WWTP effluents and have been discussed earlier under removal in sewage treatment. Due to the low volatility, air monitoring studies are not applicable in for this assessment. No monitoring studies in soil, sewage sludge or sediment have been performed.

4.1.4 PEC Calculations

The following assumptions have been made in the PEC calculations:

- no biodegradation in sewage treatment
- removal through adsorption to sludge: see Table 11
- degradation in river water:
    - Biodegradation in water: T1/2 is 395 days
    - Biodegradation in sediment: T1/2 (days)

ATMP	HEDP	DTPMP
376	471	137.5

    - Photodegradation: not taken into account
    - Hydrolysis in water: not taken into account
- Adsorption to suspended particles: set equal to adsorption to sediment: see Table 3
- Adsorption coefficients: for DTPMP estimated from the data for ATMP and DTPMP (see Table 3)
- Degradation in soil
    - Biodegradation in soil: T1/2 (days)

ATMP*	HEDP*	DTPMP
789	373	45

*Extrapolated assuming resp. 10% and 20% degradation for ATMP and HEDP in 120 days

- All PECs are expressed as active acids.

4.1.4.1 The detergent scenario

The environmental exposure was modelled with EUSES. For the HERA consumer use assessment, Industry Category 5 (Personal/domestic use) and Use Category 9 (Cleaning/washing agents and additives) were used. The local HERA scenario was applied (factor 1.5 rather than 4), but for the regional scenario the standard regional fraction (10 %) was used. Release factors (private use):

- Soil: 0
- Water: 1
- Air: 0

a) PEC Water (total) (µg/l):

	ATMP	HEDP	DTPMP
Regional	0.059	9.6	4.0
Local	0.16	26	10

b) PEC Soil (mg/kg):

	ATMP	HEDP	DTPMP
Regional (agric.)	0.003	0.21	0.013
Local (agric.) 30 days	0.024	4.3	0.46

c) PEC Sediment (mg/kg):

	ATMP	HEDP	DTPMP
Regional	0.025	3.81	0.93
Local	0.043	6.4	1.6

d) PEC STP (mg/l)

	ATMP	HEDP	DTPMP
Local	0.0011	0.17	0.063

e) Concentration in dry sewage sludge (mg/kg)

	ATMP	HEDP	DTPMP
Local	4.8	1480	391

4.1.4.2 The "5000 ton scenario"

The "5000 Ton scenario" was modelled using Industry Category 15 (Others) and Use category 11 (Complexing agents). This combination was considered to best describe in a simple manner the different exposure scenarios for the non-HERA uses.

a) PEC Water (total) (µg/l):

	ATMP	HEDP	DTPMP
Regional	4.3	5.1	4.5

b) PEC Soil (mg/kg):

	ATMP	HEDP	DTPMP
Regional (agric.)	0.20	0.11	0.015

c) PEC Sediment (mg/kg):

	ATMP	HEDP	DTPMP
Regional	1.83	2.0	1.03

4.2 Environmental Effects Assessment

4.2.1 EcoToxicity

Environmental toxicity data of phosphonates have been extensively reviewed in several recent publications. Gledhill and Feijtel (1992) summarised published and non-published company data. The ECB (2000) published several Iuclid data sets for ATMP, HEDP and DTPMP and some of their sodium salts. Also the W/M working group (1997) reviewed extensively the available data. A summary of this review was included by Jaworska et al. (2002). Finally, the Chemstar PAC Panel critically reviewed the data for ATMP, HEDP and DTPMP, including an evaluation of their reliability (Chemstar PAC, 2003).

Table 13 lists the toxicity data used in the risk assessment, with their reliability rating based on the Klimisch criteria and their source. Regarding the acute and long-term aquatic toxicity the data in Table 13 represent the most sensitive ones out of the number of reported data (Tables 14 and 15). All toxicity values are expressed as mg/l active acids. The discussion following summarises the findings.

4.2.1.1 Ecotoxicity – Aquatic: acute test results

a) Algae EC_{50}

Chelating agents can inhibit algae growth, due to complexation of essential nutrients. The 96 hours EC50 values for the species Selanastrum range from 0.45 mg/L for DTPMP up to 12 mg/L for ATMP. Very large differences have been observed between species. In an 8-day

study the effect concentration (EC50) for Chlorella was well above 10 mg/L for all phosphonates (Monsanto Research Corporation, 1972).

The effects on algae are discussed in more detail in section 4.2.1.2 (Algae – NOEC).

b) Invertebrate IC_{50}

Tests on invertebrates (Chironomus, Daphnia, Grass shrimp) show low toxicity. The most sensitive species is Daphnia magna with 24 and 48 hours LC50 values of 165 to 242 mg/L.

c) Fish LC_{50}

Phosphonates were tested on a number of fish species and demonstrated a low toxicity to fish; the 96 hours LC50 values range from 125 (48 hours) to > 2400 mg/L for freshwater fish (Bluegill Sunfish, Channel Catfish and Rainbow Trout), and from > 1000 up to 8132 mg/L for marine fish (Sheephead minnow).

All phosphonates were tested for 14 days on rainbow trout (ABC, 1978, 1979a, 1979b). LC50 values ranged from 150 to >262 mg/l. NOEC's based on mortality and behaviour ranged from 47 mg/l (ATMP) to 139 mg/l (DTPMP).

d) Other EC_{50}

Because of their chelating properties, a small effect is observed on oysters (Eastern oyster) due to interference with the shell building metabolism. The 96 hours EC50 ranges from 67 to 200 mg/L, with NOEC's of 55 to 95 mg/L (EG&G, 1977a, b and c).

The acute toxicity of ATMP and HEDP towards microorganisms relevant for sewage treatment plants was investigated in a bacterial respiration inhibition test with Pseudomonas putida showing EC0 values of $\geq$ 500 mg/l (Henkel 1983). Grohmann and Horstmann (1988) studied the toxicity to microorganisms using a photoluminescence test. The EC50 was above 2500 mg/l for ATMP and DTPMP and above 250 mg/l for HEDP.

4.2.1.2 Ecotoxicity – Aquatic: chronic test results

a) Algae NOEC

With many chelating agents, algal growth inhibition results may be strongly affected by chelation of trace metal nutrients. This is often interpreted incorrectly as a toxic effect on algae, whereas the real cause is nutrient limitation. It may also induce a high degree of variability between test labs and individual tests, due to variations in the organisms tested and small variations in the test medium composition.

The effect has been recognised for several years now and was extensively studied on a number of chelating agents used in laundry detergents (Schowanek et al, 1996). In addition, this effect has also been recognised in the OECD guidance document on aquatic toxicity testing of difficult substances (OECD, 2000). According to this document, data from tests in which complexation has been judged to have had a significant bearing on the result, are likely to be of questionable value for classifying substances and for extrapolating to a predicted no effect concentration.

One of the ways to identify the effect of nutrient limitation, is to increase the level of trace nutrients to overcome the effect of complexation. In a study sponsored by Solutia and other companies, no algal growth inhibition was observed for DTPMP up to 10 mg/litre (highest value tested) in a freshwater algae test when an equimolar quantity of Fe3+ as a trace nutrient was added (Hanstveit and Oldersma, 1996). This compares to an often observed EC50 in the range of 1 - 10 mg/l when tested without nutrient addition.

As the effect from chelating agents on algae is however due to nutrient limitation, no systemic ecotoxicological effects are to be expected at the concentration of the chelating agents below the NOEC.

In spite of the limited environmental relevance of the discussed algal effect data, the the 96 hr NOEC's (SRI International, 1980 a, b and c) were taken into account in the HERA assessment as the algae represent one of the three trophic levels data are required for in the context of PNEC derivation.

b) Invertebrate NOEC

28 day chronic studies were carried out on ATMP and HEDP (Monsanto Research Corporation 1976). NOEC's of > 25 mg/l and > 12 mg/l were found. Another chronic study was reported for HEDP (Henkel, 1984a). This study gave a NOEC of 0.1 mg/l, significantly lower than the other studies. Although it did not give a clear dose related response over the 0.1 to 10 mg/l range and the result is not consistent with the general pattern of toxicity of the phosphonates (Chemstar PAC, 2003), the study cannot be rejected in the absence of a more reliable study. To address this issue the Chemstar PAC Panel is planning to carry out a modern well-controlled chronic daphnia study. No studies are available on DTPMP. However, given that the acute toxicity is similar, and that similar 14 day fish toxicity has been observed for all four reviewed phosphonates, it is justified to assume that the NOEC values will be of the same order of magnitude.

c) Fish NOEC

60 Day early life stage studies (ABC 1980a and 1980b) were performed on ATMP and DTPMP, with NOEC's of respectively >23 and >26 mg/l. No long-term studies are available on HEDP. However, given that the acute toxicity is similar, and that similar 14 day fish toxicity has been observed for all three reviewed phosphonates, it is justified to assume that the NOEC values will be of the same order of magnitude.

d) Other NOEC including mesocosm data

No studies are available.

4.2.1.3 Terrestrial – acute test results

a) Plants LC50

Only limited test data are available. For ATMP, the test result showed no effects on emergence of Avena sativa at the highest test concentration (1000 mg/l as active ATMP acid) and consequently the 9-day EC50 was >1000 mg/l (Iuclid, 2000b). HEDP also showed

minimal toxicity. The test result showed no effects on growth at the highest test concentration (960 mg/kg dw as active HEDP acid) and consequently the 14-day EC50 was >960 mg/kg dw (Henkel, 1984a). Similar results on HEDP were found in other studies with no effect on seed germination up to 100 mg/l (Gledhill and Feijtel, 1992).

b) Earthworms LC50

Test data on Eisenia foetida are available showing low toxicity of ATMP (Henkel, 1984a) and HEDP with 14 day NOEC of 1000 mg/kg soil dw (Springborn Laboratories 1990) and > 1000 mg/kg soil dw (Henkel, 1984a).

c) Micro-organisms LC50

No studies are available.

d) Other LC50

Oral acute studies on birds showed low toxicity with oral LD50 values of more than 2500 mg/kg food for ATMP, HEDP and DTPMP (Wildlife International Ltd. 1978a, b, c, d e, and f). These dose levels were recalculated to mg/kg bodyweight (Chemstar PAC, 2003) and are given in Table 13.

4.2.1.4 Terrestrial – chronic test results

No studies are available for the following species.

a) Plants NOEC

b) Earthworms NOEC

c) Micro-organisms NOEC

e) Other NOEC

4.2.1.5 Micro-organisms e.g. in Wastewater Treatment Plants

Saeger et al. (1978) reported that no effect on COD and MBAS removal was observed in SCAS testing of ATMP and HEDP up to 160 mg/l.

4.2.1.6 Other environmental properties:

a) Bioaccumulation

Phosphonates are highly water soluble and negatively charged at the typical pH of 6 - 9 in water treatment, or at higher pH in detergent and cleaner applications. The log Kow values (octanol/water partition coefficient) are extremely low and range from -3.4 to -4.4 depending on the type of product. Tests on ATMP (EG&G Bionomics, 1976b, Steber and Wierich, 1987) and HEDP (EG&G Bionomics, 1976c; Steber and Wierich, 1986) gave BCF values of

respectively 5-17 and <2-18 (Chemstar PAC, 2003). This confirms that there is no risk of bioaccumulation in the organism and subsequently in the food chain.

b) Metal remobilisation

Metal remobilisation is the re-dissolution of metals such as zinc, copper, chromium, cadmium, mercury etc., which are precipitated in river and lake sediments. This could lead to several problems: increased exposure of water life to these metals at toxic levels, and passing through of the metal to drinking water abstracted from surface water. Recently it has been suggested that the increased metal concentrations may stimulate algal growth, leading to algae blooms in summer.

Studies (Owens et al., 1980a) have shown that phosphonates only remobilise metals at concentrations of at least 100 to 300 ppb. This is well above the predicted environmental concentration of less than 1 ppb. Even at concentrations estimated for a worst case situation of 10 to 30 ppb, no metal remobilisation is expected.

Table 13: Overview of the EC50 and NOEC values used in the PNEC calculation (all results in mg/l active acid except where otherwise indicated)

	ATMP			HEDP			DTPMP		
	Value	Rating	Reference	Value	Rating	Reference	Value	Rating	Reference
Fish LC50 (96 hours)	125 (48 hours) 150 (14d)	4 1	Iuclid 2000a ABC 1979a	200 180 (14 d)	4 1	ABC 1979b	200 (>180 <252) > 262 (14d)	2 2	EG&G 1976a ABC 1978
Daphnia EC50 (48 hours)	188 (24 hours)	2	Henkel 1972	165 (24 hours)	4	Kastner and Gode 1983	242	2	Monsanto 1981a
Fish NOEC	23 (60 days)	1	ABC 1980a	No data	-	-	26 (60 days)	1	ABC 1980b
Daphnia NOEC (21 days)	> 25 (28 days)	4	Monsanto Research Corporation 1976	0.1 6.75 (28 days)	4 4	Henkel 1984a Monsanto 1976	no data		
Algae EC50 (96 hours) Selenastrum capricornutum	12 (72 hours)	2	Monsanto Research Corporation 1992	3.0	2	SRI International 1980b	0.45 > 10 with nutrient supplement	2 2	Hanstveit & Oldersma 1996
Algae NOEC (96 hours)	7.4	4	SRI International 1980a	0.74	2	SRI International 1980b	0.63 >10 with nutrient supplement	2 2	SRI International 1980c Hanstveit & Oldersma 1996
Algae NOEC (14 days)	7.4 (18 day)	4	SRI International 1980a	13 (14 days)	2	SRI International 1980b	5.2 (14 days)	2	SRI International 1980c

Bacteria EC0	100; > 160 $\geq$ 500 mg/l	2	Saeger et al. 1978 Henkel 1983	> 160; > 580 (30 min) $\geq$ 500 mg/l	2	Saeger et al. 1978 Henkel 1983	>2500		
Bacteria EC50	>2500	4	Grohmann & Horstmann 1988	>250	4	Grohmann & Horstmann 1988	>2500	4	Grohmann & Horstmann 1988
Soil dwelling organisms NOEC	LC0 = 1000 mg/kg soil dw (earthworm)	4	Henkel 1984a	> 1000 mg/kg (14 days) (earthworm) LC0 = 1000 mg/kg soil dw (earthworm)	1 4	Springborn Laboratories 1990 Henkel 1984a	no data		

Birds LC50 (14 days)	> 565 mg/kg bw	2	Wildlife International Ltd. 1978a and b	> 284 mg/kg bw	2	Wildlife International Ltd. 1978c and d	>454 mg/kg bw	2	Wildlife International Ltd. 1978e and f
Plants NOEC	1000 mg/kg soil dw (oats)	4	Henkel 1984a	1000 mg/kg soil dw (oats)	4	Henkel 1984a	no data		
Sediments organisms EC50 (marine Corophium sp.)	> 5000 mg/kg	2	Zeneca 1995	no data			> 2500 mg/kg	1	TNO 1997

Table 14: Environmental toxicity data – acute (Chemstar Panel PAC, 2003)

Species	Test	ATMP		HEDP		DTPMP	
		EC	NOEC	EC	NOEC	EC	NOEC
Fish							
Bluegill Sunfish	96 hr LC50	>330	330	868	529	758	576
Channel Catfish	96 hr LC50	1212	924	695	529	657	432
Rainbow trout	96 hr LC50	>330	330	368	151	>180 <252	180
Rainbow trout	96 hr LC50	160		200		573	
Sheephead minnow	96 hr LC50	8132	4831	2180	104	5377	2125
Rainbow trout	14 day LC50	150	47	180	60	>262	139
Invertebrates							
Daphnia magna	48 hr EC50	297	125	527	400	242	125
Chironomus	48 hr EC50	11000	7040	8910	3925	9910	7589
Grass shrimp	96 hr LC50	7870	4575	1770	104	4849	2125
Eastern oyster	96 hr EC50	201	95	89	<52	156	56
Algae							
Selenastrum	96 hr EC50	19.6	7.4	3.0	1.3	1.9	5.2

Table 15: Environmental toxicity data – chronic (Chemstar Panel PAC, 2003)

Species	Test	ATMP		HEDP		DTPMP	
		EC	NOEC	EC	NOEC	EC	NOEC
Fish							
Rainbow trout	60 day chronic	< 47	> 23	n.a.	n.a.	< 34	>26
Invertebrates							
Daphnia magna	28 day chronic	< 54	> 25	< 25	> 12	n.a.	n.a.
Algae							
Selenastrum	14 day EC50	19.6	7.4	39.1	13.2	8.7	5.2

4.2.2 PNEC calculations

Except for the NOEC in the chronic daphnia study on HEDP, the ecotoxicity data obtained for all phosphonates are consistent and of the same magnitude for the aquatic environment. Therefore the lowest value for each species was used, even if the reliability of the test could not be established (reliability rating 4 in all cases).

Table 16: Overview of the PNEC derivation for phosphonates

	ATMP	HEDP	DTPMP
Aquatic	3 chronic tests Lowest NOEC: 23 mg/l AF = 10 PNEC: 2.3 mg/l	2 chronic tests: Lowest NOEC: 0.1 mg/l or Lowest NOEC: 6.8 mg/l AF= 50 PNEC: 0.002 or 0.13 mg/l	2 chronic tests: Lowest NOEC: 26 mg/l AF= 50 PNEC: 0.52 mg/l
Sediment	no test data Calculated PNEC: 1060 mg/kg	no test data; daphnia data used Calculated PNEC: 0.855 mg/kg or Calculated PNEC: 57.3 mg/kg	no test data Calculated PNEC: 144 mg/kg
Soil	2 acute tests NOEC used: 1000 mg/kg AF = 100 PNEC: 10 mg/kg	1 acute test NOEC used: 960 mg/kg AF = 100 PNEC: 9.6 mg/kg	no test data NOEC calculated: 45.9 mg/kg AF = 1 PNEC: 5.57 mg/kg
STP	1 acute test, no specific population NOEC used: 500 mg/l AF = 10 PNEC: 50 mg/l	1 acute test , no specific population NOEC used: 500 mg/l AF = 10 PNEC: 50	1 acute test LC50: > 2500 mg/l AF = 100 PNEC: > 25 mg/l
Secondary poisoning	LD50 used: 2000 mg/kg bw AF = 1000 PNEC: 2 mg/kg bw	LD50 used: 1000 mg/kg bw AF = 1000 PNEC: 1 mg/kg bw	LD50 used: 2000 mg/kg bw AF = 1000 PNEC: 2 mg/kg bw

The selection of the safety factor to be applied to the aquatic NOEC's presents a significant issue. Because of the deviating chronic daphnia NOEC for HEDP, it is not possible at this moment in time to set an overall NOEC covering all three phosphonates. Therefore, each

substance has to be considered on its own and the safety factor should be assigned based on the specific test data. The second relevant issue is the use of the algae data. As discussed earlier, the algae growth inhibition data are not reliable indicators of toxicity, although the effect observed may play a role in certain oligotrophic environments.

Where measured data were absent, the PNEC for the soil and sediment compartment are estimated from the aquatic data using the PNECs calculated by EUSES. This may lead to an overestimation of the ecotoxicity of ATMP and DTPMP, as it is derived from the algae NOEC's. It can be assumed that all phosphonates have a similar behaviour and toxicity towards soil and sediment organisms, in the absence of metal complexation effects.

PNEC values for the risk assessment are shown in Table 17. The two HEDP assessments are called HEDP 1 and HEDP 2 for respectively a NOEC of 0.1 mg/l and 6.7 mg/l.

Table 18: Overview of the PNEC values used in the HERA risk assessment

	ATMP	**HEDP 1 (NOEC 0.1 mg/l)**	**HEDP 2 (NOEC 6.7 mg/l)**	**DTPMP**
PNEC water mg/l	2.3	0.002	0.13	0.52
PNEC sediment mg/kg dw	1060	0.86	57.3	144
PNEC soil mg/kg dw	1	9.6	9.6	45.9
PNEC stp mg/l	50	50	50	25

4.3 Environmental Risk Characterisation HERA

a) RCR Water

	ATMP	**HEDP 1**	**HEDP 2**	**DTPMP**
Regional	$2.5*10^{-5}$	**4.7**	0.070	0.0077
Local	$7*10^{-5}$	**13.2**	0.20	0.020

b) RCR Soil

	ATMP	**HEDP 1**	**HEDP 2**	**DTPMP**
Regional	$0.28*10^{-3}$	0.021	0.021	$0.29*10^{-3}$
Local	0.0024	0.44	0.44	0.01

c) RCR Sediment

	ATMP	HEDP 1	HEDP 2	DTPMP
Regional	$2.4*10^{-5}$	**4.5**	0.067	0.006
Local	$4.0*10^{-5}$	**7.5**	0.11	0.01

d) RCR STP

	ATMP	HEDP	DTPMP
Local	$2*10^{-5}$	0.003	0.003

4.4 Environmental Risk Characterisation 5000 ton

a) RCR Water

	ATMP	HEDP 1	HEDP 2	DTPMP
Regional	$1.8*10^{-3}$	**2.52**	0.037	0.008

b) RCR Soil

	ATMP	HEDP 1	HEDP 2	DTPMP
Regional	0.020	0.012	0.012	0.0003

c) RCR Sediment

	ATMP	HEDP 1	HEDP 2	DTPMP
Regional	$1.7*10^{-3}$	**2.39**	0.036	0.007

4.5 Discussion and conclusions

The HERA environmental assessment showed that the use of ATMP and DTPMP in consumer detergent products does not cause concerns. For ATMP and DTPMP all RCR's are below 1, both for the regional and the local assessments. The use of these phosphonates in other applications will increase the continental and regional backgrounds, but no to such an extent that it leads to RCR's (PEC/PNEC ratios) exceeding 1.

For the time being, an environmental risk is indicated for HEDP. However, the overall pattern of toxicity of the three assessed phosphonates is quite similar, and the single low NOEC forming the basis for the currently resulting PEC/PNEC ratio > 1 is expected to be an outlier. If the environmental risk assessment of HEDP was assessed on the same basis as for ATMP and DTPMP (algae 96 hrs NOEC), no at risk situation would result. To allow a clear conclusion in terms of the HEDP environmental risk assessment, a repetition of the chronic daphnia study is recommended.

The PEC values of the three different phosphonates relate closely to the volumes assessed. The RCR's on the other hand vary much more due not only to the different quantities of substance used, but also due to the variability in PNEC, driven by the algal growth inhibition data and the HEDP daphnia data. The use of the algae data to estimate the PNEC is extremely conservative, as in normal environmental conditions, the trace nutrients essential for algal growth are abundant and their availability will not be influenced by the phosphonate concentrations. In addition, the PNECs for the soil and sediment compartment are also estimated from the aquatic data, again leading to a conservative approach in the absence of metal complexation effects.

Further refinement of the PEC estimations is possible. Laboratory data show that the most important degradation mechanism is photodegradation. Although this has been confirmed in several laboratory studies, not all phosphonates have been studied to the same extent, and only limited evidence is available from field. Photodegradation has not been taken into account as a separate mechanism. It has been assumed to be covered water/sediment microcosm biodegradation studies. On the other hand, further degradation mechanisms have been identified such as hydrolysis and manganese catalysed oxidation. Hydrolysis was not taken into account either as an explicit removal mechanism, although it may be, in particular at low concentrations, an important degradation mechanism of phosphonates.

Although no overall risk was identified for phosphonates in consumer detergent products, further studies on the degradation and fate of phosphonates in the environment and at environmental levels remain useful, but difficult to perform in the absence of a well established analytical method. In addition, the results of a repeat daphnia chronic test have to be awaited to finalise the risk assessment.

5. HUMAN HEALTH ASSESSMENT

5.1 Consumer exposure

5.1.1 Product types

In line with the objectives of the HERA initiative, this human health assessment will focus on the use of phosphonic acid compounds ("phosphonates") ATMP, HEDP, and DTPMP in household cleaning products. While HEDP and DTPMP are widely used in these applications, the use of ATMP is limited to surface cleaners. Table 18 lists household cleaning applications and typical finished product concentration ranges of HEDP, DTPMP and ATMP.

Table 18: Household applications and finished product concentrations of the different phosphonates under review

Product application	Type of phosphonate used	Range of phosphonate level in finished product	Typical content of phosphonate in finished product
Regular laundry detergents	HEDP, DTPMP	0.02-1.3 %	0.05-0.71 %
Compact laundry detergents	HEDP, DTPMP	0.00-2.3 %	0.05-1.6 %
Fabric conditioners	HEDP, DTPMP	0.02-0.05 %	0.03-0.05 %
Laundry additives	HEDP, DTPMP	0.10-1.9 %	0.12-0.34 %
Hand dishwashing detergent	HEDP, DTPMP	0.002-0.04 %	0.02 %
Machine dishwashing detergent	HEDP, DTPMP	0.0-2.0 %	0.20-1.5 %
Surface cleaners	HEDP, DTPMP, ATMP	0.05-0.50 %	0.18-0.50 %
Carpet cleaner	HEDP, DTPMP	2.0-4.0 %	2.0 %
Toilet cleaner	HEDP, DTPMP	0.05-0.15 %	0.05 %

5.1.2 Consumer contact scenarios

For the use of ATMP, HEDP, and DTPMP the following consumer exposure scenarios were identified and assessed:

1. Direct skin contact with neat (*e.g.*, laundry pre-treatment) or diluted consumer product (*e.g.*, hand-washed laundry, hand dishwashing, surface cleaning)
2. Indirect skin contact via release from clothes fibres to skin
3. Inhalation of detergent dust or aerosols generated by spray cleaners
4. Oral ingestion of residues deposited on dishes
5. Oral ingestion of residues in drinking water
6. Accidental or intentional overexposure

5.1.3 Consumer exposure estimates

There is a consolidated overview concerning habits and practices of use of detergents and surface cleaners in Western Europe which is tabulated and issued by the European Soap and Detergent Industry Association, AISE (AISE, 2002). This table reflects consumers' use of detergents in g/task, tasks/week, duration of task and other uses of products and is largely the basis for the exposure estimates in the following paragraphs. In some instances (*e.g.*, habits & practices (H&P) of pre-treatment of laundry), additional H&P information for a targeted exposure assessment is directly provided by the member companies of AISE. The calculations of the estimated consumer exposures are based on the highest relevant concentrations that consumers can be exposed to.

5.1.3.1 Direct skin contact from hand-washing laundry

Hand-washing laundry is identified as a common consumer habit. In this task, the phosphonate containing laundry solution comes in direct contact with the skin of hands and forearms. A hand washing task is typically expected to take 10 minutes (AISE, 2002). The dermal systemic exposure (Exp_{sys}) to phosphonates is estimated according to the following algorithm from the HERA guidance document:

$$\mathbf{Exp_{sys} = F_1 \times C \times Kp \times t \times S_{der} \times n / BW} \qquad \mathbf{(1)}$$

For this exposure estimate, the terms are defined with following values for the calculation of a worst case scenario:

F_1	percentage weight fraction of substance in product	***2.3 %*** (laundry gel) (AISE unpublished data)
C	product concentration	***10 mg/cm^3*** (AISE, 2002)
Kp	dermal penetration coefficient	***7.6 x 10^{-6} cm/h**** (Henkel KGaA, 1983a)
t	duration of exposure or contact	***10 min*** (AISE, 2002)
S_{der}	surface area of exposed skin	***1980 cm^2*** (TGD, 1996)
n	product use frequency (tasks per day)	***1.4*** (AISE, 2002)
BW	body weight	***60 kg*** (TGD, 1996)

$$\mathbf{Exp_{sys}} = [0.023 \times (10\ mg/cm^3) \times (7.6 \times 10^{-6}\ cm/h) \times (0.17\ h) \times 1.4 \times (1980\ cm^2)] / 60\ kg$$
$$\mathbf{= 0.014\ \mu g/kg\ bw/day}$$

* Due to their similar chemical structure and physico-chemical characteristics, the dermal penetration coefficient for the three phosphonic acid compounds under consideration is assumed to be similar. It was calculated on the basis of an *in vivo* dermal penetration

experiment with ^{14}C-labeled ATMP sodium salt in rats (Henkel KGaA, 1983a) from the dermal flux (*i.e.*, 0.0016 mg/cm^2) according to the following algorithm: Kp = dermal flux / exposure time x concentration of test solution; Kp = 0.0016 mg/cm^2 / 24 h x 8.8 mg/cm^3 = 7.6 x 10^{-6} cm/h. Generally, ionic substances such as salts of organic acids are assumed to penetrate the skin less readily compared to the respective organic acids. It can, however, be assumed that at the pH under in-use conditions, the phosphonates will be present to a large extent in their neutralized forms (an overview of the different phosphonic compounds at physiological pH is provided in Chapter 3). Thus, the dermal penetration experiment with the sodium salt of ATMP is assessed to appropriately reflect the in-use exposure scenarios occurring in laundry and cleaning applications. A further level of conservatism is warranted by the fact that rat skin is typically more permeable to chemicals compared to human skin (Schaefer and Redelmeier, 1996; van Ravenzwaay and Leibold, 2004).

5.1.3.2 Direct skin contact from laundry tablets and powder

Filling laundry tablets into the dispenser of the washing machine involves only a very short direct skin contact with the neat material. Due to the short contact time, the very small skin contact area and the low concentration in finished product, the dermal exposure to phosphonates from this use is considered to be insignificant. Loading the dispenser with liquid or powder also results in negligible exposure as mostly this is done via a measuring ball such that the consumer never comes in contact with the product.

5.1.3.3 Direct skin contact from pre-treatment of laundry

Consumers typically spot-treat stains on the laundry by hand with the help of either a detergent paste (*i.e.*, water/laundry powder = 1:1) or a laundry liquid which is applied directly on the garment. In this exposure scenario, at most the skin surface of both hands is exposed and the time taken for the task is typically less than 10 minutes. Algorithm (1) is used to calculate the systemic exposure resulting form the pre-treatment of laundry. The following assumptions are considered to represent a realistic reflection of this scenario:

F_1	percentage weight fraction of substance in product	***2.3 %*** (laundry gel) (AISE unpublished data)
C	product concentration	***1000 mg/cm^3*** (AISE, 2002)
Kp	dermal penetration coefficient	***7.6 x 10^{-6} cm/h*** (Henkel KGaA, 1983a)
t	duration of exposure or contact	***10 min*** (AISE, 2002)
S_{der}	surface area of exposed skin	***840 cm^2*** (TGD, 1996)
n	product use frequency (tasks per day)	***1*** (AISE, 2002)
BW	body weight	***60 kg*** (TGD, 1996)

Exp$_{sys}$ = [0.023 x (1000 mg/cm^3) x (7.6 x 10^{-6} cm/h) x (0.17 h) x (840 cm²) x 1] / 60 kg
= **0.42 µg/kg bw/day**

The above exposure estimate can be regarded to be very conservative. Typically, consumers pre-wet the laundry before applying the detergent for pre-treatment or conduct the pre-treatment under running tap water. Both practices lead to a significant dilution which is not reflected in this exposure estimate. The assumption that the consumer is exposed to the concentrated laundry product is therefore a worst case assumption. It should also be considered that only a fraction of the hands' skin will actually be exposed to the product. The assumption that both hands will be fully immersed in the product is a likely overestimate of the true exposure.

5.1.3.4 Direct skin contact from hand dishwashing

To calculate the dermal systemic exposure from direct contact of the skin to dishwashing detergent algorithm (1) is adapted. The determination of phosphonate exposure from hand dishwashing is conducted in a manner very similar to that of hand-washed laundry. The following assumptions have been made to address a reasonable worst case scenario:

F_1	percentage weight fraction of substance in product	***0.04 %*** (regular liquid) (AISE unpublished data)
C	product concentration	**2 *mg/cm^3*** (AISE, 2002)
Kp	dermal penetration coefficient	***7.6 x 10^{-6} cm/h*** (Henkel KGaA, 1983a)
t	duration of exposure or contact	***45 min*** (AISE, 2002)
S_{der}	surface area of exposed skin	***1980 cm^2*** (TGD, 1996)
n	product use frequency (tasks per day)	***3*** (AISE, 2002)
BW	body weight	***60 kg*** (TGD, 1996)

$$\mathbf{Exp_{sys}} = [0.0004 \times (2\ mg/cm^3) \times (7.6 \times 10^{-6}\ cm/h) \times (0.75\ h) \times (1980\ cm^2) \times 3] / 60\ kg$$
$$= \mathbf{4.5 \times 10^{-4}\ \mu g/kg\ bw/day}$$

5.1.3.5 Direct skin contact from machine dishwashing products

Filling dishwashing powder or tablets into the dispenser of the dishwasher involves only a very short direct skin contact with the neat material. Due to the short contact time and the very small skin contact area, the dermal exposure to phosphonates from this use is considered insignificant. No skin contact should occur when filling the dispenser with gel or liquid product.

5.1.3.6 Direct skin contact from hard surface cleaners

During this task, the phosphonate containing hard surface cleaning solution comes in direct contact with the skin of the hands. A hard surface cleaning task takes at maximum 20 minutes (AISE, 2002). The algorithm (1) is used to calculate the dermal systemic exposure (µg/kg BW/day) to hard surface cleaning products.

The terms are defined with following values for the calculation of a worst case exposure estimate:

F_1	percentage weight fraction of substance in product	***0.5 %*** (liquid) (AISE unpublished data)
C	product concentration	***22 mg/cm^3*** (AISE, 2002)
Kp	dermal penetration coefficient	***7.6 x 10^{-6} cm/h*** (Henkel KGaA, 1983a)
t	duration of exposure or contact	***20 min*** (AISE, 2002)
S_{der}	surface area of exposed skin	***840 cm^2*** (TGD, 1996)
n	product use frequency (tasks per day)	***1*** (AISE, 2002)
BW	body weight	***60 kg*** (TGD, 1996)

Exp_{sys} = [0.005 x (22 mg/cm^3) x (7.6 x 10^{-6} cm/h) x (0.33 h) x 1 x (840 cm²)] / 60 kg
= **0.004 µg/kg bw/day**

5.1.3.7 Direct skin contact from carpet cleaners

Typically, dermal exposure to carpet cleaners would occur when cleaning the carpet with the neat product or a solution thereof. Carpet cleaners were considered separate from hard surface cleaners. The maximum concentration at which phosphonates in liquid carpet cleaners are used for spot cleaning is 0.2 %. This task takes around a maximum of 20 minutes and the skin of the hands is the exposed. Carpet cleaners are considered to be used at maximum once a day. Algorithm (1) is used to estimate the worst case dermal exposure scenario. The variables are defined below:

F_1	percentage weight fraction of substance in product	***0.2 %*** (liquid spot cleaner) (HERA 2004; oral communication)
C	product concentration	***1000 mg/cm^3*** (AISE, 2002)
Kp	dermal penetration coefficient	***7.6 x 10^{-6} cm/h*** (Henkel KGaA, 1983a)
t	duration of exposure or contact	***20 min*** (HERA 2004; oral communication)
S_{der}	surface area of exposed skin	***840 cm^2*** (TGD, 1996)
n	product use frequency (tasks per day)	***1*** (HERA 2004; oral communication)
BW	body weight	***60 kg*** (TGD, 1996)

Exp_{sys} = [0.002 x (1000 mg/ml) x (7.6 x 10^{-6} cm/h) x (0.33 h) x 1 x (840 cm²)] / 60 kg
= **0.071 µg/kg bw/day**

5.1.3.8 Indirect skin contact from wearing clothes

The consumer can also be exposed to detergent residues via the skin by wearing clothes that have been laundered. No data are available measuring the phosphonate deposit on the fabric following a wash process. Due to their high water solubility phosphonates are assumed to be removed to a large extent with the wash water and not absorbed to the fabric fibres. In this exposure scenario, it is assumed that the concentration of substance available for deposition before spinning is decreased to less than 2.5 % of the initial concentration in the wash-liquor (ZVEI and IKW, 1999). The indirect skin exposure resulting from phosphonate residues in clothes can be estimated with algorithm (2) listed below. This algorithm has been slightly modified versus the algorithm for calculation of the dermal exposure to detergent residues in the fabric recommended in the HERA guidance document (AISE, 2002) to account for the absence of real phosphonate deposition data.

$$\mathbf{Exp_{sys} = F_1 \times (M \times (F' / V) \times FD \times FL) \times S_{der} \times F_2 \times F_3 \times F_4 / BW} \qquad \mathbf{(2)}$$

The terms used in this algorithm are defined as follows:

F_1	percentage weight fraction of substance in product	***2.3 %*** (laundry gel) (AISE, 2002)
M	amount of undiluted product used	***140 g*** (laundry gel) (AISE, 2002)
F'	Concentration of water soluble ingredient in wash liquor	***2.5 %*** (ZVEI and IKW, 1999)
V	volume of wash liquor	***15 l*** (assumption)
FD	fabric density	**10 mg/cm^2** (Procter and Gamble, 1996)
FL	percentage liquor after final spinning	***60 %*** (AISE, internal data)
S_{der}	surface area of exposed skin	***17600 cm^2***
F_2	percent weight fraction transferred to skin	***1 %*** (Vermeire *et al*., 1993)
F_3	percent weight fraction remaining on skin	***100 %*** (worst case)
F_4	percent weight fraction absorbed via skin	***0.9 %*** (Henkel KGaA, 1983a)
BW	body weight	***60 kg*** (TGD, 1996)

Exp$_{sys\ (indirect\ skin\ contact)}$ = [0.023 x [(140000 mg) x (0.025 / 15000000 mg) x (10 mg/cm^2) x 0.6] x (17600 cm^2) x 0.01 x 1 x 0.009] / 60 kg
= **0.0009 µg/kg bw/day**

5.1.3.9 Inhalation of detergent dust during washing processes

Filling powder into the washing machine dispenser can result in some detergent dust being generated. Studies determined an average release of about 0.27 µg dust per cup of product

(*i.e.*, laundry powder) used for machine laundering (van de Plassche *et al*., 1998). Phosphonates are present in laundry powder detergents at a maximum level of 1.25 %. Exposure to detergent dust particles containing phosphonates can be calculated by algorithm (3) derived from the HERA guidance document.

$$\mathbf{Exp_{sys\ (inhalation)} = F_1 \times n \times F_5 \times F_6 / BW} \qquad \mathbf{(3)}$$

The variables are explained below with the relevant values which represent worst case exposure for this task:

F_1	percentage weight fraction of substance in product	***1.25 %*** (laundry powder) (AISE unpublished data)
n	product use frequency (tasks per day)	***2.6*** (AISE, 2002)
F_5	amount of inhalable dust per task	***0.27 µg*** (van de Plassche *et al*., 1998)
F_6	percentage weight fraction absorbed or inhaled	***100 %*** (worst case)
BW	body weight	***60 kg*** (TGD, 1996)

$$\mathbf{Exp_{sys\ (inhalation)}} = [0.0125 \times 2.6 \times (0.27\ \mu g) \times 1] / 60\ kg$$
$$= \mathbf{1.5 \times 10^{-4}\ \mu g/kg\ bw/day}$$

5.1.3.10 Inhalation of aerosols from cleaning sprays

Phosphonates are also present in surface cleaning sprays. The HERA guidance document specifies the algorithm (4) to be used for calculation of consumers' worst-case exposure to phosphonate containing aerosols generated by the spray cleaner:

$$\mathbf{Exp_{sys} = F_1 \times C` \times Q_{inh} \times t \times n \times F_7 \times F_8 / BW} \qquad \mathbf{(4)}$$

The terms used in this algorithm are defined as follows:

F_1	percentage weight fraction of substance in product	***0.48 %*** (AISE unpublished data)
C`	product concentration in air	***0.35 mg/m^3*** * (Procter and Gamble, 1996)
Q_{inh}	ventilation rate	***0.8 m^3/h*** (TGD, 1996)
t	duration of exposure	***10 min*** (AISE, 2002)
n	product use frequency (tasks per day)	***1*** (AISE, 2002)
F_7	weight fraction of respirable particles	***100 %*** (worst case)

F_8	weight fraction absorbed or bioavailable	***75 %*** (TGD, 1996)
BW	body weight	***60 kg*** (TGD, 1996)

* this value was obtained by experimental measurements of the concentration of aerosol particles smaller than 6.4 microns in size which are generated upon spraying with typical surface cleaning spray products.

$Exp_{(inhalation)}$ = [0.0048 x (0.35 mg/m^3) x (0.8 m^3/h) x (0.17 h) x 1 x 1 x 0.75] / 60 kg
= **0.0029 µg/kg bw/day**

5.1.3.11 Oral exposures to phosphonates

Oral exposure to phosphonates can originate from residues on eating utensils and dishes as well as from exposure to residues found in water.

The daily exposure to phosphonates from eating utensils and dishware that were washed in a dish washer using phosphonates-containing tablets can be estimated according to algorithm (5) from the HERA guidance document. Due to the low level of phosphonates in dishwashing liquids (*i.e.*, < 0.04 %), the contribution of oral exposures to phosphonates from residues on eating utensils via hand dishwashing was considered to be negligible.

$$\mathbf{Exp_{sys} = [F_1 \times C` \times T_{a'} \times S_a / BW] \times A} \qquad \mathbf{(5)}$$

For the machine dishwashing exposure estimate, the terms are defined with following values for the calculation considering a worst case scenario:

F_1	percentage weight fraction of substance in product	***2.0 %*** (tablet) (AISE unpublished data)
C`	concentration of product in dish wash solution	***1 mg/cm^3*** (AISE, 2002)
$T_{a'}$	amount of water left on dishes after rinsing	***5.5 x 10^{-5} ml/cm^2*** (Schmitz, 1973)
S_a	area of dishes in daily contact with food	***5400 cm^2*** (TGD, 1996)
BW	body weight	***60 kg*** (TGD, 1996)
A	oral absorption	***10 %*** (Heaney and Saville, 1976)

$Exp_{sys\ (oral\ dish\ deposition)}$ = [[0.02 x (1 mg/cm^3) x (5.5 x 10^{-5} ml/cm^2) x (5400 cm^2)] / 60 kg] x 0.1 = **0.01 µg/kg bw/day**

With regard to the uptake of phosphonates from the drinking water, the Environmental Risk Assessment discussed in chapter 5 estimated a worst case regional predicted environmental concentration of HEDP in surface water of about 0.0094 mg/l. However, it has been estimated that more than 90% of the phosphonates will be removed during the drinking water treatment process using e.g., sand or activated carbon filtration techniques. This estimation is supported by flocculation and chlorination studies which reported a removal of phosphonates by ferrous sulphate, lime and aluminium at concentrations well above the predicted environmental concentration by more than 95% (Gledhill and Feijtel, 1992). A further contribution to the removal of phosphonates occurs through the phosphonates adsorption properties on adsorbents such as soil, sediments and mineral surfaces.

Taking the conservative assumption that an adult person drinks 2 litres of water per day (TGD, 1996), the daily human exposure to phosphonates via drinking water can be estimated as:

$$\mathbf{Exp_{sys} = [C` \times V / BW] \times R \times A} \qquad \mathbf{(5)}$$

For the machine dishwashing exposure estimate, the terms are defined with following values for the calculation considering a worst case scenario:

C`	concentration of phosphonates in drinking water	***0.0094 mg/L*** (ERA)
V	Volume of water consumed by adult	***2 l/day*** (TGD, 1996)
BW	body weight	***60 kg*** (TGD, 1996)
R	Removal in drinking water treatment process	***90%*** (Gledhill and Feijtel, 1992)
A	oral absorption	***10 %*** (Heaney and Saville, 1976)

$$\mathbf{Exp_{sys\ (oral\ via\ drinking\ water)}} = [((0.00944\ mg/l) \times (2\ l)) / 60\ kg] \times 0.1 \times 0.1$$
$$= \mathbf{0.0032\ \mu g/kg\ bw/day}$$

5.1.3.12 Accidental or intentional overexposure

Accidental or intentional overexposure to phosphonates may occur via accidental swallowing of solid detergents or drinking of liquid washing solutions. Typically, one would estimate that no more than 5 g of powder detergent (equals about 62.5 mg of phosphonate) or 20 ml of dishwashing liquid (equals about 8 mg of phosphonate) would be swallowed. Studies of acute oral toxicity demonstrate that the toxic dose of phosphonates is many times higher than this, even for a toddler (see 5.2.1.1.1 Acute oral toxicity).

The German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV, 1999) published a report on products involved in poisoning cases. No fatal case of poisoning with detergents is reported. Detergent products are not mentioned as dangerous products with a high incidence of poisoning. Accidental exposure to eyes is possible by splashes of dilute washing solutions or to low amounts of the detergent powder from hands into the eyes.

Equally, in the UK, the Department of Trade and Industry (DTI) produces an annual report of the home accident surveillance system (HASS). The data in this report summarizes the information recorded at accident and emergency (A&E) units at a sample of hospitals across the UK. It also includes death statistics produced by the Office for National Statistics for England and Wales. The figures for 1998 show that for the representative sample of hospitals surveyed, there were 33 reported accidents involving detergent washing powder (the national estimate being 644) with none of these resulting in fatalities (DTI, 1998). In 1996 and 1997, despite their being 43 and 50 reported cases, respectively, no fatalities was reported either.

5.1.3.13 Total exposure

In the unlikely event of maximum worst case exposure from all sources, the total exposure to phosphonates from its use in cleaning products would be 0.31 µg/kg bw/day. The major contribution stems from the phosphonate exposures during pre-treatment of laundry. The individual sources of exposures leading to the overall exposure are summarized in Table 19.

Table 19: Worst case exposure estimates for the different consumer contact scenarios

Task	**Worst case exposure estimate (EXP_{sys}) [µg/kg bw/day]**
Direct contact from hand washing laundry	0.014
Direct skin contact from pre-treatment of laundry	0.42
Direct skin contact from hand dishwashing	0.00045
Direct skin contact from hard surface cleaners	0.004
Direct skin contact from carpet cleaners	0.071
Indirect skin contact from wearing laundered clothes	0.0009
Inhalation of laundry powder dust	0.00015
Inhalation of aerosol particles	0.0029
Oral exposure to phosphonates	0.0132
Total exposure	**0.53 µg/kg bw/day**

5.2 Hazard assessment

5.2.1 Summary of the available toxicological data

The following hazard assessment will cover the toxicological properties of ATMP, HEDP and DTPMP in their acid and/or salt form and has been prepared on the basis of peer reviewed SIDS Initial Assessment Reports as part of the OECD HPV programme and the underlying IUCLID data sets for the different phosphonates considered. Both, SIDS reports and IUCLID data sets can be made available upon request.

An overview of the pH range of the different phosphonic acid compounds and their salts is given in Table 20.

Table 20: pH values of aqueous solution of the different phosphonate groups

Group	Substance	CAS No.	pH at typically 1 %
ATMP group	ATMP acid	6419-19-8	< 2
	ATMP.xNa	20592-85-2*	
	ATMP.4Na	94021-23-5	~ 7
	ATMP.5Na	2235-43-0	~ 11
HEDP group	HEDP acid	2809-21-4	< 2
	HEDP.xNa	29391-71-3*	
	HEDP.4Na	3794-83-0	11-12
DTPMP group	DTPMP acid	15827-60-8	< 2
	DTPMP.xNa	22042-96-2	
	DTPMP.7Na	68155-78-2	7-9

* Different salts are closely related and can be covered in one CAS No. for the set of salts

5.2.1.1 Acute toxicity

5.2.1.1.1 Acute oral toxicity

ATMP acid (CAS No. 6419-19-8) and ATMP salts (CAS No. 20592-85-2)

The acute oral toxicity of ATMP acid was determined in Sprague-Dawley rats (Younger Laboratories, 1967). Five rats per group (*i.e.*, 2-3 per sex) received dose levels of 2000, 2510, 3160 and 3980 mg active acid/kg bw, administered by gavage. The rats were observed for mortalities and clinical symptoms following treatment. Animals that died during the test underwent necropsy for macroscopic examination. Clinical signs of toxicity included weakness in the first 2 hours after exposure, diarrhea, salivation and tremors. All animals died at the highest treatment level of 3980 mg active acid/kg bw, one female and one male at 3160 mg active acid/kg bw and one female at 2510 mg active acid/kg bw. Upon necropsy, inflammation of gastrointestinal mucosa and liver and renal hyperaemia were observed. The acute oral LD_{50} was reported to be 2910 mg active acid/kg bw. Although the study was pre-GLP and not in full compliance with OECD guidelines, the study was scientifically sound and judged to be reliable.

The mouse acute oral LD_{50} of ATMP acid was determined to be 2790 mg active acid/kg bw (Gloxhuber, unpublished data). More detailed study information was not available. Therefore the quality and reliability of this investigation could not be evaluated.

Two acute oral toxicity studies were conducted with the tetrasodium salt of ATMP, both in compliance with GLP and following the principles of the OECD guideline 401. Ten Sprague-Dawley rats (*i.e.,* 5 of each sex) were administered by gavage a single dose of 10 mL/kg bw of an aqueous solution containing 41 % active salt (Safepharm Laboratories Ltd., 1982a). This equalled a dose of 5740 mg active salt/kg bw. Following treatment, the rats were observed daily for clinical symptoms and mortality. Animals that died during the test and surviving animals at the end of the study underwent necropsy for macroscopic examination. General signs of toxicity observed in all animals included pilo-erection, hunched posture, lethargy, and decreased respiration rate. Two females died on the day of dosing. Ptosis was also observed in three animals (*i.e.,* 2 males and 1 female) 2-3 hours after dosing, and pallor in extremities in one female 3 hours after dosing. The female animal in which these two symptoms were observed died in the 4^{th} hour after dosing. No abnormal symptoms were observed in the surviving animals from day 2. At necropsy, congestion in the lungs was observed in the 2 animals that died during the test. One animal exhibited haemorrhage in the intestine. No abnormal signs of toxicity were observed in the surviving animals. The acute oral LD_{50} in rats was determined to be greater than 5740 mg active salt/kg bw. In the follow-up study, a higher dose of 15 mL/kg was administered, equivalent to 8610 mg active salt/kg body weight (Safepharm Laboratories Ltd., 1982b). Six out of 10 animals (*i.e.,* 3 males and 3 females) died on the day of dosing. General signs of toxicity observed in all animals included pilo-erection, hunched posture, lethargy, and decreased respiratory rate and ptosis. At necropsy, congestion in the lungs and haemorrhage of lungs, stomach, small and large intestine, and pallor of spleen and kidneys were observed in the animals that died during the study. On the basis of this study, the acute oral LD_{50} was estimated to be approximately 8610 mg/active salt/kg bw.

One acute oral toxicity study was conducted with the pentasodium salt of ATMP (Younger Laboratories, 1962). Five rats per group (*i.e.,* 2-3 per sex) were administered by gavage aqueous solutions the salt at 5040, 6320, 8000 and 10040 mg active salt/kg bw. The rats were observed for clinical symptoms and mortalities following treatment. Animals that died during the test underwent necropsy for macroscopic examination. Clinical signs included severe diarrhea, loss of appetite, lethargy and increasing weakness. All animals died at the highest treatment level of 10040 mg active salt/kg bw, four at 8000 mg active salt/kg bw and one at 6320 mg active salt/kg bw. At necropsy renal, liver and pulmonary hyperaemia were observed. On the basis of this investigation, the acute oral LD_{50} was determined to be 7120 mg active salt/kg bw. The study was conducted prior to GLP and OECD guidelines, but it was well conducted and documented and therefore judged to be reliable.

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

The acute oral toxicity of HEDP acid was tested in Sprague-Dawley rats (Younger Laboratories, 1965a). In this study, five rats per group (*i.e.,* 2-3 per sex) were administered by gavage an aqueous solutions of HEDP acid at dose levels of 1200, 1506, 1896 and 2388 mg active acid/kg bw. Following treatment, rats were observed for clinical signs of toxicity and mortalities. Animals that died during the test underwent necropsy for macroscopic examination. Clinical signs included weakness within the first minutes, followed by dyspnoea and collapse. Four animals died at the highest treatment level of 2388 mg active acid/kg bw,

three at 1896 mg active acid/kg bw and one at 1506 mg active acid/kg bw. Survival time was 1 to 8 hours after treatment with most deaths occurring within 2 hours. At necropsy, inflammation of the gastric mucosa and hemorrhagic areas in the lungs were observed. The acute oral LD_{50} of HEDP acid was determined to be 1878 mg active acid/kg body weight.

In a similar study rats were dosed by gavage at levels of 948, 1200, 1506 and 1896 mg active acid/kg bw (Younger Laboratories, 1977). The acute oral LD_{50} was determined to be 1440 mg/active acid/kg bw. Signs of toxicity included decreased appetite and activity, increasing weakness, diarrhoea, tremors, collapse and death. Four animals died at the highest treatment level of 1896 mg active acid/kg bw, 3 at 1506 mg active acid/kg bw, and 1 at 1200 mg active acid/kg bw. All deaths occurred 1-2 days after dosing. Animals that died showed symptoms lung and liver hyperaemia and acute gastrointestinal inflammation. Although both studies were pre-GLP and not in full compliance with OECD guidelines, they appeared to be scientifically sound and well conducted and were therefore judged to be reliable.

The mouse acute oral LD_{50} of HEDP acid was 1100 mg/kg bw (unpublished data). More detailed study information was not available. Therefore the quality and reliability of this investigation could not be evaluated.

The acute oral toxicity of disodium salt of HEDP was tested in a non-GLP and guideline compliant study in rats and rabbits. Sprague-Dawley rats were dosed by gavage up to 1600 mg HEDP disodium salt/kg bw (Nixon *et al.*, 1972). Dentifrice formulations and mouthwash formulations containing HEDP disodium salt were also tested at 750 mg and 250 mg active acid/kg bw, respectively. The rats were observed for clinical symptoms and mortalities following treatment. Surviving animals were necropsied after 14 days. Blood and tissue samples were taken from selected individuals for examination. Signs of toxicity and number of deaths were not reported. At necropsy, tubular damage in the kidneys and mucosal irritation in the stomach was observed in surviving animals that had been dosed with high levels (*i.e.*, 1140 and 1600 mg/kg bw). The investigators determined the acute oral LD_{50} in rats to be 1340 mg active salt/kg bw.

In the second part of the afore mentioned acute oral toxicity study, New Zealand white rabbits, including pregnant females, were administered the disodium salt of HEDP by oral gavage (Nixon *et al*., 1972) The dose levels were not reported. The rabbits were observed daily for clinical symptoms and mortalities after the treatment. Surviving animals underwent necropsy for macroscopic examination. Blood and tissues were taken from all surviving animals. An unreported number of animals died in the first 4 days with most deaths occurring within the first 24 hours. The only treatment-related toxic symptom in surviving animals reported was diarrhoea. At necropsy, mild gastric irritation was observed in animals from each test group. About 50 % of surviving animals from all groups presented kidney lesions resembling chronic interstitial nephritis. However, the latter may have been non-treatment related as this is a common condition in rabbits. The lowest LD_{50} found for mature male rabbits was 581 mg active salt/kg bw, while immature animals appeared less sensitive with an LD_{50} of 1050 mg/kg bw.

The mouse acute oral LD_{50} of HEDP disodium salt ranged from >1000 to > 5000 mg/kg bw (Henkel KGaA unpublished data a, b; Henkel KGaA, 1975). Due to the absence of more detailed study information, the quality and reliability of this investigation could not be evaluated.

Two acute oral toxicity studies were conducted with the tetrasodium salt of HEDP in rats. In the first study, ten rats (*i.e.*, 5 per sex) were administered an aqueous solutions of the salt by

gavage at dose levels of 660, 825, 1056, 1320 and 1650 mg active salt/kg bw. The rats were observed for clinical symptoms and mortalities following treatment. All animals were necropsied after 14 days. Clinical signs included ataxia and/or tremors, oral and nasal discharge, hypo-activity, soft stool and faecal and/or urinary staining. All 10 animals died at the highest treatment dose of 1650 mg active salt/kg bw, 8 at 1320 mg active salt/kg bw, 7 at 1056 mg active salt/kg bw, 4 at 825 mg active salt/kg bw and 1 at 660 mg active salt/kg bw. At necropsy, discoloration of the lungs, gastrointestinal changes like red or black walls and red or black fluid present, and pallor of kidneys were observed. The acute oral LD_{50} in rats was determined to be 940 mg active salt/kg bw. The study was not in compliance with GLP regulations, but conducted using a protocol following the principles of OECD guideline 401 (Bio/Dynamics Inc., 1985a).

In the 2nd acute oral toxicity study, rats were administered by gavage aqueous solutions of the tetrasodium salt of HEDP at dose levels of 915, 1152, 1451 and 1826 mg active salt/kg bw (Younger Laboratories, 1965b). Following exposure, signs of toxicity included rapid weakness with collapse within 10 to 30 minutes, diarrhoea and convulsions. All animals died at the two highest treatment levels (*i.e.,* 1451 and 1826 mg active salt/kg bw) and one male and one female died at the 1152 mg active salt/kg bw dose level. At necropsy renal, liver and pulmonary hyperaemia were observed. The acute oral LD_{50} was determined to be 1219 mg active salt/kg bw. The study was not in compliance with GLP or OECD guidelines, but was evaluated to be scientifically sound and therefore judged to be reliable.

DTPMP acid (CAS No. 15827-60-8) and DTPMP salts (CAS No. 22042-96-2)

The acute oral toxicity of DTPMP acid was evaluated in Sprague-Dawley rats (Younger Laboratories, 1971a). Five rats per group (*i.e.*, 2-3 per sex) were administered by oral gavage aqueous solutions of DTPMP acid at dose levels equivalent to 2906, 3660, 4605 and 5800 mg active acid/kg bw. The rats were observed for clinical symptoms and mortalities following treatment. All animals underwent necropsy after 7 days. Clinical signs of toxicity included reduced appetite and activity, slight lethargy, rapidly increasing weakness, collapse and death. All animals died at the highest treatment level of 5800 mg active acid/kg bw, 3 females at 4605 mg active salt/kg bw and 2 females at 3660 mg active salt/kg bw. No deaths occurred at a treatment level of 2906 mg active acid/kg bw. At necropsy, slight liver discoloration and acute gastro-intestinal inflammation were observed. The acute oral LD_{50} of DTPMP acid was reported to be 4164 mg active acid/kg bw. Although the study was pre-GLP and not in full compliance with OECD guidelines, the study provided sufficient information and was assessed to be reliable.

Three acute oral toxicity studies were conducted on the heptasodium salt of DTPMP. Ten Sprague-Dawley rats, 5 per sex were administered aqueous solutions of the salt by oral gavage at doses of 3 and 10 mL/kg bw. This equals a dose of 1751 and 5838 mg active salt/kg bw respectively. Rats were observed daily for mortalities and clinical symptoms following treatment. No deaths occurred at any dose level. All surviving animals underwent necropsy for macroscopic examination at the end of the study (*i.e.,* day 14). General signs of toxicity which were observed in all animals in the 10 mL/kg bw treatment group included pilo-erection, abnormal body carriage (*i.e.*, hunched posture), lethargy, and decreased respiration rate. All males and two females also suffered ptosis. At necropsy, no macroscopic abnormalities were observed. The acute oral LD_{50} in rats was determined to be greater than 5838 mg active salt/kg bw (Safepharm Laboratories Ltd., 1982c). In a follow-up study, rats were administered a higher dose of 15 mL/kg, equivalent to 8757 mg active salt/kg

(Safepharm Laboratories Ltd., 1982d). Nine out of 10 animals died within two days after dosing. General signs of toxicity included pilo-erection, hunched posture, lethargy, and decreased respiratory rate and ptosis. At necropsy, congestion in the lungs and haemorrhage of lungs, stomach, small intestine, and pallor of spleen, kidneys and liver were observed in the animals that died during the study. The acute oral LD_{50} was estimated to be less than 8757 mg active salt/kg bw. Both studies were GLP-compliant and broadly compatible with OECD guidelines.

Another acute oral toxicity study, non-GLP but also following the principles of OECD guidelines, was conducted with the heptasodium salt of DTPMP (Hazleton Laboratories Europe Ltd., 1979). Rats were administered an aqueous solutions of the salt by gavage at dose levels of 82.5, 165, 330, 660 and 1650 mg active salt/kg bw. Rats were observed daily for mortalities and clinical symptoms following treatment. In this investigation, none of the animals were necropsied at the end of the study. None of the animals died or showed signs of toxicity throughout the study. The acute oral LD_{50} was therefore determined to be greater than 1650 mg active salt/kg bw.

The acute oral LD_{50} of octasodium salt of DTPMP in Sprague-Dawley rats was reported to be greater than 3870 mg active salt/kg (Monsanto, 1979a). Fifty rats (*i.e.*, 25 per sex) were administered an aqueous solution containing 43 % active salt at dose levels of 2150, 2580, 3010, 3440 and 3870 mg active salt/kg bw by gavage. The rats were observed for mortalities and clinical symptoms following treatment. No deaths occurred. Twenty animals underwent necropsy for macroscopic examination. Diarrhea was observed in one animal and many animals exhibited urine-stained fur 1 day after dosing. At necropsy, unilateral hydronephrosis in one animal and hemorrhagic thymus in another were observed. Although the study was pre-GLP and not in full compliance with OECD guidelines, the study was assessed to be scientifically well conducted and therefore judged to be reliable.

Table 21: Summary of acute oral toxicity data

Test material	Species	Doses tested (active acid/salt)	LD_{50} values (active acid/salt)	Reference
ATMP acid	rat	2000-3980 mg/kg bw	2910 mg/kg bw	Younger Laboratories (1967)
ATMP acid	mouse	Not reported	2790 mg/kg bw	Gloxhuber, unpublished data
ATMP.4Na	rat	5740 mg/kg bw	>5740 mg/kg bw	Safepharm Laboratories Ltd. (1982a)
ATMP.4Na	rat	8610 mg/kg bw	~8610 mg/kg bw	Safepharm Laboratories Ltd. (1982b)
ATMP.5Na	rat	5040-10040 mg/kg bw	7120 mg/kg bw	Safepharm Laboratories Ltd. (1962)
HEDP acid	rat	1200-2388 mg/kg bw	1878 mg/kg bw	Younger Laboratories (1965a)
HEDP acid	rat	948-1896 mg/kg bw	1440 mg/kg bw	Younger Laboratories (1977)
HEDP acid	mouse	Not reported	1100 mg/kg bw	Henkel KGaA unpublished data (a)
HEDP.2Na	rat	Up to 1600 mg/kg bw	1340 mg/kg bw	Nixon *et al*. (1972)

Test material	Species	Doses tested (active acid/salt)	LD_{50} values (active acid/salt)	Reference
HEDP.2Na	rabbit	Not reported	581 mg/kg bw	Nixon *et al*. (1972)
HEDP.2Na	mouse	Not reported	>1000->5000 mg/kg bw	Henkel KGaA unpublished data (a, b) Henkel KGaA (1975)
HEDP.4Na	rat	660-1650 mg/kg bw	940 mg/kg bw	Bio/Dynamics Inc. (1985a)
HEDP.4Na	rat	915-1826 mg/kg bw	1219 mg/kg bw	Younger Laboratories (1965b)
DTPMP acid	rat	2906-5800 mg/kg bw	4164 mg/kg bw	Younger Laboratories (1971a)
DTPMP.7Na	rat	1751-5838 mg/kg bw	>5838 mg/kg bw	Safepharm Laboratories Ltd. (1982c)
DTPMP.7Na	rat	8757 mg/kg bw	<8757 mg/kg bw	Safepharm Laboratories Ltd. (1982d)
DTPMP.7Na	rat	82.5-1650 mg/kg bw	>1650 mg/kg bw	Hazleton Laboratories Europe Ltd. (1979)
DTPMP.8Na	rat	2150-3870 mg/kg bw	3870 mg/kg bw	Monsanto (1979a)

Conclusion

The phosphonic acid compounds ATMP, HEDP, DTPMP and their salts evaluated in this review can be considered to be of low to moderate acute oral toxicity. ATMP acid was of moderate acute toxicity to mammals. The acute oral LD_{50} in rat was determined to be 2910 mg active acid/kg bw. In comparison, the tetrasodium and pentasodium salt of ATMP were less acutely toxic with LD_{50} values of 8610 and 7120 mg active salt/kg bw, respectively. HEDP acid and its salts are of moderate acute oral toxicity LD_{50}'s in rats and mice ranging from 1100 to 1878 mg active acid/kg bw. The oral LD_{50} values of HEDP salts were in a slightly wider range from 581 mg active salt/kg bw to greater than 5000 mg active salt/kg. DTPMP acid and salts are of low toxicity with oral LD_{50} values from 3870 mg active salt/kg bw to less than 8757 mg active salt/kg bw.

5.2.1.1.2 Acute inhalation toxicity

There were no test data available to evaluate the acute inhalation toxicity of the phosphonic compounds ATMP, HEDP and DTMP, both in the acid and salt forms.

5.2.1.1.3 Acute dermal toxicity

ATMP acid (CAS No. 6419-19-8) and ATMP salt (CAS No. 20592-85-2)

Five rabbits (*i.e.*, one per group) received a single 24-hour dermal application of ATMP acid at dose levels of 1000, 1580, 2510, 3980 and 6310 mg active acid/kg (Younger Laboratories Ltd., 1967). None of the treated animals died or showed signs of toxicity as a result of exposure. No necropsies were performed at the completion of the studies. In this

investigation, the acute dermal LD_{50} was determined to be greater than 6310 mg active salt/kg bw. The study was not in compliance with OECD guideline 402 and GLP regulations. A critical weakness is related to the fact that only one animal was used per dose level. However, as there was no toxicity observed and no other studies with the acid were performed this value serves to fill a data gap and gives an indication of the low dermal toxicity of this substance.

In a GLP-compliant study, a group of ten rats (*i.e.*, five per sex), was given a single 24-hour dermal application of an aqueous solution of the tetrasodium salt of ATMP at a dose level of 10 mL/kg bw, equivalent to 5740 mg active salt/kg bw (Safepharm Laboratories Ltd., 1982e). The rats were observed for mortalities and clinical symptoms following treatment. After 14 days, the surviving animals underwent necropsy for macroscopic examination. No deaths occurred during the test and clinical signs were limited to lethargy and increased lachrymation (with red colouring) in one animal. The acute dermal LD_{50} was determined to be greater than 5740 mg active salt/kg bw. The study followed the principles of OECD guideline 402 and was assessed to be scientifically reliable.

The pentasodium salt of ATMP was evaluated in a further acute dermal toxicity study (Younger Laboratories, 1962). In this investigation, six female New Zealand white rabbits (*i.e.*, one per group) received a single 24-hour dermal application under occlusive patch of an aqueous solution of the pentasodium salt of ATMP at doses levels of 632, 1004, 1592, 2524, 4000 and 6320 mg active salt/kg. No animals died and no symptoms of toxicity were observed during the study. The acute dermal LD_{50} was therefore determined to be greater than 6320 mg active salt/kg bw. Although the study was pre-GLP and not in full compliance with OECD guideline 402, the study provided sufficient information to be reliable. Similarly to the study on the acid form of ATMP, the fact that only one animal was used per does level is a weakness. However, also here the study was scientifically well conducted and indicates the low dermal toxicity of the pentasodium salt of ATMP.

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

The acute dermal toxicity of HEDP acid was tested in New Zealand white rabbits in a non-GLP study following OECD guidelines 402 (Younger Laboratories, 1965a). In this study, six rabbits (*i.e.*, one per treatment) received a single 24-hour dermal application of an aqueous solutions of HEDP acid at dose levels of 600, 948, 1506, 2388, 3786 and 6000 mg active acid/kg bw. No necropsies were performed. Clinical signs included moderate weakness and much discomfort at the higher dose levels, but no paralysis developed. No deaths occurred during the study. The LD_{50} of HEDP acid was determined to be greater than 6000 mg active acid/kg bw. A similar study determined an acute dermal LD_{50} value for the acid of greater than 4764 mg active acid/kg bw (Younger Laboratories, 1977). In this study, three rabbits received a single 24-hour dermal application of an aqueous solution of HEDP acid at dose levels of 3006 mg active acid/kg bw (*i.e.*, 1 animal) and 4764 mg active acid/kg (*i.e.*, 2 animals). After 14 days, surviving animals were necropsied and no abnormalities were observed. Clinical signs included reduced appetite and activity within 2-3 days. Although both studies testing the acid were pre-GLP and not in full compliance with OECD guidelines with significant methodological deficiencies (e.g., few animals per dose) they provided evidence of the low dermal toxicity of HEDP acid in rabbits.

Two acute dermal toxicity studies were conducted with the tetrasodium salt of HEDP in New Zealand white rabbits. In the first study, ten female rabbits (*i.e.*, 5 per sex) received a single

24-hour dermal application of an aqueous solution of the salt at a dose level of 5000 mg/kg bw. This equals a dose of 1650 mg active salt/kg bw (Bio/Dynamics Inc., 1985b). The rabbits were observed for mortalities and clinical symptoms following treatment. All animals were necropsied after 14 days. Slight weight loss and severe persistent dermal effects at the dose site, defined as necrosis followed by eschar formation and /or exfoliation of the eschar tissue, were observed in most animals. One female animal died on day 13 of the observation period. Necropsy suggested this death was not treatment-related. The acute dermal LD_{50} was determined to be greater than 1650 mg active salt/kg bw. The study was non-GLP but conducted using a protocol which follows the principles of OECD guidelines 402.

In the second study with the tetrasodium salt of HEDP, six rabbits (*i.e.*, 1 per group) received a single 24-hour dermal application of 230, 363, 577, 915, 1451 or 2300 mg active salt/kg bw (Younger Laboratories, 1965b). Signs of toxicity included considerable weakness in the two highest-dose animals (*i.e.*, 1451 and 2300 mg active salt/kg bw), lethargy and reduced appetite for several days. No deaths occurred at any dose level. The acute dermal LD_{50} was therefore determined to be greater than 2300 mg active salt/kg bw. Although the study had significant methodological deficiencies, it supports the low dermal toxicity of HEDP tetrasodium salt in rabbits determined in the more scientifically robust study above.

DTPMP acid (CAS No. 15827-60-8) and DTPMP salts (CAS No. 22042-96-2)

The acute dermal toxicity of DTPMP acid was tested in New Zealand white rabbits (Younger Laboratories, 1971a). Four rabbits (*i.e.*, 2 per sex) received a single 24-hour dermal application of an aqueous solution of DTPMP acid at dose levels of 4605 mg active acid/kg bw (2 animals), 1833 and 2906 mg active acid/kg bw (1 animal). No deaths were observed during the study period. Surviving animals were necropsied after 14 days for macroscopic examination and no signs of toxicity were observed. Clinical signs included reduced appetite and activity for 1-2 days. The LD_{50} of DTPMP acid was reported to be greater than 4605 mg active acid/kg bw. The study was pre-GLP and not in full compliance with OECD guideline 402 but provided sufficient information to be reliable. The low number of animals used in this study is a weakness. Nevertheless, this investigation indicates a low level of dermal toxicity of DTPMP acid.

Two acute dermal toxicity studies in rats were conducted with the heptasodium salt of DTPMP (Safepharm Laboratories Ltd., 1982f). In the first study, ten Sprague-Dawley rats (*i.e.*, 5 of each sex) received a single 24-hour dermal application of an aqueous solution of the salt at a dose level of 10 mL/kg bw. This equals a dose of 5838 mg active salt/kg bw. The rats were observed daily for mortalities and clinical symptoms following treatment. No deaths occurred at any dose level. At the end of the study (day 14), all animals underwent necropsy for macroscopic examination. Sign of toxicity observed in this study were limited to lethargy observed in one male and female animal shortly after dosing. From 4 hours after dosing, no further abnormal symptoms were observed. At necropsy, no macroscopic abnormalities were determined. On the basis of this study, the acute dermal LD_{50} in rats was determined to be greater than 5838 mg active salt/kg bw. The study was GLP and OECD compliant and considered to be of good quality.

In the second acute dermal toxicity study with the heptasodium salt, ten rats (*i.e.*, 5 per sex) received a single 24-hour dermal application of 2145 mg active salt/kg bw. The rats were observed daily for mortalities and clinical symptoms following treatment. No deaths occurred during the study, and no clinical signs of toxicity were observed. The acute dermal LD_{50} was

evaluated to be greater than 2145 mg active salt/kg bw (Hazleton Laboratories Europe Ltd., 1979). The study was not in compliance with GLP or OECD guidelines, but supports the low dermal toxicity of salt.

The acute dermal toxicity of octasodium salt of DTPMP acid was tested in white New Zealand rabbits (Monsanto, 1979b). Ten rabbits (*i.e.*, 5 per sex) received a single 24-hour dermal application of the octasodium salt of DTPMP at a dose of 860 mg active salt/kg bw. No deaths occurred during the study. All test animals underwent necropsy at the end of the study. Only signs of toxicity were related to erythema on the application site which was observed in five animals. On the basis of this evaluation, the study investigators concluded that the acute dermal LD_{50} octasodium salt of DTPMP is greater than 860 mg active salt/kg bw. Although the study was pre-GLP and not in full compliance with OECD guideline 402, the study provided sufficient information and was judged to be reliable.

Table 22: Summary of acute dermal toxicity data

Test material	Species	Doses tested (active acid/salts)	LD_{50} values (active acid/salt)	Reference
ATMP acid	rabbit	1000-6310 mg/kg bw	>6310 mg/kg bw	Younger Laboratories (1967)
ATMP.4Na	rat	5740 mg/kg bw	>5740 mg/kg bw	Safepharm Laboratories Ltd. (1982e)
ATMP.5Na	rabbit	632-6320 mg/kg bw	>6320 mg/kg bw	Safepharm Laboratories Ltd. (1962)
HEDP acid	rabbit	600-6000 mg/kg bw	>6000 mg/kg bw	Younger Laboratories (1965a)
HEDP acid	rabbit	3006-4764 mg/kg bw	>4764 mg/kg bw	Younger Laboratories (1977)
HEDP.4Na	rabbit	1650 mg/kg bw	>1650 mg/kg bw	Bio/Dynamics Inc. (1985a)
HEDP.4Na	rabbit	230-2300 mg/kg bw	>2300 mg/kg bw	Younger Laboratories (1965b)
DTPMP acid	rabbit	1833-4605 mg/kg bw	>4605 mg/kg bw	Younger Laboratories (1971a)
DTPMP.7Na	rat	5838 mg/kg bw	>5838 mg/kg bw	Safepharm Laboratories Ltd. (1982f)
DTPMP.7Na	rat	2145 mg/kg bw	>2145 mg/kg bw	Hazleton Laboratories Europe Ltd. (1979)
DTPMP.8Na	rabbit	860 mg/kg bw	>860 mg/kg bw	Monsanto (1979a)

Conclusion

The acids and salts of ATMP, HEDP, and DTPMP evaluated in this review, can be considered to be of low acute dermal toxicity.

ATMP acid and its tetra- and pentasodium salt were practically non-toxic with LD_{50} values exceeding the concentrations tested. Dermal LD_{50} values were determined to be greater than 6310 mg active acid/kg bw. No dermal toxicity was observed for HEDP acid and its salts at the highest tested concentrations tested of 1650 mg active salt/kg bw. DTPMP compounds

were practically non-toxic with dermal LD_{50} values in rabbit greater than 2145 mg active salt/kg bw, respectively.

Although there were some weaknesses in some of the studies because of the use of a low number of test animals per dose group, the overall picture that these phosphonates are of very low acute dermal toxicity is very consistent. This assessment is supported by a few high quality studies which complied with OECD guidelines 402 and GLP regulations.

5.2.1.1.4 Acute toxicity-other routes

Intraperitoneal application of HEDP acid and *in vitro* administration of HEDP disodium salt in mice resulted in LD_{50} values of 285 mg/kg bw and 61 mg/kg bw (Henkel KGaA unpublished data c, d). No further details on both studies were reported.

5.2.1.2 Corrosiveness/irritation

5.2.1.2.1 Skin irritation

ATMP acid (CAS No. 6419-19-8) and ATMP salt (CAS No. 20592-85-2)

The potential of ATMP acid to cause skin irritation in rabbit was evaluated in a high quality, GLP and OECD guideline 404 compliant study (Safepharm Laboratories Ltd., 1982g). An aqueous solution containing 50 % of active ATMP acid and 1 % HCl was applied as a single dose of 0.5 mL, equivalent to 333 mg active acid, under an occlusive dressing for 4 hours to the shorn intact skin of three New Zealand white rabbits. Skin reactions were graded 1, 24, 48 and 72 hours after removal of the patch and residual test substance. Mild erythema (score 1), but no oedema was observed up to 24 hours after treatment. A primary skin irritation index (PII) of 0.4 was calculated, indicating that ATMP acid is minimally irritating to rabbit skin. The PII is determined by evaluating the skin site at 24, 48 and 72 hours for erythema and oedema. The applied scores can range from 0 (*i.e.*, no erythema/oedema) to 4 (*i.e.*, severe erythema/oedema). The assigned scores were then averaged over the observation period and added up to give a total out of the maximum of a PII of 8.

In a further study using the same methodology, undiluted ATMP acid powder and a 25 % aqueous solution of ATMP acid was applied as a single dose under an occlusive dressing for 4 hours to the shorn intact skin of six rabbits (*i.e.*, 3 animals per dose group) (Younger Laboratories, 1967). The skin responses were evaluated over a period of 7 days. While the powder did not cause any visible irritation (*i.e.*, no observation of erythema or oedema), exposure to the 25 % aqueous formulation of ATMP acid resulted in moderate erythema and defined oedema (overall scores of 4-5 per animal) which resolved after 7 days. A PII of 4.6 was calculated. On the basis of these results, the powder form of ATMP acid was considered to be not irritating, while the 25 % aqueous solution of ATMP acid can be considered to be moderately irritating.

The skin irritation potential of the tetrasodium salt of ATMP in New Zealand white rabbits was evaluated in a high quality GLP and OECD guideline 404 compliant study (Safepharm Laboratories Ltd, 1982h). A volume of 0.5 mL of an aqueous solution containing 41 % active salt was applied as a single dose under an occlusive dressing for 4 hours to the shorn intact

skin of three rabbits. The skin reactions were graded 1, 24, 48 and 72 hours after removal of the patch and washing off the residual test substance. Mild erythema in one animal (score 1), but no oedema were observed 1 hour after treatment. On the basis of the results present, a primary skin irritation index (PII) of 0.1 was calculated. This PII indicates that tetrasodium salt ATMP is only minimally irritating to rabbit skin.

The pentasodium salt of ATMP was tested with rabbits as a 40 % aqueous solution. The test material was applied as a single dose under an occlusive dressing for 24 hours to the shorn intact skin of 3 rabbits. Further details about the test conditions were not available. The skin response was evaluated over a period of 7 days. Two of the three animals showed a mild response with barely perceptible redness (*i.e.*, erythema) and no oedema. The effects were fully reversible by 72 hours. On the basis of the PII which was calculated to be 0.6, the pentasodium salt of ATMP was considered to be slightly irritating. The study was not performed according to GLP standards and did not follow OECD guidelines. In addition the study report makes several omissions that call in question the scientific reliability of the study. However, the data reported provide further indication of the low irritation potential of the ATMP salts (Younger Laboratories, 1962).

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

HEDP acid was applied to rabbit's skin as a single aqueous solution containing 60 % active acid and 0.02 % HCl. Six New Zealand white rabbits were exposed to 0.5 mL of the solution under an occlusive dressing for 24 hours. No responses indicative of skin irritation were observed throughout the study (Younger Laboratories, 1977). This study was not conducted in compliance with OECD guidelines and GLP regulations.

The potential of HEDP acid to cause skin irritation in New Zealand white rabbits was evaluated by applying an aqueous solution (concentration not stated) under an occlusive dressing for 24 hours to both intact and abraded skin of 3 animals (Younger Laboratories, 1965a). Skin responses were evaluated for a period of 7 days. The results indicate that the substance was a moderate irritant to rabbit skin. However, this non GLP/OECD study was judged unreliable due to insufficient documentation; results from intact and abraded skin were not shown separately and there was no distinction between erythema/eschar and oedema formation.

The disodium salt of HEDP has been reported to be slightly irritating to rabbit skin, but not irritating in the hairless mouse (Henkel KGaA unpublished data e, f; Henkel KGaA, 1975)(Procter and Gamble, 1999; 2000; Soap and Detergent Association, 2002). Due to the absence of further detailed information, the reliability of the studies could be evaluated.

The skin irritation potential of the tetrasodium salt of HEDP in rabbits was tested in two studies. In the first, a good quality GLP and OECD guideline 404 compliant study, an aqueous solution containing 30 % active salt was applied as a single dose of 0.5 mL under a semi-occlusive dressing for 4 hours to the skin of three New Zealand white rabbits (Safepharm Laboratories Ltd., 1995a). Skin reactions were graded 1, 24, 48 and 72 hours after removal of the patch. Very mild erythema (score 1) was observed at 1 hour, but this had resolved within 24 hours and no other effects were seen. On the basis of this investigation, the tetrasodium salt of HEDP was essentially non-irritating.

In the second study, an aqueous solution containing 33 % active tetrasodium salt of HEDP was applied as a single dose of 0.5 mL under a semi-occlusive dressing for 4 hours or under

an occlusive dressing for 24 hours, to the skin of six New Zealand white rabbits (Bio/Dynamics, 1985c). Skin reactions were graded 1, 24, 48 and 72 hours after removal of the patch and after removal of the residual test substance. In the group treated under semi-occlusive conditions for 4 hours, very mild erythema was seen in a few animals up to day 7 (scores 0-2) after exposure. One animal exhibited very mild oedema (score 1) at the 24 hour reading only. On the basis of these findings, a PII of 0.3 was calculated. In the group treated under occlusive conditions for 24 hours, erythema, oedema, superficial necrosis, necrosis, desquamation and/or exfoliation were observed at some test sites and some symptoms had not cleared within 14 days (day 14 erythema and eschar scores 4 in three animals). Based on these observations, the PII was calculated to be 5. Hence, under semi-occlusive patching conditions tetrasodium salt can be classified as minimally irritating while under fully occlusive conditions it should be considered as a moderate irritant. This study was not in compliance with GLP, but partly following the principles of OECD guideline 404 and judged to be reliable.

DTPMP acid (CAS No. 15827-60-8) and DTPMP salts (CAS No. 22042-96-2)

The potential of DTPMP acid to cause skin irritation in rabbits was evaluated in a good quality, GLP/OECD compliant study (Safepharm Laboratories Ltd., 1982i). In this investigation, an aqueous solution containing 50 % active DTPMP acid and 12.8 % HCl was applied as a single dose of 0.5 mL under an occlusive dressing for 4 hours to the shorn intact skin of three New Zealand white rabbits. Skin reactions were graded 1, 24, 48 and 72 hours after removal of the patch and after removal of residual test substance. Very slight erythema in one animal up to 72 hours post-dosing and very slight oedema in one animal in the first hour after dosing were observed. A PII of 0.5 was calculated, indicating that under the testing conditions DTPMP acid is only minimally irritating for rabbit skin, despite the presence of 12.8 % HCl in the solution.

Similar observations were made in two further non-GLP studies (Younger Laboratories, 1971a, 1971b). In the first study 0.5 mL of a solution containing 58 % active acid was applied as a single dose under an occlusive dressing for 24 hours to skin of three rabbits. The skin responses were evaluated over a period of 7 days. After 24 hours very slight erythema were observed in all animals, which was resolved after 48 hours. On the basis of this study, DTPMP acid can be considered as slightly irritating to rabbit skin (*i.e.*, PII = 1) (Younger Laboratories, 1971a).

In the second study, 0.5 mL of an aqueous solution containing 50 % active acid and 8-10 % HCl was applied to intact and abraded rabbit skin. No further details about test conditions were provided. In both, abraded and intact, skin erythema (score 1) was observed in all animals 24 hours after dosing, but resolved by 48 hours. Based on this investigation, DTPMP acid can be considered as minimally irritating to skin (*i.e.*, PII = 0.5) (Younger Laboratories, 1971b).

Two skin irritation studies were conducted with the heptasodium salt of DTPMP. An aqueous solution containing 42 % active salt was applied as a single dose of 0.5 mL under an occlusive dressing for 4 hours to the shorn intact skin of three New Zealand white rabbits. Skin reactions were graded 1, 24, 48 and 72 hours after removal of the patch. No skin reactions or deaths were observed in any of the tested animals. The heptasodium salt was therefore not considered to be irritating to rabbit skin. The study was performed in compliance GLP and OECD guidelines (Safepharm Laboratories Ltd., 1982j).

In another good quality study which was, however, not in compliance with GLP or OECD guidelines, an aqueous solution containing 33 % heptasodium salt of DTPMP, the test substance was applied as a single dose of 0.58 mL under a semi-occlusive dressing for 24 hours to the shorn abraded and intact skin of six rabbits (Hazleton Laboratories Europe Ltd., 1979). Skin responses were evaluated 24 and 72 hours after treatment. Very slight erythema in both intact and abraded skin and oedema in abraded skin only, were observed at 24 hours after treatment. Effects were fully resolved at 72 hours. Based on these observations a PII of 0.4 was calculated, indicating heptasodium salt of DTPMP was minimally irritating.

The octasodium salt of DTPMP was reported to be minimally irritating to rabbit skin (Monsanto, 1979c). An aqueous solution containing 26 % active salt was applied as a single dose of 0.5 mL to the shorn intact skin and abraded skin of six New Zealand white rabbits. The skin responses were evaluated 24 and 72 hours after treatment. Very slight erythema (*i.e.*, on intact and abraded skin) and oedema (*i.e.*, on abraded skin only) were observed at 24 hours after treatment. Effects were fully resolved at 72 hours. Based on these observations a PII of 0.4 was calculated, indicating octasodium salt of DTPMP to be minimally irritating. Although the study was pre-GLP and not in full compliance with OECD guidelines, the study was scientifically sound and appeared to be well conducted.

Conclusion

On the basis of the studies presented, the phosphonic acid compounds ATMP, HEDP, DTPMP and their salts, can generally be considered to be mildly irritating to skin at most. The PII in most cases did not exceed 3 which indicate a compound to be mildly irritating to skin. In one study a more severe reaction was observed, when an aqueous solution containing 25 % of ATMP acid was applied to intact rabbit skin for 4 hours under occluded conditions (PII = 4.6). The same result was obtained when an aqueous solution containing 33 % active tetrasodium salt of HEDP was applied to rabbit skin for 24 hours under occlusive dressing (PII = 5). The longer application time of 24 h caused more irritation then when the acid or salt product was only applied over 4 h where no irritation response was observed in most cases regardless of the strength of the product tested. Applying the neat acid or salt did not seem to produce a consistently greater effect, rather in some cases the neat powder product was less irritating than some tested formulations, indicating reduced potential of the applied powder product for skin reactivity.

5.2.1.2.2 Eye irritation

ATMP acid (CAS No. 6419-19-8) and ATMP salt (CAS No. 20592-85-2)

The eye irritation potential of ATMP acid was evaluated following the Draize method (Draize *et al*., 1944). In this study, 100 mg ATMP acid powder was placed into the conjunctival sac of the right eye of three rabbits (Younger Laboratories, 1967). The other eye remained untreated to serve as a control. An initial pain reaction to the product was recorded and the eyes were examined for ocular reactions up to 7 days after the instillation of the test material. The Kay and Calandra rating was used to assign an Eye Irritation Index (EII) from 0 to 110 (Kay and Calandra, 1962). This method uses the criteria of incidence, extent and persistence of injury to determine the irritation potential of a product on the cornea, iris, and conjunctivae. Immediately after instillation, oedema, lid closure, copious discharge, moderate redness of the conjunctivae and mild corneal cloudiness were observed in all three rabbits.

Twenty four hours after product instillation, the eyes were rinsed and observations included also lid closure and iris congestion (overall scores per animal after 24 hours: 57, 49 and 55). Responses decreased, but were still present after 7 days. The EII was calculated to be 43.6; indicating ATMP acid to be moderately irritating to the rabbit eye. The study was not in compliance with GLP and OECD guidelines, but was considered to be scientifically robust and reliable.

The tetrasodium salt of ATMP was reported to be practically non-irritating to rabbit eye in a good quality, OECD and GLP compliant study (Safepharm Laboratories Ltd., 1982k). An aqueous solution (*i.e.*, 0.1 mL) containing 41 % active salt was placed into the conjunctival sac of the right eye of 3 New Zealand white rabbits. Responses were evaluated after 1, 24, 48 and 72 hours. Minor symptoms were observed, primarily in the first hour after treatment (*i.e.*, dulled cornea, iritis, mild conjunctival inflammation, and conjunctival chemosis) which were fully reversible by 24 hours. The EII was calculated to be 0.7.

A 40 % aqueous solution of the pentasodium salt of ATMP (*i.e.*, 0.1 mL) was placed into the conjunctival sac of one eye of 3 rabbits (Safepharm Laboratories Ltd., 1962). After 24 hours the eyes were rinsed and eye irritation effects were evaluated over a period of 7 days. Responses observed in the first hour after application included slight discharge and redness, very slight oedema and a trace of corneal dullness. All responses decreased and were fully reversed in two animals by 72 hours and in all animals by 120 hours. An EII of 3.8 was calculated. The study was pre-GLP and not in compliance with OECD guideline 405, but it was considered to be scientifically sound and reliable.

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

The eye irritation potential of HEDP acid was evaluated in two studies. In the first study, 0.1 mL of an aqueous solution containing 60 % active acid and 0.02 % HCl was placed into the conjunctival sac of the right eye of three New Zealand white rabbits (Younger Laboratories, 1965a). In one animal, the eye was rinsed after 4 seconds while the treated eyes of the other two animals were rinsed after 24 hours. The responses were evaluated up to 7 days after dosing. In the eye that was rinsed immediately after introduction of the test compound moderate lachrymation, mild oedema and erythema and mild corneal cloudiness were observed after 1 hour (overall score 31). These effects were reduced to very slight redness after 7 days. In this exposure scenario, with an EII of 29, the acid was considered to be moderately irritating. However, without immediate rinsing, both animals showed at the one hour reading copious discharge, translucent cornea with iris details moderately obscured and swelling with partial eversion of lids (overall eye irritation scores after 1 hour exposure: 42 and 49). These effects increased in severity over the study period (overall scores after 72 hours in both animals: 90). At day 7, the iris did not respond to light and the lower half of the cornea was opaque. With a calculated EII of 77.3, HEDP acid can be considered as severely irritating when rabbit eyes were only rinsed after 24 hours. However, this effect was shown to be reduced by immediate rinsing of the eye after application. Although predating GLP and OECD guidelines, the study was conducted using an acceptable protocol and provided reliable information on the eye irritation potential of HEDP acid.

In the second study, 0.1 mL of an aqueous solution, containing 60 % HEDP acid and 0.02 % HCl was instilled into the eyes of six rabbits. The eyes of the animals were observed for up to 21 days after installation (Younger Laboratories, 1977). Observations included initial pain, severe erythema and copious discharge 10 minutes after introduction of the test material.

These symptoms were more pronounced after 24 hours and then improved slightly over the next 10 days. However, after 14 days ulceration were observed in all rabbits and a ruptured cornea in one instance. At 21 days, corneal ulceration, slight erythema and copious discharge were still observed in 4 animals. The EII was calculated to be 38.8, indicating HEDP acid was moderately to severely irritating to rabbit's eyes. Based on the persistence of the observed response, the acid can be considered to cause irreversible effects to rabbit's eyes.

The disodium salt of HEDP was reported to be moderately irritating to rabbit eyes (Nixon *et al*., 1972). However, the study used a non-standard scoring system and the lack of full presentation place a low reliability on the outcome.

The eye irritation potential of the tetrasodium salt of HEDP was evaluated in three eye irritation studies. In the first study, a good quality GLP and OECD guideline 405 compliant study, 0.1 mL of an aqueous solution containing 30 % active salt, was placed into the conjunctival sac of the right eye of three New Zealand white rabbits (Safepharm Laboratories Ltd., 1995b). The treated eye was rinsed 24h after dosing and responses were evaluated 1, 24, 48 and 72 hours after product installation. Mild conjunctival redness, chemosis and discharge were observed 1 hour after dosing but these effects had completely cleared within 24h. An eye irritation index of 0.4 was calculated. Based on these results, the test material can be considered as practically non-irritant.

In another OECD guideline compliant study, 0.1 mL of an aqueous solution containing 33 % active tetrasodium salt of HEDP was placed into the conjunctival sac of the right eye of six rabbits (Bio/Dynamics Inc., 1985d). The treated eyes were rinsed 24h after dosing and observed for 7 days. An initial pain response was observed in 2 females and mild conjunctival redness and chemosis was observed 24 h after dosing. Seven days after dosing, all animals were free of symptoms and the EII was calculated to be 2.9. On the basis of these results the tetrasodium salt of HEDP can be considered to be only minimally irritating.

In another less robust and less well reported study summary, an aqueous solution containing 23 % active salt of HEDP was placed into the conjunctival sac of the right eye of 3 animals (Younger Laboratories, 1965b). After 24 hours, the eye was rinsed and responses were evaluated up to 7 days after dosing. A moderate pain response was observed in all animals following dosing and irritant effects after 24 h (*e.g.*, discharge, oedema, moderate redness of conjunctivae, diffuse corneal areas with details slightly obscured) which were mainly resolved by day 7. The test substance was considered to be a moderate eye irritant.

DTPMP acid (CAS No. 15827-60-8) and DTPMP salts (CAS No. 22042-96-2)

The eye irritation potential of DTPMP acid was evaluated in two studies. In the first study, 0.1 mL of an aqueous solution of 50 % active acid and 8-10 % HCl was placed into the conjunctival sac of the right eye of three New Zealand white rabbits (Younger Laboratories, 1971a). Responses to the test material were observed up to 7 days after dosing. Observations included moderate to severe initial pain, moderate erythema, slight oedema and moderate discharge, with full recovery after 7 days. DTPMP acid was found to be minimally irritating to rabbit eye, with an EII of 8.2.

In a subsequent study, using the same test substance and protocol, more severe effects were reported (Younger Laboratories, 1971b). Observations included severe initial pain, corneal cloudiness, necrosis in conjunctival sac, slight oedema and copious discharge, with slight improvement after 7 days. The Eye Irritation Index was calculated to be 48.8, indicating that

DTPMP acid was moderately irritating to rabbit eyes. In the same study, six rabbits were also observed after application of 0.1 mL of the test substance, followed by rinsing after 1 minute. Again there was severe initial pain, necrosis in conjunctival sac, slight oedema, copious discharge and corneal cloudiness. The reaction had not completely cleared at study termination on day 7 and the compound was judged to be moderately irritating to rabbit's eyes (EII = 40.9). On the basis of this investigation, DTPMP acid was considered to be moderately irritating to rabbit eye. Although both studies were pre-GLP and not in full compliance with OECD guidelines, they provided sufficient information to be reliable.

The heptasodium salt of DTPMP has been reported to be minimally irritating in two studies. In a GLP and OECD compliant study, 0.1 mL of an aqueous solution containing 42 % active salt was placed into the conjunctival sac of the right eye of each animal. Responses were evaluated up to 7 days after exposure. One hour after dosing, dulling of the cornea was observed along with conjunctival redness and chemosis. All these symptoms had cleared by 24 hours (EII = 0.0) (Safepharm Laboratories Ltd., 1982l). The same result was obtained in another non-GLP study, which broadly OECD guideline 405 (Hazleton Laboratories Europe Ltd., 1979). The test substance (*i.e.*, 0.1 mL) was placed into the conjunctival sac of six rabbits. Conjuntival redness and chemosis were observed in all six animals 1 day after dosing but these symptoms had cleared by the second day post-dosing.

The octasodium salt of DTPMP was reported to be practically non-irritating to rabbit's eyes (Monsanto, 1979d). An aqueous solution containing (*i.e.*, 0.1 mL) 43 % active salt was placed into the conjunctival sac of one eye of six rabbits. Responses were observed up to 72 hours after dosing. Based on an EII of 2.3, the octasodium salt was considered to be practically non-irritating. The study was pre-GLP and OECD, but considered to be reliable.

Conclusion

The observed eye irritation potential of the phosphonic acid compounds ATMP, HEDP, DTPMP and their salts, ranged from practically non-irritating to severely irritating with irreversible effects.

ATMP acid tested as neat product was considered to be moderately irritating to rabbit eyes, whereas the tetra- and pentasodium salt which were tested in aqueous solutions containing around 40 % active salt were found to be practically non-irritating. These products were evaluated without immediate rinsing the eye following application. All test animals were free of symptoms by the end of the observation period. The studies were of good quality and either in compliance with the OECD guideline 405 or followed its principles.

HEDP acid was tested as a formulation containing 60 % active acid and minimal amounts of HCl with and without rinsing immediately after application. In the study without rinsing, the formulation caused severe irritation and persistent effects. Rinsing the eye directly after application, lessened the severity of the response and all effects disappeared by the end of the observations. All studies were conducted on the basis of acceptable protocols and provided reliable information. The HEDP salts were less irritating to the rabbit eyes in studies with pure salts and formulations thereof tested without rinsing. The tetrasodium salt (*i.e.*, tested as solution containing up to 30 % active salt) was only minimally irritating to the rabbits eyes. The studies with the tetrasodium salt were reliable and partly following OECD guidelines. The studies with the disodium salt were, however, from secondary literature and could therefore not be judged for their reliability. However, also these studies provided some evidence for the low irritation potential of the tested salts.

For the acid of DTPMP the results were equivocal with two studies testing the same product (*i.e.*, a 50 % aqueous solution of the acid) without rinsing. In one study the acid was determined to be moderately irritating whereas the other study found the acid to be only minimally irritating. Both studies were considered as reliable as they provided sufficient information to determine if an adequate protocol was followed. The salts (*i.e.*, up to 50 % active salt) were much less irritating to the rabbit eyes and all effects had cleared by 72 hours post dosing in studies that were judged to be reliable.

In general the same trend as was found with skin irritation was found for eye irritation. The acid compounds were more irritating then tested salts and duration of exposure (*i.e.*, as mimicked by rinsing/non-rinsing immediately after product installation) increased the observed symptoms.

5.2.1.3 Sensitization

ATMP acid (CAS No. 6419-19-8) and ATMP salt (CAS No. 20592-85-2)

There is only very limited information available on the skin sensitization potential of ATMP acid and ATMP salts. In a poorly reported variation of the Magnusson and Kligman guinea pig maximization test, ATMP acid did not result in any evidence for skin sensitization (Henkel KGaA, 1984b). In this study, twenty guinea pigs were exposed to an aqueous solution of the test substance by an initial intradermal injection. No further study details were available on the second part of the induction (*i.e.*, topical application) and the challenge phase. Due to the absence of detailed study information, the study cannot be considered to provide reliable information. However, the study still gives some indications that ATMP acid does not cause skin sensitisation.

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

The disodium salt of HEDP was evaluated in a variation of the Magnusson and Kligman guinea pig maximization test (Henkel KGaA, 1982). In the induction phase, 20 animals from the treatment group were injected intracutaneously with 0.1 mL of three different solutions in duplicate (*i.e.*; injection 1: Freud's Adjuvant/water; injection 2: 5 % aqueous solution of HEDP salt; injection 3: 5 % aqueous solution of HEDP salt and Freud's Adjunct in a 1:1 mixture) in the shoulder region. A further 20 animals served as the control group, receiving also intracutaneous injections with three different solutions in duplicate (*i.e.*; injection 1: Freud's Adjuvant; injection 2: water; injection 3: 5 % water and Freud's Adjunct in a 1:1 mixture). A week later, a mixture containing 5 % HEDP salt in Vaseline was placed on the injection site under occluded conditions for 48 hours in the treatment group. Two weeks after the induction phase, the flanks of the treated and the control animals were shaved and a challenge patch containing 25 % HEDP salt in Vaseline was applied to one flank of the animals for 21 hours. No occlusive patch appeared to have been used. Approximately 24 hours from the start of the challenge application, the skin reaction was observed and recorded according to the Magnusson-Kligman grading scale. Treatment of the control group was not described. Under the test conditions, HEDP acid did not cause skin sensitization in guinea pigs. The study was not in compliance with GLP nor OECD standards but was reported in enough detail to judge it as scientifically reliable.

DTPMP acid (CAS No. 15827-60-8) and DTPMP salts (CAS No. 22042-96-2)

DTPMP acid and its salts did not show any evidence of skin sensitization in a Buehler study and in the Magnusson and Kligman guinea pig maximization test (Procter and Gamble, 1976; Unilever unpublished data). No details on test conditions and study conduct were available. The quality and reliability of these two studies could therefore not be assessed.

Conclusion

The tested phosphonic acid compounds have been demonstrated not to induce skin sensitization in guinea pigs. None of the studies were following OECD guidelines or were GLP compliant. However, only the investigation on the disodium salt of HEDP was recorded to a standard sufficient to support the robustness and reliability of the study design and conduct. Most studies were not reported in great detail, but they stated the adherence to well established protocol such as Buehler or Magnusson and Kligman. The information available provided, however, a coherent picture in that these compounds should not be considered skin sensitizers.

5.2.1.4 Repeated dose toxicity

ATMP acid (CAS No. 6419-19-8) and ATMP salt (CAS No. 20592-85-2)

In a 28-day subacute oral gavage study (Manley, 1981), 12 male rats were dosed with an aqueous solution of ATMP acid at a dose level of 600 mg active acid/kg bw/d. During the study, the animals were observed for any signs of toxicity and mortality. Investigations included measurements of body weights, food consumption, urinalysis as well as biochemical examinations. The study provided limited evidence for a lack of toxicological effects. Duodenal damage (*i.e.*, likely due irritation) observed in a few animals was considered to be due to high osmolarity. However, the number of test animals was too low for a full evaluation of these observations. The investigators suggested the no observed adverse effect level (*i.e.*, NOAEL) to be greater than 600 mg active acid/kg bw/d. In the absence of more detailed study information on this non-GLP and non-guideline compliant study, the quality of the study protocol and conduct cannot be verified.

In a dose-range finding study preceding a 2-year oral feeding study, sixty Long-Evans rats (*i.e.*, 5 per sex and dose group) were fed at dose levels of 0, 125, 250, 500, 750 and 1000 mg/kg bw/d ATMP acid over a period of 34 days (Bio/Dynamics Inc., 1976). No treatment-related effects were observed on body weights, food consumption, or in the survival and gross necropsy. The NOAEL for the dose-range finding study was therefore determined to be > 1000 mg/kg bw/d for both sexes.

A sub-chronic study was conducted with the sodium salt of ATMP. The study was not in full compliance with GLP and OECD guidelines, and provided only limited information. Ten Sprague-Dawley rats (*i.e.*, 5 per sex) were fed for 90 days with a diet containing 161 mg active salt/kg bw/day (*i.e.*, for males) and 175 mg active acid/kg bw/day (*i.e.*, for females) (Safepharm Laboratories Ltd., 1982m). During the study, the animals were observed for any signs of toxicity and mortality. Investigations included measurements of body weights, food consumption, opthalmoscopic examination, urinanalysis as well as biochemical examinations. No treatment-related changes for mortality, body weights, food consumption,

clinical chemistry, haematology and gross pathology were observed. Due to the absence of any treatment related effects, the NOAEL was established at greater than 161 mg active acid/kg bw/d for males and greater than 175 mg active acid/kg bw/d for females.

In a 2-year chronic feeding study, Long-Evans rats were fed with ATMP acid containing diet a dose levels of 0, 50, 150 and 500 mg ATMP acid/kg for a period of 24 months (Bio/Dynamics Inc., 1979a). Seventy animals were used per sex and dose group. During the study, the animals were observed for any signs of toxicity and mortality. Investigations included measurements of body weights, food consumption, opthalmoscopic examination, haematology, clinical chemistry, urinanalysis as well as biochemical examinations. At 6, 12 and 24 months selected organs and tissues were examined at necropsy. No treatment-related effects were observed for mortality, opthalmoscopy, clinical chemistry, histology, haematology and urinalysis. Reduced body weights, changes in absolute and relative liver, spleen and kidney weights or weight ratios, and some erratic increases in testes and kidney weights were observed only in the high dose group. However, these effects were not consistent with time, were minor in extent, were not consistently altered in relation to body weight and were not accompanied by any histopathological findings. There were no differences in the incidence of neoplasia in the different treatment groups (see also section 5.2.1.6). Overall, none of these findings are considered to be indicative of toxicologically-relevant effects. The NOAEL, based on 2-year feeding study can therefore considered to be 500 mg/kg bw/d, the highest tested dose. Taking a conservative approach, based on the 2-year feeding study one could consider the NOEL to be 150 mg ATMP acid/kg bw/d for both sexes. Although the study was not in compliance with GLP regulations, it conformed in all major aspects to OECD method 453.

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

The sub-chronic toxicity of HEDP acid was evaluated in non-GLP but OECD compliant studies in dogs and rats conducted by Industrial Biotest Labs Inc. The quality of the studies is of unknown reliability.

In the dog study (Industrial Biotest Labs Inc., 1975), Beagle dogs (*i.e.*, 4 per sex and dose group) were dosed HEDP acid via the diet for 90 days at dose levels of 0, 1000, 3000 and 10000 ppm. This equals dose levels of about 0, 191, 554 and 1746 mg active acid/kg bw/d for males and 0, 202, 553 and 1620 mg active acid/kg bw/d active acid for females. During the study, the animals were observed for any signs of toxicity and mortality. Investigations included measurements of body weights, food consumption, opthalmoscopic examination, urinanalysis as well as biochemical examinations. At study termination, animals underwent necropsy and histopathological examinations. No treatment-related effects for body weights, organ weights or gross pathology were observed. Erythrocyte counts were slightly elevated in all test groups except the low dose male group, and mean corpuscular volume was slightly decreased in the two highest levels. A dose-related increase in leukocytes in the urine was seen at all dose levels. Histopathology did not show any correlation between the changes in the haematopoietic or urinary systems and the increase in erythrocyte counts and urinary leukocytes and the decrease in mean corpuscular volume. The observed changes are not considered to be of toxicological concern and therefore the NOAEL was determined to be greater than 1746 mg active acid/kg bw/d for males and greater than 1620 mg active acid/kg bw/d for females.

In the rat study (Industrial Biotest Labs Inc., 1979), Charles River albino rats (*i.e.*, 15 per sex and dose group) were administered HEDP acid via the diet for 90 days at dose levels of 0, 1000, 3000 and 10000 ppm. This is equivalent to an exposure of 0, 154, 524 and 1583 mg active acid/kg bw/d for males and 0, 166, 545 and 1724 mg active acid/kg bw/d for females. During the study, the animals were observed for any signs of toxicity and mortality. Observations included measurements of body weights, food consumption, haematology, urinanalysis as well as biochemical examinations. At study termination, animals underwent necropsy and histopathological examinations. No treatment-related effects on clinical chemistry, urinanalysis and gross pathology were observed. Body weights and absolute and relative liver weight were slightly decreased in the highest dose groups. Haematology revealed the following observations at the highest dose: increased erythrocyte counts in males only, reduced haemoglobin concentration and decreased haematocrit. The report further stated that there were no treatment-related histopathological effects. However, there were 2 changes observed in the treated groups and not in the controls: bilateral mineralized microconcretions in kidney tubules (*i.e.*, 3 highest dose males affected) and extramedullary haematopoiesis in the spleen (*i.e.*, 3 highest dose females affected). The presence of extramedullary haematopoiesis was probably related to alterations in iron homeostasis and bilateral mineralized microconcretions may be a result of altered calcium homeostasis. The NOAEL was determined to be approximately 1583 mg active acid/kg bw/d for males and 1724 mg active acid/kg bw/d for females.

The disodium salt of HEDP was tested in a 90-day oral feeding study in Sprague-Dawley rats (Huntingdon Research Centre, 1977). The study, which was not in compliance with GLP but of a good standard, was part of a carcinogenicity study. The rats (*i.e.*, 10 per sex per group) were fed with the test substance in the diet at dose levels of 0, 500, 2000 and 10000 ppm. This equals an exposure of about 0, 41, 169 and 817 mg active salt/kg bw/d for males, and 0, 50, 195 and 1000 mg active salt/kg bw/d for females. Satellite animals were used for laboratory observations to avoid stress on animals being investigated for neoplastic endpoints. Observations included mortality, food consumption, body weights, haematology, biochemistry and urinalysis. No necropsies were performed at study termination. Severe pallor of skin in rats from the 10000 ppm dose group and slight pallor in rats receiving 2000 ppm were observed. Higher alkaline phosphatase and higher plasma glucose levels were seen in the highest dose levels in males and females respectively. A decrease in red cell parameters was seen in the highest dose group for both sexes, and for males at 2000 ppm by week 12. There was evidence of prolonged anaemia in both sexes at 10000 ppm, with a slight retardation of bone marrow development. The observed anaemia, reduction in red cell parameters and pallor were consistent with the perturbation of iron homeostasis. Some of the effects were also observed at 2000 ppm and therefore the NOAEL was determined to be 41 mg active salt/kg bw/d for males and 50 mg active salt/kg bw/d for females.

In another 90-day oral feeding study, Charles River rats (*i.e.*, 20 per sex per group) were fed HEDP disodium salt at doses of 200 and 1000 ppm, equivalent to 260 and 1300 mg/kg bw/d (Nixon *et al*., 1972). The study was non-GLP and only briefly reported with few details. During the study animals were observed for clinical signs and mortalities. Observations included body weights, food consumption and haematology. At necropsy, 15 major organs underwent a histopathological examination. The only effect noted was a slight increase in relative kidney weight in the high dose females which occurred in the absence of histopathological findings. The NOAEL was established at greater than 260 mg/kg bw/d for both sexes, based on the limited information available. The paper indicates a preceding study (unavailable for evaluation) in which rats were fed HEDP sodium salt up to 5000 ppm.

Severe weight loss and a high rate of mortality were observed, with gross findings of gastritis and generalized erosion of the glandular mucosal epithelium.

The chronic toxicity of disodium salt of HEDP was evaluated in a 2-year oral feeding study in rats (Huntingdon Research Centre, 1979). The study was of good quality and performed broadly following OECD method 452. In this study, Sprague-Dawley rats (*i.e.*, 40 per sex and dose group) were administered the test substance via the diet at dose levels of 0, 500, 2000 and 10000 ppm. This equals dose levels of about 0, 19, 78, 384 mg active salt/kg bw/d for males and 0, 24, 96, 493 mg active salt/kg bw/d for females, for 104 weeks. During the study, animals were observed for clinical signs of toxicity and mortality. Observations included body weight, food and water consumption, opthalmoscopic examination, haematology, clinical chemistry and urinanalysis. During the study and at study terminations animals underwent necropsies and histopathological examinations. No mortality or treatment-related effects were observed on ophtalmoscopy, clinical chemistry or gross pathology. Several haematological perturbations were seen during the study, but there were no treatment-related effects which persisted until the termination at 24 months. In animals of the high dose groups (*i.e.*, 2000 and 10000 ppm) anaemia was observed. No histopathological changes were found at study termination. A lack of iron in the spleen was seen in the two highest dose levels at 26 weeks, although this resolved by 104 weeks. The iron deficiency was probably related to the chelating properties of the test substance. No increased incidence of neoplastic lesions was observed in the treated groups at study termination (see also 5.2.1.6). Due to the occurrence of anaemia at the higher doses, the NOAEL was determined to be 19 mg active salt/kg bw/d for males and 24 mg active salt/kg bw/d for females.

DTPMP acid (CAS No. 15827-60-8) and DTPMP salts (CAS No. 22042-96-2)

No repeated dose toxicity studies with DTPMP acid were identified.

The sub-chronic toxicity of DTPMP sodium salt was evaluated in a high quality 90-day oral feeding study (Central Toxicology Laboratory, 1988). In this investigation, Wistar rats (*i.e.*, 12 per sex and dose group) were fed with a diet containing DTPMP salt at a dose level of 0, 100, 1000 and 10000 ppm, equivalent to an exposure level of about 0, 8, 83 and 850 mg active salt/kg bw/d for male rats and 0, 9.2, 92.3, and 903 mg salt/kg bw/d for female rats. During the study, animals were observed for signs of toxicity and mortality. Observations included body weights, food consumption, opthalmoscopic examination, haematology, biochemistry and urinanalysis. At study termination animals underwent necropsies, histopathological and bone mineral examination. No treatment-related effects on mortality, body weights, urinanalysis and gross pathology were observed. Minor non-dose related changes in clinical chemistry were reported but not considered to be toxicologically significant. Mean group absolute liver weights in males at the highest test dose were significantly decreased. Several changes in haematological parameters were seen at the highest dose tested: red blood cell levels increased, mean cell volume and haemoglobin decreased in both sexes. Total serum iron was decreased in high dose females only, while total serum iron binding capacity was increased in high dose males only. A reduction in iron complexes and reduced pigmentation for age was noted in the spleens in highest dose animals of both sexes. The changes in haematological parameters and serum iron and binding capacity were considered by the study authors to be perturbations of iron homeostasis. There was no effect on density of cortical bone. Total bone density and that of trabecular bone was increased in both sexes at the highest dose, but no effects were seen at other dose levels. The incidence of microlithiasis in kidneys of females was reduced at all dose levels. The effects

on bone density and the reduced level of microlithiasis in the kidneys were considered to be indicative of effects of calcium homeostasis, without causing any changes of calcium plasma levels. Therefore the minor changes that occurred in rats fed doses up to 10000 ppm (*i.e.*, about 850 mg/kg bw/d males and 902 mg/kg bw/d females) over a 90-day period were not considered to be of toxicological significance by the study authors. However, considering the changes in haematological parameters at the highest dose level to be of toxicological relevance, the NOAEL was established to be at 83 mg salt/kg bw/d for males and 92.3 mg salt/kg bw/d for females. The study was of high quality and in compliance with GLP and OECD method 408.

In another 90-day oral feeding study, Sprague-Dawley rats were fed with a diet containing the salt of DTPMP at dose levels equivalents to 0, 4, 45 and 511 mg salt/kg bw/d (males) and 0, 6, 57 and 656 mg salt/kg bw/d (females). No details on the study protocol were available. In the mid dose group body weights and liver weights were decreased in males, while spleen hemosiderin was decreased in both sexes. Decreased body weights and liver weights were attributed to chelation of iron in the diet, and were reversed during the 60-day post-exposure period. The absence of any histopathological or haematological involvement suggests that these changes were of limited toxicological relevance. At the top dose, signs of anaemia were observed in both sexes (pale extremities, decreased haematocrit, decreased haemoglobin, decreased red blood cells, decreased plasma iron, heart weight and spleen hemosiderin), in males body weights, plasma calcium and liver weights were decreased. All effects induced during the 90 day exposure period were found to be readily reversible. A NOEL of 4 mg/kg bw/day was determined by the study authors based on the decrease in male body weights and liver weights at the mid-dose. It was discussed, however, that the absence of histopathological involvement and reversibility of the observed effects indicate that they were not of toxicological significance suggesting the NOAEL to be 45 mg/kg bw/day for males and 57 mg/kg bw/day for females (Procter and Gamble, 1978). Due the absence of more detailed study information on this study, the quality of the study could not be verified. The reliability of the study was therefore not assignable.

A neutralized solution of DTPMP was also tested in a chronic 1-year oral feeding study in rats (Procter and Gamble, 1982). Fischer 344 rats were administered via the diet an aqueous solution containing 50 % DTPMP salt, in dose levels equivalent to 0, 4, 20, 100 and 500 mg salt/kg bw/d. At 100 mg/kg bw/d, a decrease in spleen hemosiderin was noted in males only. In the highest dose, decreased haematocrit, haemoglobin, plasma iron and magnesium, liver and spleen weight, and spleen hemosiderin were observed, together with unspecified changes in liver histopathology. The study authors established a NOEL of 20 mg/kg bw/d for both sexes based on the changes in spleen hemosiderin in males. However, the observed decrease was considered to be without toxicological effect. The observed effects at the highest dose were considered to be of toxicological relevance. The available IUCLID summary of this study was performed in compliance with GLP and OECD method 452. However, similarly to afore mentioned 90-day study, due to the absence of more detailed study information, the reliability of the study was not assignable.

A 2-year combined chronic toxicity/carcinogenicity study has been performed with a neutralized solution of DTPMP sodium salt (Procter and Gamble, 1987). Fisher rats (*i.e.*, 50 per sex and dose group) were fed with a diet containing the test substance at dose levels of 0, 4, 20 or 100 mg/kg bw/d. During the study, the animals were observed for clinical signs of toxicity and mortality. No other observations were reported. Although some effects on bone magnesium and phosphate were noted in all dose levels, no associated changes were found in bone mineralization, chondral ossification and bone formation or resorption. There were no

biologically significant differences in neoplastic findings between control and treated groups. In the absence of more detailed study information on this study, the quality of the study protocol and conduct cannot be verified. The available information was too limited to assign a NOAEL.

Table 23: Summary of repeated dose toxicity data

Test material	Species	Doses tested (active acid/salt)	Route	Duration	Estimated NOAEL (active acid/salt)	Reference
ATMP acid	rat	600 mg/kg bw/d	Oral gavage	28 days	>600 mg/kg bw/d (m)	Manley (1981)
ATMP acid	rat	0, 125, 250, 500, 750, 1000 mg/kg bw/d	Oral feeding	34 days	>1000 mg/kg bw/d (m/f)	Bio/Dynamics Inc. (1976)
ATMP salt	rat	0, 161 mg/kg bw/d (m); 0, 175 mg/kg bw/d (f)	Oral feeding	90 days	>161 mg/kg bw/d (m); >175 mg/kg bw/d (f)	Safepharm Laboratories Ltd. (1982m)
ATMP acid	rat	0, 50, 150, 500 mg/kg bw/d	Oral feeding	2 years	>500 mg/kg bw/d (m/f)	Bio/Dynamics Inc. (1979a)
HEDP acid	dog	0, 191, 554, 1746 mg/kg bw/d (m); 0, 202, 553, 1620 mg/kg bw/d (f)	Oral feeding	90 days	>1746 mg/kg bw/d (m); >1620 mg/kg bw/d (f)	Industrial Biotest Labs Inc. (1975)
HEDP acid	rat	0, 154, 524, 1583 mg/kg bw/d (m); 0, 166, 545, 1724 mg/kg bw/d (f)	Oral feeding	90 days	>1583 mg/kg bw/d (m); >1724 mg/kg bw/d (f)	Industrial Biotest Labs Inc. (1979)
HEDP.2 Na	rat	0, 41, 169, 817 mg/kg bw/d (m); 0, 50, 195, 1000 mg/kg bw/d (f)	Oral feeding	90 days	41 mg/kg bw/d (m); 50 mg/kg bw/d (f)	Huntingdon Research Centre (1977)
HEDP.2 Na	rat	260 and 1300 mg/kg bw/d	Oral feeding	90 days	> 260 mg/kg bw/d (m/f)	Nixon *et al*. (1972)
HEDP.2 Na	rat	0, 19, 78, 384 mg/kg bw/d (m); 0, 24, 96, 493 mg/kg bw/d (f)	Oral feeding	2 years	19 mg/kg bw/d (m); 24 mg/kg bw/d (f)	Huntingdon Research Centre (1979)
DTPMP acid	Not evaluated					
DTPMP salt	Rat	0, 8, 83, 850 mg/kg bw/d (m); 0, 9.2, 92.3, 903 mg/kg bw/d (f)	Oral feeding	90 days	83 mg/kg bw/d (m); 92.3 mg/kg bw/d (f)	Central Toxicology Laboratory (1998)
DTPMP salt	rat	0, 4, 45, 511 mg/kg bw/d (m);0, 6, 57, 656 mg/kg bw/d (f).	Oral feeding	90 days	4 mg/kg bw/d (m); 6 mg/kg bw/d (f)	Procter and Gamble (1978)
DTPMP salt	rat	0, 4, 20, 100, 500 mg/kg bw/d	Oral feeding	1 year	> 20 mg/kg bw/d (m/f)	Procter and Gamble (1982)
DTPMP salt	rat	0, 4, 20, 100 mg/kg bw/d	Oral feeding	2 years	Not assigned	Procter and Gamble (1987)

(m) males; (f) females; (m/f) both sexes

Conclusion

In a good quality 2-year chronic feeding study, the NOAEL of ATMP acid in rats was established to be at the highest dose level of 500 mg/kg bw/d. At this dose level, food consumption was slightly increased in male rats and male and female rats exhibited changes in relative organ weights (*i.e.*, spleen, liver, testes, and kidney). The effects were evaluated to be an adaptive response to exposure and not considered to be adverse in nature. Taking a more conservative approach, a NOEL can be considered to be 150 mg active acid/kg bw/d. The NOAEL for the sodium salt of ATMP was established to be greater than 161 mg active salt/kg bw/d for males on the basis of a 90-day single dose oral feeding study. No treatment related effects were observed at this exposure level under the conditions of the study.

Repeated dose toxicity of HEDP acid was tested in dogs and rats. NOAELs determined in these studies are in the same range; > 1620 mg/kg bw/d (dog/females) and > 1583 mg/kg bw/d (rat/males). Because these studies were conducted by Industrial Biotest Labs Inc., the studies are considered to be of unknown reliability. The disodium salt of HEDP was tested in a good-quality 90-day oral feeding study in rat on which basis a NOAEL 41 mg active salt/kg bw/d (males) was established. Anaemia, reduction in red cell parameters and pallor which were observed at higher dose levels, could be related to a potential perturbation of iron homeostasis as a result of the chelating properties of HEDP. The chronic toxicity of the disodium salt of HEDP was evaluated in a good quality 2-year oral feeding study. On the basis of this investigation, a NOAEL of 19 mg active acid/kg bw/d (males) was established on the basis of this investigation. Similarly to the subchronic feeding study, anaemia was observed in the high-dose groups.

While there are no studies available on DTPMP acid, a number of subchronic and chronic oral feeding studies evaluated the repeated dose toxicity of DTPMP salts. Unfortunately, only one of these studies, a subchronic oral feeding study complying with OECD method 408 and GLP regulations, was evaluated to be reliable. In this investigation, exposure to DTPMP resulted in perturbations of iron and calcium homeostasis indicated by changes in haematological parameters, serum iron and binding capacity as well as increased bone density and reduced incidence of microlithias in female kidneys. The NOAEL for DTPMP was therefore established at 83 mg salt/kg bw/d for males and 92.3 mg/kg bw/d for females. In another 90-day study similar effects were seen at the mid-dose levels (i.e., 45 mg/kg bw/d for males, 57 mg/kg bw/d for females) suggesting a NOEL of DTPMP salt as low as 4 mg/kg bw/d. Similar changes in haematological parameters were observed in a chronic 1-year study at a dose level of 100 mg/kg bw/d were seen at dose levels of 100 mg/kg bw/d for males and females respectively. This led to the authors to establish a NOEL of 20 mg/kg bw/d. The reliability of the findings of the latter 2 studies could not be assigned as the original study reports were not available for review. There were further no biologically significant differences in neoplastic findings between control and treated groups in a 2-year chronic feeding study in rats. The available study information was too limited to assign a NOAEL.

5.2.1.5 Genetic toxicity

5.2.1.5.1 *In vitro*

Bacterial tests

ATMP acid (CAS No. 6419-19-8) and ATMP salts (CAS No. 20592-85-2)

The mutagenic activity of ATMP acid was evaluated in a bacterial reverse mutation assay, the so-called Ames test (Monsanto, 1981b). The study was in compliance with GLP regulations and OECD method 471 and thus assessed to be reliable. ATMP acid was tested as an aqueous solution containing 50 % active acid in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 in a standard plate incorporation and spot test both with and without metabolic activation (*i.e.*, via addition of Aroclor induced S9 mix). The dose levels in the plate incorporation test were 0.01, 0.04, 0.2, 1, 3 and 10 µL/plate, equivalent to 0.0065, 0.026, 0.13, 0.65, 1.95 and 6.5 µL active acid/plate; and in the spot test a dose of 25 µL/plate, equivalent to 9 µL active acid/plate, was tested. No significant increase in revertants in any strain/metabolic activation system was observed. It was therefore concluded that under the conditions chosen, ATMP acid is not a bacterial mutagen.

The potential of the tetrasodium salt of ATMP to induce reverse mutations in an *in vitro* bacterial system was evaluated in a non-GLP or guideline compliant study (Manley, 1981). In this study, an aqueous solution containing 30 % active ATMP salt, neutralized with NaOH was investigated in *Salmonella typhimurium* strains TA98, TA100 both with and without metabolic activation (*i.e.*, via addition of Aroclor induced S9 mix) at dose levels of 0-40 mg/plate, which was equivalent to a dose of 12 mg salt/plate. Under the conditions of the study, the tetrasodium salt of ATMP was neither mutagenic nor cytotoxic to the bacterial test system.

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

HEDP acid was not mutagenic when tested for its mutagenic potential in the Ames test using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with and without metabolic activation (*i.e.*, via addition of Aroclor induced S9 mix) (Monsanto, 1977). The substance was tested as an aqueous solution at a dose level of 10 µL solution/plate which was the limit with regard to its cytotoxicity and solubility. Due to the absence of further study details, the reliability of the study could not be evaluated.

Two entries into the previous ECB IUCLID file on the disodium salt of HEDP stated that this material was not mutagenic in an *in vitro* bacterial gene mutation assay which was not defined further (Henkel KGaA unpublished data g). In the absence of more details on the protocol and also the results, the reliability of the study could not be evaluated.

DTPMP acid (CAS No. 15827-60-8) and DTPMP salts (CAS No. 22042-96-2)

The mutagenic activity of DTPMP acid was evaluated in a high quality Ames test which was in compliance with GLP regulations and OECD method 471 (Monsanto, 1981c). DTPMP acid was tested as an aqueous solution containing 50 % active acid in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 in a standard plate incorporation

and spot test at dose levels up to 10 µL/plate with metabolic activation (*i.e.*, via addition of Aroclor induced S9 mix) and up to 0.3 µL/plate without metabolic activation. No significant increases in revertants in any strain/metabolic activation system were observed, based on which it was concluded that under the test conditions DTPMP acid was considered not a bacterial mutagen.

The salt of DTPMP was tested for its mutagenic potential in an *in vitro* bacterial system, both in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2 uvr$^-$ (Japan Oilstuff Inspectors Corporation, 2001). DTPMP salt was tested as an aqueous solution containing 23.7 % active salt at dose levels up to 1185 µg active salt/plate, both with and without metabolic activation (*i.e.*, via addition of Aroclor induced S9 mix). The study was of good quality and conducted in compliance with GLP and OECD method 471.

Non-bacterial tests

ATMP acid (CAS No. 6419-19-8) and ATMP salts (CAS No. 20592-85-2)

ATMP acid was tested in two *in vitro* mammalian cell gene mutation assays. Both studies were of good quality and in compliance with GLP regulations and OECD method 476. In the first study (SRI International, 1982a), an aqueous solution of the active acid was dosed at 0 up to 780 µg active acid/L in a standard mouse lymphoma L5178Y TK+/- plate assay both with and without metabolic activation (*i.e.*, Aroclor induced S9 mix). The top dose was eliminated due to precipitation. No increase in mutation frequency was seen in the absence of S9 at any dose. However, in the presence of S9 a dose-related positive response reaching four times the control values was observed.

A follow-up *in vitro* mammalian cell gene mutation assays was conducted to investigate the positive response seen in the previous study (SRI International, 1988). In this study, an aqueous solution containing 50 % active acid neutralized with NaOH was tested at a dose of 0 up to 1.2 µL, equivalent to 0 up to 0.78 µL active acid/mL. The experiment was performed on 2 occasions in the presence of S9 only, but the first was discounted because of low growth of the control cultures. In the second study, no increase in mutant frequency and no cytotoxicity were observed. Compared to the previous study where the same concentrations of the un-neutralized acid resulted in a positive response, this indicates that the positive result was an artefact due to a pH perturbation. As a result of this investigation, ATMP acid was not considered to be mutagenic under the test conditions.

The pentasodium salt of ATMP was tested in an *in vitro* mammalian cytogenicity test with Chinese Hamster Ovary (CHO) cells for its potential to induce structural chromosomal aberrations (Covance Laboratories, 1998a). An aqueous solution containing 40 % active salt was tested at a dose level up to 4400 µg active salt/mL, both with and without metabolic activation (S9 mix). In the presence of S9, an increase in aberrations was seen at a single low dose in the first test. This result was not observed at higher doses and not repeated in the second test. No other effects were observed. Since the effect was not reproducible and due to the absence of a dose-related response, it was concluded that under the conditions of the study ATMP pentasodium salt did not induce chromosome aberrations *in vitro* when tested up to acceptable limits for this assay. The study was of good quality and in compliance with GLP regulations and OECD guideline 473.

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

An aqueous solution of HEDP acid was evaluated for induction of mutants at the thymidine locus in a mouse lymphoma L5178Y assay (Litton Bionetics, 1978). The study was based on OECD method 476 but used a non-standard selective agent, bromodeoxyuridine. The testing was performed on two occasions in the presence and absence of S9 mix. In the first test, a dose-related increase in the presence of S9 was observed; however the relative growth was less than 10 % and therefore considered to be unreliable and not providing evidence of mutagenic potential. Because of high control values the test was repeated. In the second test, the control frequency was 10 fold lower than in the first, with a 3 fold variation between the control and solvent control. No dose-related responses were observed and HEDP acid was not considered to have any mutagenic potential. Although study was guideline compliant it was considered to be of low reliability because of a high variability of control values, a lack of details on test conditions and the use of a non-standard selective agent, bromodeoxyuridine.

DTPMP acid (CAS No. 15827-60-8) and DTPMP salts (CAS No. 22042-96-2)

DTPMP acid was tested in three *in vitro* mammalian cell gene mutation assays, all GLP-compliant, good quality studies. In the first study (SRI International, 1982b), an aqueous solution of the active acid was tested in a standard mouse lymphoma L5178Y TK+/- plate assay at a dose level of 70 up to 1400 µg active acid/mL without metabolic activation and 35 to 1050 µg active acid/mL with metabolic activation. The top doses were selected on the basis of the toxicity limit. A dose-related increase in mutation frequency at the thymidine kinase locus was seen in the presence and the absence of S9. A further experiment was conducted to investigate if the response was due to pH change by neutralizing the test substance with NaOH (SRI International, 1983b). Mutations were induced by the neutralized test substance at the thymidine kinase (TK) locus in mouse lymphoma cells in the presence of S9 mix, indicating, the positive response seen in the preceding study was not related to pH perturbation. Furthermore, it was shown that these mutants are stable and not lacking in TK enzyme activity. In a third *in vitro* mammalian cell gene mutation assay, the results obtained in the previous study were confirmed (Microbiologicals Associates, 1983). A neutralized aqueous solution of DTPMP acid was tested in a standard mouse lymphoma L5178Y TK+/- plate assay at a dose level of 1.2 up to 17.2 µL/mL without metabolic activation (*i.e.*, Aroclor induced S9 mix) and 3 to 9 µL/mL with metabolic activation. It was shown that the mutations induced at the TK locus in previous studies were not due to selection of pre-existing mutants.

DTPMP acid was also was tested in an *in vitro* mammalian cell gene mutation assay with Chinese Hamster Ovary (CHO) cells using mutation at the HPRT locus (Pharmakon Research International, 1984). The study was of good quality in compliance with GLP regulations and OECD guideline 476. An aqueous solution of DTPMP acid was tested at a dose level of >8000 µg/mL, which is higher than recommended in OECD guidelines, but it was not stated whether this is expressed as solution or as active salt, both with and without metabolic activation (S9 mix). No evidence of mutagenic potential in CHO cells at HPRT locus in the absence or presence of S9 was observed.

The salt of DTPMP was assessed in a standard mouse lymphoma L5178Y TK+/- plate assay (Central Toxicology Laboratory, 1997). An aqueous solution of the salt was dosed at 0 up to 2200 µg/mL both with and without metabolic activation (S9 mix). The DTPMP salt did not cause mutations in L5178Y TK+/- cells following *in vitro* treatment either in the presence or absence of S9 mix at concentrations up to 2200 µg/mL. This concentration was chosen to

avoid any osmolality effects. However, since only a relative toxicity of >75 % was achieved, higher dose levels could have been employed. The study was of good quality and conducted in compliance with GLP and OECD method 476.

5.2.1.5.2 *In vivo*

ATMP acid (CAS No. 6419-19-8) and ATMP salts (CAS No. 20592-85-2)

The pentasodium salt of ATMP was tested in a well-conducted GLP and OECD method 474 compliant mouse micronucleus test (Covance Laboratories, 1998b). CD-1 (ICR)BR male mice were administered by gavage an aqueous solution containing 50 % ATMP pentasodium salt at dose level of 500, 1000 and 2000 mg active salt/kg at 24 hours, and 2000 mg active salt/kg at 48 hours. No clinical toxicity in any treated animals and no cytotoxicity to the bone marrow were observed at the highest dose of 2000 mg active salt/kg. No increases in micronucleated polychromatic erythrocytes were seen in the study.

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

No mutagenic activity was found for HEDP disodium acid when tested in a mouse micronucleus assay at dose levels of 18.75 and 150 mg/kg, administered at two doses at 0 and 24 hours (Henkel KGaA unpublished data h). No further details on the study protocol or quality were available.

DTPMP acid (CAS No. 15827-60-8) and DTPMP salts (CAS No. 22042-96-2)

DTPMP acid was tested in an *in vivo* cytogenicity study with Sprague-Dawley rats (Monsanto, 1983). Twelve animals (*i.e.*, 6 per sex and dose group) were administered by gavage a single dose of an aqueous solution containing 19.7 % DTPMP acid which was neutralized to pH 7 at dose levels equivalent to 0, 200, 660 and 1970 mg active acid/kg bw. Animals were sacrificed at 6, 12, 24 and 48 hours. At 1970 mg active acid/kg bw, 25 % of the animals died, mild clinical signs were observed and both sexes lost body weights. There was no decrease in mitotic index and no increase in chromosome aberrations in any group at any sacrifice time. Under the conditions of the study, DTPMP acid did not show any evidence for clastogenic activity in rats when administered by gavage at doses up to the maximum tolerated dose.

Conclusion

Generally, from a structure activity standpoint, none of the phosphonates evaluated in this review possess structural elements that indicate the potential for genotoxicity.

Neither ATMP acid nor the salt induced gene mutations in bacterial systems. When testing ATMP acid in the acid form, it induced dose-dependent gene mutations in mouse lymphoma cells. However, this positive result was demonstrated to be an artefact of pH which was not observed when neutralized ATMP acid was tested in the *in vitro* mouse lymphoma assay up

to the solubility limit. The pentasodium salt of ATMP did not induce chromosome damage either *in vitro* or *in vivo*.

The available data on *in vivo* and *in vitro* genotoxicity of HEDP and its salts are only of limited quality and reliability. Those studies that are available indicate no potential of HEDP and its salts to cause mutagenicity in bacterial mutagenicity assays. Conflicting results were obtained in an *in vitro* mouse lymphoma assay. In this assay, a dose-dependent positive response was seen in the presence of metabolic activation which was, however, discounted because of high control values. In the second assay, absence of mutation was claimed by the study investigators which led to the overall conclusion that HEDP acid did not cause mutagenicity in this assay. In the absence of more detailed study information this conclusion could not be verified. It seems appropriate to point out that the positive response could also be artefactual due to a pH effect (*e.g.*, as seen for ATMP acid) or possibly due chelation of essential ions or induction of oxidative species in the presence of S9 mix. These issues may require further investigations prior to being able to draw final conclusions. Although the genetic toxicology data package is lacking robustness, the available data set and the absence of structural alerts for genotoxicity suggests that neither HEDP acid, nor its salts are genotoxic.

Both, DTPMP acid and the salt were negative in well performed and guideline compliant bacterial mutagenicity assays. DTPMP acid was further negative for gene mutations at the HPRT locus in CHO cells. Similarly to HEDP acid, the evidence for mutagenic potential is conflicting. While the salt of DTPMP was negative for mammalian gene mutations, DTPMP acid, even when neutralised, induced mutations at the thymidine kinase locus in mouse lymphoma L5178Y cells. It is difficult to rationalise the difference in the outcome between the tests on the neutralised acid and on the salt. In principle, the chemical species tested should be similar for both test substances. Since pH effect has been excluded and increased osmolality is an unlikely cause (positive response was only seen in presence of S9 mix), it is possible that chelation of essential ions may have caused the positive response in the presence of S9 (see also Chapter 3 for chelating potential). Iron chelation appears to play a role in contributing to positive responses in the mouse lymphoma assay. In a study conducted by Whittaker et al., eight out of 12 iron chelators induced mutagenic responses in the L5178Y mouse lymphoma cells (Whittaker et al., 2001). However, if the effects are due to chelation, there would need to be a complex interplay between the test substance and the S9 to alter chelation properties or ionization state, resulting in effects in the presence but not the absence of S9 mix. An alternative explanation could be that the test substance interacts with S9 resulting in the formation of oxidative species. Thus, while there is no single definitive rationale for the positive responses in this assay, there is considerable evidence that this is an isolated and artefactual response, for which mechanistic bases can be proposed. Some further evidence for the absence of genotoxic potential of DTPMP *in vivo* is provided by a well conducted chromosome aberration study in rats following gavage with doses up to 1970 mg/kg. In conclusion, the weight of the evidence suggests that DTPMP and its salt should not be considered to pose a genotoxic hazard.

5.2.1.6 Carcinogenicity

ATMP acid (CAS No. 6419-19-8) and ATMP salts (CAS No. 20592-85-2)

A 2-year combined chronic toxicity/carcinogenicity study in rat was conducted with ATMP acid (Bio/Dynamics Inc., 1979a; see also section 5.2.1.4). Long-Evans rats were exposed via the diet to a dose level of 0, 50, 150 and 500 mg ATMP acid/kg for a period of 24 months. Seventy animals were used per sex and dose. During the study, animals were observed for any signs of toxicity and mortality. Investigations included measurements of body weights, food consumption, opthalmoscopic examination, haematology, clinical chemistry, urinanalysis and biochemical examinations. At 6, 12 and 24 months, necropsies were performed. Approximately 50 animals per group survived until study termination. Similar frequencies of tumours were found in all groups. The tumour incidence for tumour bearing animals was: 28.6 % for control males, 28.6 % for low dose, 30 % for mid dose; and 21.4 % for high dose males; 31.4 % for control females, 32.9 % for low dose, 24.3 % for mid dose; and 28.6 % for high dose females No major differences in tumour types were observed between treated and control animals. Most common tumours in all test groups after 2-years were pituitary and mammary tumours. Miscellaneous isolated tumours were observed in all treatment groups but none was treatment-related. One high dose male, which died after one year, had an unusual tumour (*i.e.*, osteosarcoma axilla), but this observation was considered to be a chance finding by the investigator. On the basis of this investigation, it can be concluded ATMP acid was not carcinogenic when tested up to 500 mg active acid/kg. The study conformed in all major aspects to OECD method 453. It was, however, not in compliance with GLP regulations

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

No indications of an increased incidence in tumours was noted in a 2-year combined chronic toxicity/carcinogenicity study in Sprague-Dawley rats in which HEDP disodium salt was administered via the diet at doses of 0, 500, 2000 and 10000 ppm for 104 weeks. This was equivalent to doses of about 0, 19, 78, 384 mg active salt/kg bw/d for males and 0, 24, 96, 493 mg active salt/kg bw/d for females, for 104 weeks (Huntingdon Research Centre, 1979; see also 5.2.1.4). During the study, animals were observed for clinical signs of toxicity and mortality. Observations included body weight, food and water consumption, opthalmoscopic examination, haematology, clinical chemistry and urinanalysis. During the study and at study termination animals underwent necropsies and histopathological examinations. No treatment-related effects were observed on mortality, ophtalmoscopy, clinical chemistry or gross pathology. Several haematological perturbations were seen during the study, but there were no treatment-related effects which persisted until the termination at 24 months. Anaemia was observed in the high dose animals. No histopathological changes were found at study termination. A lack of iron in the spleen was seen at the two highest dose levels at 26 weeks, although resolved by 104 weeks. This was assumed to be related to the chelation properties of the test substance. No increased incidence of neoplastic lesions was observed in the treated groups at study termination and HEDP disodium salt was considered not to be a carcinogen. The study was non-GLP but of good quality and performed broadly following OECD method 452.

DTPMP acid (CAS No. 15827-60-8) and DTPMP salts (CAS No. 22042-96-2)

A 2-year chronic toxicity/carcinogenicity study was conducted to determine the carcinogenic potential of the sodium salt of DTPMP (Procter and Gamble, 1987; see also 5.2.1.5). A neutralized solution containing 50 % of the sodium salt, was administered via diet to Fischer 344 rats (*i.e.*, 50 per sex and dose group) at doses of 0, 4, 20 and 100 mg/kg bw/d. During the study animals were observed for clinical signs of toxicity and mortality. No other observations have been reported. No biologically significant differences in neoplastic findings were found between control and treated groups. In the absence of more details on the protocol and also the results, the reliability of the study could not be evaluated.

Conclusion

The acids or salts of ATMP, HEDP and DTPMP did not show any carcinogenic activity when tested in rodents.

5.2.1.7 Toxicity for reproduction

5.2.1.7.1 Effects on fertility

ATMP acid (CAS No. 6419-19-8) and ATMP salts (CAS No. 20592-85-2)

In a 3-generation reproduction study, Long-Evans rats (*i.e.*, 12 males and 24 females and group) were fed with ATMP acid containing diet at dose levels of 0, 300, 1000 or 3000 ppm. The treatment started 60 days prior to first mating of the F_0 generation (Bio/Dynamics Inc., 1979b). The offspring of the first litter of each generation (F_{1a}, F_{2a}, F_{3a}) were taken for necropsy on lactation day 21 (LD 21) after which the parents were re-mated and the offspring used to breed the F_{1b} and F_{2b} parent generations. Non-breeding F_{1b} and F_{2b} animals, as well as the F_{3a} and F_{3b} generation were taken for necropsy. Based on food consumption and body weight data collected during the study, the overall intake of ATMP acid by the high dose animals could be calculated: high dose males consumed the equivalent of 275 mg active acid/kg bw/d and females 310 mg active acid/kg bw/d. During the study, parents were observed for mortality, body weight changes, food consumption, clinical observations and pregnancy data (*i.e.*, mating indices, pregnancy indices, fertility percentage, and pre-coital interval). Litter observations included mortalities, body weights, food consumption, clinical observations, gestation length, survival index at birth, offspring body weight, postnatal growth, pup survival and other effects on offspring. At necropsy, all animals underwent histopathological examinations. No adverse treatment-related effects on reproduction parameters and no pathologic and histopathologic lesions were observed in either parental animals or pups at any dose level. The NOAEL for parental, reproductive and litter effects was therefore established to be at the highest dose tested, *i.e.* 275 mg ATMP acid/kg bw/d in males and 310 mg ATMP acid/kg bw/d in females. Although the study was pre-GLP and not in full compliance with OECD guidelines, the study provided sufficient information and was assessed to be scientifically reliable. As the study predates current guidelines, it did not evaluate the oestrus cycle, sperm parameters and developmental milestones.

Erratic alterations in testis weight were apparent in male Long Evans rats fed diets designed to deliver up to 500 mg/kg bw/d for up to 2 years (Bio/Dynamics Inc., 1979a, see also

5.2.1.4). However, these changes were not consistent with time, minor in extent and showed no consistent relationship with body weight and therefore considered unrelated to treatment. Microscopic evaluation of ovaries and testes from these animals revealed no histopathological abnormalities.

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

In a dog study (Industrial Biotest Labs Inc., 1975; see also 5.2.1.4), Beagle dogs (*i.e.*, 4 per sex and dose) were fed for 90-days with a HEDP acid containing diet at dose levels of 0, 191, 554 and 1746 mg active acid/kg bw/d for males and 0, 202, 553 and 1620 mg active acid/kg bw/d for females. No treatment related effects on the reproductive organs were observed and the NOAEL was determined to be >1746 mg active acid/kg bw/d for males and >1620 mg/kg bw/d for females. The study was pre-GLP and in compliance with OECD guideline 409. The quality of the studies is of unknown reliability.

Similarly, Charles River albino rats (*i.e.*, 15 per sex and dose) were administered via the diet for 90 days an aqueous solution containing HEDP acid, at dose levels of 0, 1000, 3000 and 10000 ppm, equivalent to 0, 154, 524 and 1583 mg active acid/kg bw/d for males and 0, 166, 545 and 1724 mg active acid/kg bw/d active acid for females (Industrial Biotest Labs Inc., 1979; see also 5.2.1.4). During the study animals were observed for any signs of toxicity and mortality. Observations included measurements of body weights, food consumption, haematology, urinanalysis as well as biochemical examinations. At study termination, animals underwent necropsy and histopathological examinations. No treatment-related effects were observed on gonads, seminal vesicles, uterus of prostate and the NOAEL was established at the highest dose tested, *i.e.*, 1583 mg/kg bw/d for males and 1724 mg active acid/kg bw/d for females. The study was pre-GLP and in compliance with OECD guideline 409. The quality of the studies is of unknown reliability.

The disodium salt of HEDP was fed in a 2-generation study via the diet to Charles River rats at dose levels of 0, 112 or 447 mg active salt/kg bw/d (Nolen and Buehler, 1971). The test material was dosed continuously to both sexes, or to pregnant females between gestation days 5-15. No treatment-related effects on pregnancy rate were observed. The F_0 females were allowed to deliver two litters (i.e., F_{1a}, F_{1b}) while a third (F_{1c}) was used for a teratology evaluation. For the mating procedure, one male and female rat was placed in a mating cage. The start of pregnancy was determined on the basis of a positive vaginal smear. Five weanlings of F_{1a} were subject to necropsy and histological examinations. The remaining pups of F_{1a} were discarded after weaning. Litters of F_{1b} were used for breeding the F_2 generation. The group sizes were 22/sex/treatment for F_0 and F_1 and 20/sex/treatment for F_{1b} and F_2. In-life observations such as body weights and food intake were recorded from 8 weeks pre-mating on. The litter observations were not described. Generally, growth, feed consumption and feed efficiency from weaning to maturity were not significantly affected by the treatment. There were also no treatment-related effects on pregnancy rate, number of live litter size and mean number of stillborn per litter. The NOAEL for reproductive toxicity was determined to be greater than 447 mg/kg bw/d, the highest dose tested (see also 5.2.1.7.2). There was only limited information available with regard to the study design. However, a few deviations from the OECD guideline protocol were apparent.

DTPMP acid (CAS No. 15827-60-8) and DTPMP salts (CAS No. 22042-96-2)

DTPMP acid was administered via the diet to Long-Evans rats (*i.e.*, 20 per dose group) at doses of 0, 300, 1000 and 3000 ppm (Bio/Dynamics Inc., 1979c). Females were treated over 2 generations and males over 1 generation. Administration of the test substance started after mating of the untreated parents on day 0 of gestation and at weaning until necropsy of F_{2b} generation. Based on food consumption and body weight data collected during the study, the overall intake of DTPMP acid in the growth phase was calculated to be 0, 28, 97 or 294 mg active acid/kg bw/d for males and 0, 32, 108 or 312 mg active acid/kg bw/d for females. During the study, parents were observed for mortality, body weight changes, food consumption, clinical observations and pregnancy data (*i.e.*, mating indices, pregnancy indices, fertility percentage, and pre-coital interval). Litter observations included mortalities, body weights, food consumption, clinical observations, gestation length, survival index at birth, offspring body weight, postnatal growth, pup survival and other effects on offspring. At necropsy, all animals underwent histopathological examinations. F_0 females fed with the high dose delivered fewer live pups with a decreased body weight. Both alterations were not statistically significant. A statistically insignificant lower pregnancy rate and a statistically significant reduced pup body weight were observed in the F_{2a} litters from dams fed at the high dose of 3000 ppm DTPMP. These changes were, however, either not observed in the F_1 litter or replicated in the F_{2b} litter. This suggests that these effects were unrelated to treatment with DTPMP acid. On the basis of this investigation, the NOAEL for reproductive toxicity of DTPMP acid was established to be 294 mg active acid/kg bw/d for males and 312 mg active acid/kg bw/d for females. The study was not in compliance with GLP regulations, but followed the principles of OECD guidelines and was therefore judged to be reliable.

The evaluation of reproductive parameters was also included in the design of a 90-day oral feeding study. In this GLP and OECD guideline 408 compliant study, Wistar rats (*i.e.*, 12 per sex and dose group) were fed with a diet containing DTPMP sodium salt at dose levels equivalent to 0, 8, 83 and 850 mg active salt/kg bw/d for male rats, and 0, 9.2, 92.3 and 903 mg active salt/kg bw/d for female rats (Central Toxicology Laboratory, 1998; see also 5.2.1.4). There were no treatment-related microscopic changes apparent in ovary, oviduct, epididymis, cervix, seminal vesicle, testis and uterus.

5.2.1.7.2 Developmental toxicity/teratogenicity

ATMP acid (CAS No. 6419-19-8) and ATMP salts (CAS No. 20592-85-2)

As discussed before, the reproductive and developmental toxicity of ATMP acid was evaluated in a rat 3-generation reproductive toxicity study (Bio/Dynamics Inc., 1979b: see also 5.2.1.7.1). In this study, no adverse treatment-related effects on reproduction parameters, gross abnormalities or histopathological lesions were observed in either parental animals or pups at any dose level. Under the conditions of this study, the NOAEL for developmental toxicity can be considered to be greater than 275 mg ATMP acid/kg bw/day for males and 310 mg ATMP acid/kg bw/day for females. The study was pre-GLP and not in full compliance with OECD guidelines, but its conduct and reporting was judged to provide scientifically reliable information.

ATMP acid was administered by gavage to pregnant Sprague-Dawley rats at dose levels of 0, 100, 500 or 1000 mg active acid/kg bw/d from day 6 to 15 of gestation (Bio/Dynamics Inc., 1979d). Amongst others, the parameters evaluated during the study included mortality,

pregnancy rate, body weight and clinical/necropsy findings, uterine implantations (*i.e.*, mean number, efficiency, mean number of resorptions), foetal data, sex ratios, variation in ossification and teratological findings (*i.e.*, external, skeletal, soft tissue and visceral malformations). A slight overall reduction in maternal body weight gain was observed at the highest treatment rate of 1000 mg/kg bw/day, while no significant reduction was observed in the lower treatment groups. ATMP acid was neither embryotoxic nor teratogenic at dose of 100 or 500 mg/kg bw/day. However at the dose of 1000 mg/kg bw/d, 6 of 16 foetuses from a single high dose litter showed common multiple malformations (including flexed forepaws, shortened and thickened torso, abdominal distension, exaggerated flexure of the head and malformation defect of the heart) in the presence of a 50 % decrease in individual maternal body weight gain. This indicates that maternal toxicity was present at this exposure level. The clear absence of any comparable effect on the other high dose litters and the lack of dose-response indicate that ATMP acid should not be considered to be fetotoxic or teratogenic. Based upon these observations, the NOAEL was established at 1000 mg active acid/kg bw/d. However, a possible effect on maternal performance could not be excluded. The study was well conducted using a FDA segment II protocol.

The same test protocol was used to evaluate developmental toxicity properties of neutralized ATMP acid in mice (Bio/Dynamics Inc., 1980). Pregnant CD-1 mice were administered by gavage an aqueous solution containing 20 % active acid at dose levels of 0, 100, 500 and 1000 mg active acid/kg bw/d from day 6 to 15 of gestation. No embryonic or teratogenic effects were observed in parents or foetuses at any doses. The only remarkable finding was a statistically significant increase in malformations (including misshapen tibia, fibula bones, angulated ribs and defective sternebrae) present in six foetuses from one litter of the 500 mg/kg bw/d treatment group. Since no comparable effect was observed in any of the other treatment groups (*i.e.*, even in the higher dose group), this observation was considered to be a spontaneous effect, unrelated to treatment with the ATMP acid. The NOAEL was determined to be greater than 1000 mg active acid/kg bw/d, under the test conditions.

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

In a 2-generation study, the disodium salt of HEDP was fed via the diet to Charles River rats at dose levels of 0, 0.1 and 0.5 %, equivalent to 0, 112 or 447 mg active salt/kg bw/d (Nolen and Buehler, 1971; see also 5.2.1.7.1). HEDP disodium salt was dosed continuously to both sexes, or to pregnant females between gestation days 5-15. No treatment-related effects on pregnancy rate were observed. The F_0 females were allowed to deliver two litters (*i.e.*, F_{1a}, F_{1b}) while a third was used for a teratology evaluation. Five weanlings of F_{1a} were subject to necropsy and histological examinations. The remaining pups of F_{1a} were discarded after weaning. Litters of F_{1b} were used for breeding the F_2 generation. The group sizes were 22/sex/treatment for F_0 and F_1 and 20/sex/treatment for F_{1b} and F_2. In-life observations such as body weights and food intake were recorded from 8 weeks pre-mating on. The only effect seen was a significant decrease in the number of live pups born to dams given the 0.5 % level, and a non-significant increase in stillborns, suggesting fetotoxicity. Due to some evidence of fetotoxicity at the high doses, the NOAEL for fetotoxicity was established to be 112 mg active salt/kg bw/d. There was only limited information available with regard to the study design. However, apparent deviations from the OECD guideline protocol were observed. These deficiencies limit the reliability of this investigation.

Pregnant rabbits were fed with a diet containing HEDP disodium salt at dose levels of 0, 25, 50 or 100 mg active salt/kg bw/d during day 2 to 16 of gestation (Nolen and Buehler, 1971).

The start of pregnancy was determined on the basis of positive vaginal smears. The in-life observations included recording of body weight on gestation days 0 and 29 and daily food intake between gestation days 2 and 16. The dams were sacrificed on gestation day 29. The evaluated maternal parameters included the number of resorptions, corpora lutea and implantations and the foetuses were evaluated for sex, body weight, gross abnormalities, skeletal effects (*i.e.* in only one third of foetuses) and soft tissue anomalies. There were no treatment-related effects observed in either the parents or the foetuses. The study was not described in sufficient details to assess the study's reliability, but suggests a NOAEL for maternal, foetal and teratogenic effects in rabbit to be greater than100 mg active salt/kg bw/d, the highest dose tested in this investigation.

DTPMP acid (CAS No. 15827-60-8) and DTPMP salts (CAS No. 22042-96-2)

As discussed in section on toxicity on fertility, the reproductive and developmental toxicity of DTPMP acid was evaluated in a well conducted 2-generation reproductive toxicity study (Bio/Dynamics Inc., 1979c). In this study, Long Evan rats were fed with DTPMP acid containing diet at dose levels of 0, 300, 1000, and 3000 ppm. There were no treatment-related effects observed in the offspring and thus a NOAEL of 312 mg active acid/kg bw/d for fetotoxicity and teratogenicity was established.

Sprague-Dawley rats were dosed by gavage with an aqueous solution containing DTPMP sodium salt at dose levels of 0, 500, 1000 or 2000 mg active salt/kg bw/d on days 6 to 19 of gestation (Monsanto, 1982). The dams were sacrificed on gestation day 20. Gross post-mortem (*i.e.*, external surfaces, thoracic and abdominal cavities), uterus (*i.e.*, live/dead foetuses, early/late resorptions) and implantation site (*i.e.*, ovaries: corpora lutea per ovary) examinations were conducted in the parental animals. The foetuses were examined for body weights, sex, and external/skeletal/soft tissue malformations. Maternal toxicity indicated by a 30 % decrease in body weights were observed at the high dose level of 2000 mg/kg bw/d. No significant increase in the number of malformations was observed at any dose level. Increased incidences in vertebral anomalies, including missing, reduced or fused vertebral arches, were observed in the 1000 and 2000 mg/kg bw/d treatment group, but these findings were considered to be of equivocal toxicological relevance and the incidence did not differ significantly from the control. On the basis of this investigation, the NOAEL for maternal toxicity was established to be 1000 mg/kg bw/d, for teratogenicity at 2000 mg/kg bw/d and for fetotoxicity at 1000 mg active salt/kg bw/d. The study was performed in compliance with GLP and considered to be of good quality.

Conclusion

The effects of ATMP acid and its salts on the reproductive system can be evaluated on the basis of a well conducted 3-generation reproductive toxicity study. Although the study predated current guidelines (*e.g.*, no evaluation of the oestrus cycle, sperm parameters and developmental milestones), the overall evidence suggests that ATMP acid and its salts are not selectively toxic to the male or female reproductive system. The absence of effects on the reproductive organs in well conducted subchronic and chronic toxicity studies with ATMP provides further support to this assessment. On the basis of afore mentioned 3-generation reproductive toxicity study and also a well conducted FDA segment II study, there is further no evidence for foetotoxic or teratogenic effects of ATMP. No adverse effect levels for reproductive toxicity were established to be greater than 275–310 mg acid/kg bw/d and for that of fetotoxicity and teratogenicity of 1000 mg/kg bw/d.

In the absence of any guideline compliant reproductive toxicity studies, the reproductive toxicity of HEDP acid can be evaluated on the basis of subchronic oral feeding studies in rats and dogs which did not reveal any effects on the reproductive system at exposures up to 1500-1800 mg/kg bw/d. There were also no effects on fertility (*i.e.*, indicated by the pregnancy rate) of the disodium salt of HEDP when fed at doses up to 447 mg/kg bw/d to rats in a 2-generation study. Subsequently, the NOAEL for fertility was established to be 447 mg/kg bw/d. The reliability of the latter study could not be determined as there was an insufficient description of the methodology used. Some apparent deviations from OECD guidelines could, however, be determined. In the same 2-generation rat study, some fetotoxicity was observed in the high dose group which led to the establishment of a NOAEL at 112 mg/kg bw/d. No foetotoxic effects were observed in a poorly reported rabbit study for which a NOAEL was established to be greater than the highest tested dose of 100 mg salt/kg bw/d.

The reproductive toxicity of DTPMP acid and its salts can be evaluated on the basis of a well conducted 2-generation study in which Long Evan rats fed with DTPMP containing diet at levels up to 312 mg acid/kg bw/d. Although in this study, some alterations were observed with regard to a lower pregnancy rate in F_2 (*i.e.*, not statistically significant) and reduced pup body weight in F_{2a} (*i.e.*, statistically significant), these effects were not considered to be of biological significance as they were either not observed in F_1 or could not be replicated in F_{2b}. The absence of effects on the reproductive system could further be confirmed in an OECD guideline compliant subchronic toxicity study. The NOAEL for reproductive toxicity was therefore established to be 294 mg/kg bw/d for male rats and 312 mg/kg bw/d for female rats. The fetotoxicity of DTPMP can be assessed on the basis of afore mentioned 3-generation reproductive toxicity study in rats and also a guideline compliant developmental toxicity study. Fetotoxicity was not observed in the 3-generation study leading to the establishment of a NOAEL greater than 294 mg/kg bw/d for male rats and 312 mg/kg bw/d for female rats. On the basis of a developmental toxicity study, the NOAEL for fetotoxicity was established to be 1000 mg/kg bw/d and that for teratogenicity 2000 mg/kg bw/d.

5.2.1.8 Additional data

Toxicokinetics

ATMP acid (CAS No. 6419-19-8) and ATMP salts (CAS No. 20592-85-2)

The toxicokinetics of ATMP acid were studied in Charles River rats in a well designed and reported 10-day oral gavage study (Hotz *et al.*, 1995). Eight male rats were administered a single dose of 150 mg ^{14}C-ATMP/kg bw/d by gavage. The investigators demonstrated that faecal excretion was the principal route of elimination of ATMP/kg bw/d. Seventy four % of the dose was eliminated in 24h, 83 % in 48h and 84.4 % by day 10 following exposure. Trace amounts of radioactivity were present in urine (approx. 1 % of dose) and blood, tissues and carcass (total approx. 0.3 %) but not in exhaled air. Overall mean recovery from all sources was 85.9 %. Quantification of ^{14}C in individual tissues revealed moderate increases in kidney (consistent with urinary excretion) and carcass relative to that in blood, whereas levels in bone and bone marrow were increased approximately 100-fold. Autoradiography demonstrated deposition of ^{14}C throughout all bones of the body (*i.e.*, most intense in epiphyseal plate of the long bones and in nasal turbinates). Ten days after treatment,

deposition of ^{14}C was still high in bone, especially in the epiphyseal plate of the long bones. Some low level deposition of ^{14}C was present in stomach lining and the kidneys. No other tissues were affected. The rate of elimination of radioactivity in urine, as well as whole body elimination, showed a rapid initial phase (*i.e.*, half-life approximately 5 hrs) and a much slower terminal phase (*i.e.*, half-life approximately 70 hrs for urine and 300 hrs for whole body). The study was of good quality and GLP-compliant.

In the same study, the investigators studied the toxicokinetics of ATMP acid in Charles River rats after intravenous (i.v.) administration for 10 days (Hotz *et al*., 1995). Eight male rats were given a single i.v. injection of 1.93 µCi/kg bw, reflecting a dose of 15 mg ^{14}C-ATMP/kg bw/d. One day after treatment and at study termination, two animals were sacrificed for whole body autoradiography. Expired gases (up to 48 hr) and urine/faeces (up to 72 hr) were collected from a further subset of 2 animals. Urine, faeces end blood samples from a further 4 rats were taken every 24 hours until sacrifice. At necropsy various tissues and organs were examined. Radioactivity present in urine and plasma were quantified using HPLC with radio-detection. Contrarily to the gavage study, with about 53 % of the administered dose, the majority of the radioactivity was recovered in the urine (46 % within 6h post-dosing). About 21 % of the administered dose was determined in carcass, 4.56 % was detected in faeces, 2.4 % in tissues and organs and less than 0.1 % was found in blood. The overall mean recovery was 88.9 %. Quantification of ^{14}C in individual tissues revealed moderate increases in kidney (consistent with urinary excretion), spleen and carcass relative to that in blood, whereas levels in bone and bone marrow were increased up to a 1000-fold. Autoradiography demonstrated deposition of ^{14}C throughout all bones of the body (*i.e.*, most intense in epiphyseal plate of the long bones and in nasal turbinates). Ten days after treatment, deposition of ^{14}C was still high in bone, especially in the epiphyseal plate of the long bones. Some low level deposition of ^{14}C was present in stomach lining and the kidneys. No other tissues were affected. The rate of elimination of radioactivity in urine, as well as whole body elimination, showed a rapid initial phase (*i.e.*, half-life approximately 2 hrs for urine and 4 hrs for whole body) and a much slower terminal phase (*i.e.*, half-life approximately 130 hrs for urine and 770 hrs for whole body). Based upon relative urinary excretion after gavage and i.v. administration, gastrointestinal uptake was calculated as 2.15%.The study was of good quality and GLP-compliant.

Information from limited secondary sources suggests that uptake of orally administered ^{14}C-ATMP (10 or 50 mg/kg bw, neutralized to pH 7 with NaOH, hence formation of ATMP sodium salt) is low, with 1.8-2.0 % absorbed from the gut by male rats (Henkel KGaA, 1983b) and 10 % of the dose present in exhaled air as ^{14}C-CO_2. No details on test conditions or study conduct were available. It was therefore not possible to assess the data quality and reliability of this study.

Kinetic analyses indicate that ATMP is excreted in a biexponential manner by the rat, with urinary half-lives of 5 hr or 70 hr after oral exposure, and 2 hr or 127 hr after i.v. treatment (Hotz *et al*., 1995).

Unchanged ATMP accounts for 25 % of material recovered from rat urine 0-24 hr after oral administration (150 mg/kg bwt, by gavage), with 46 % present as an N-methyl derivative and 29 % as an unknown metabolite. In contrast, the parent substance predominated (64 % of total) in urine after i.v. dosing (15 mg/kg bwt), with approximately equivalent amounts of the N-methyl derivative (21 %) and the unknown metabolite (14 %) also present (Hotz *et al*., 1995).

The *in vivo* dermal absorption, distribution and excretion of ^{14}C-labeled ATMP sodium salt was evaluated in Wistar rats (Henkel KGaA, 1983b). In this investigation, 0.2 mL of 0.88 % neutralized aqueous solution of ^{14}C-ATMP acid was applied for 48 hours to a 10 cm^2 area of skin under fully occlusive conditions. This reflected an exposure of 0.18 mg/cm^2. Radioactivity was detected and quantified in urine, faeces, exhaled air, skin (*i.e.*, at application site) and quantified over 48 hours after treatment. The majority of the dose, *i.e.*, 60-70 % of the administered dose, was recovered from the application site and therefore considered to be not bioavailable. Trace amounts of radioactivity was found in urine, faeces in exhaled air or in the carcass. On the basis of this study, the percutaneous absorption of the sodium salt of ATMP was estimated to be 0.6 % in male rats and 0.9 % in females. Absorbed radioactivity was excreted mainly in urine within the first 24 hours following dermal application.

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

The distribution of intravenously administered ^{14}C-labeled HEDP acid was studied in MRI mice (Monkkonen *et al*., 1987). Forty male mice were given a single i.v. injection of 25 mg ^{14}C-HEDP/kg bw. Samples of blood, skin, liver, kidneys, adrenal glands, spleen, pancreas, small intestine, lungs, thymus, testis, thyroid, muscle, brain, bone and vertebra were collected at regular time-intervals until 360 days after treatment in subgroups of 4 animals per time point. Radioactivity was removed rapidly from the plasma (*i.e.*, less than 10 % of the injected dose remained 5 minutes post-dose), transferred to kidney (*i.e.*, about 5 % at 5 minutes post-dose) and tibia/femur (*i.e.*, about 13 % at 2 hours post-dose) with trace levels present in spleen (*i.e.*, 1 % at 5 minutes post-dose) and liver (*i.e.*, about 1 % or less up to 2 hours post-dose). Levels in kidneys decreased rapidly (*i.e.*, about 8 % at 30 minutes, 1-2 % at 2 hours), consistent with rapid urine excretion. Only in the long bones small amounts of ^{14}C (*i.e.*, about 5 %) remained at 360 days after treatment.

The distribution of ^{14}C-labeled HEDP acid was studied in Sprague-Dawley rats for up to 16 days after intraperitoneal (i.p.) injection (Larsson and Rohlin, 1980). One-day and 5-day old rats (sex not stated) were given a single i.p. injection of 50 mg ^{14}C-HEDP/kg bw. A time-dependent increase in radioactive material in peripheral bone surfaces and epiphyseal cartilage of long bones was observed. Trace amounts of radioactive material was found in kidneys, liver, stomach and renal medulla. When animals were pre-treated with unlabeled HEDP before the i.p. injection of 50 mg ^{14}C-HEDP/kg bw, deposition of ^{14}C-HEDP was increased in bone, stomach and kidney. The study was not in compliance with GLP nor OECD standards, but was reported in enough detail to judge it to be scientifically reliable.

The *in vivo* absorption, distribution and excretion of ^{14}C-labeled HEDP disodium salt after oral administration in rat, dog, monkey and rabbit was evaluated in a pre-GLP study of good quality (Michael *et al*., 1972).

In the rat study, animals were administered by gavage a single dose of 50 mg ^{14}C-HEDP/kg bw or one dose for 5 consecutive days. With about 80-95 %, the majority of the radioactivity recovered after 72 hours was found in faeces, less than 4 % in urine and trace amounts in soft tissues (*i.e.*, up to 0.5 % of the administered dose). Less than 0.2 % was exhaled as ^{14}C-CO_2. Absorption of disodium salt of HEDP was less than 10 % of which half is excreted in urine, and the remainder deposited in the skeleton. Disposition was essentially similar following a single- or repeated dose. There was virtually no enterohepatic recirculation. Preconditioning

with unlabeled disodium HEDP (*i.e.*, 0.5 % in diet for 30 days prior to gavage administration of labelled HEDP sodium salt) did not affect disposition or elimination in the rat.

In the dog study, three female Beagle dogs were administered by gavage a single dose of 20 mg ^{32}P-HEDP/kg bw or 50 mg ^{14}C-HEDP/kg bw. Total absorption of disodium HEDP over 72 hours was approximately 21 % in young dogs and 14 % in older dogs. Absorption occurred primarily from the stomach. The majority of the radioactivity recovered after 72 hours was found in faeces (*i.e.*, approximately 80 %) and urine (*i.e.*, 10 %). Unabsorbed ^{14}C or ^{32}P were present in faeces, gastrointestinal contents and vomitus. A similar disposition pattern was observed in ^{14}C and ^{32}P labelled test substance. Higher levels present in bones from younger dogs reflects increased skeletal remodelling.

In the monkey study, one male and one female Rhesus monkey were administered via gavage a dose of 20 mg ^{32}P-HEDP/kg bw and one male monkey was given 50 mg ^{14}C-HEDP/kg bw by gavage. The majority of ^{14}C recovered was found in faeces (*i.e.*, 97 %), and less than 1 % in urine, carcass and soft tissues. The same pattern was observed for ^{32}P: 92 % recovery in faeces, 3.6 % in skeleton, 1.7 % in carcass and less than 1 % in urine and soft tissues.

In the rabbit study, three male New Zealand rabbits were administered by gavage a dose of 50 mg ^{14}C-HEDP/kg bw. The majority of ^{14}C recovered was found in faeces (*i.e.*, 78 %), 26 % in gastrointestinal contents, 3.3 % in urine and less than 1 % in urine, liver and kidney. Less than 4 % of the administered does was absorbed of which 87 % was excreted in the urine.

DTPMP acid (CAS No. 15827-60-8) and DTPMP salts (CAS No. 22042-96-2)

Very limited information is available on the elimination of ^{14}C-DTPMP following oral or dermal administration to Sprague-Dawley rats (Procter and Gamble, 1978). The information is a secondary reference from the IUCLID data sheet for DTPMP acid with CAS No 15827-60-8, although the test substance was described as a neutralized sodium salt.

Three male rats were administered by gavage a dose of 10 mg ^{14}C-labeled DTPMP sodium salt/kg bw. The majority of the radioactivity recovered at 72 hours post-dosing was found in the faeces (*i.e.*, 94 % of administered dose), 1.3 % was detected in urine and less than 1 % in expired CO_2 and tissues.

In the dermal absorption study, three male rats were exposed for 72 hours to 0.1 mL of ^{14}C-labeled DTPMP sodium salt which equals a dose of 0.6 mg/kg bw. Seventy-two hours after application, 89 % of the applied test material was recovered at the test site. Negligible amounts were found in faeces (*i.e.*, less than 0.01 %), urine (*i.e.*, less than 2 %) and carcass (*i.e.*, less than 1.5 %).

Conclusion

The physicochemical properties of phosphonic acid compounds, notably their high polarity, charge and complexing power, suggests that they will not be readily absorbed from the gastrointestinal tract. This is supported by experimental data which confirm that absorption after oral exposure is low, averaging 2-7% in animals and 2-10% in humans. Faecal elimination of unabsorbed material predominates after ingestion (up to 90% of dose). Renal clearance of any material absorbed from the gut is rapid, with urinary half-lives of 5 hr and 70 hr reported. This second phase of excretion may represent mobilization of material

initially sequestered by bone, since deposition studies have shown preferential accumulation of these substances in the epiphyseal plate and other regions of the long bones *in vivo*. Around 25% of material absorbed following an oral dose is excreted unchanged in urine, with the reminder converted to an N-methyl derivative or unidentified product(s). Inconsistent data indicate conversion to carbon dioxide is negligible. More pronounced accumulation is observed in bone after i.v. or i.p. injection, reflecting enhanced bioavailability following exposure by these non-physiological routes. Based on the available data, no major differences appear to exist between animals and humans with regard to the absorption, distribution and elimination of phosphonic acid compounds *in vivo*.

ATMP acid and ATMP salts are poorly absorbed from the gut and rapidly eliminated after oral and i.v. administration. Faeces represent the principal route of excretion after oral administration with trace amounts present in urine and carcass. Faeces elimination was, in contrast, comparatively insignificant after i.v. injection, with the majority of the dose present either in urine or carcass. Bone is the only tissue that exhibits deposition of test-substance derived radioactivity. Absorption after dermal exposure was very low and only trace amounts were found in urine, faeces and carcass. The main route of excretion was via the urine in the first 24 hours following application.

Gastro-intestinal absorption of HEDP acid and HEDP salts is rat, dog, rabbit and monkey is low, with the majority of the dose excreted in faeces and a substantial amount excreted via the urine. The remainder of the test substance derived radioactivity deposited mainly in the bones. After i.v. or i.p. injection, internal body burdens increased, presumably reflecting greater systemic availability.

Very limited information is available on the absorption, distribution, metabolism and elimination of DTPMP acid and DTPMP salts. In a secondary reference, it was demonstrated that only minor amounts of sodium salt of DTPMP enter the body after oral administration. The majority of the radioactivity recovered was found in faeces. When applied dermally, only negligible amounts were recovered from faeces, urine and carcass.

5.2.1.9 Experience with human exposure

Toxicokinetics

HEDP acid (CAS No. 2809-21-4) and HEDP salts (CAS No. 29391-71-3)

In a human intestinal absorption study, nine adult male volunteers ingested 30 mg HEDP disodium salt/kg bw in 3 divided doses for 2-3 weeks (Recker and Saville, 1973). One hour after ingestion of the final dose, individuals received a single i.v. injection of 10 µCi ^{14}C-labeled HEDP disodium salt. Blood samples were taken at regular intervals until 300 min post-dose. Urine was collected from 4 individuals over 24 hours. Forty eight hours after the i.v. injection, 4 subjects ingested 5 mg ^{14}C-disodium salt/kg bw as a powder in water following an overnight fasting. Venous blood was sampled at regular intervals up to 8 hours post-dose. Five subjects ingested 30 mg ^{14}C-disodium salt/kg bw as dry powder in water plus 20 mL of a 10 % PEG4000 solution (*i.e.*, faecal marker) 1 hour later. Venous blood samples were collected up to 5 hours post-dose, urine was collected over 24 hours and stools for 5 days post-dose. Mean urinary recovery was 52 % after i.v. injection and 1.8 % after oral administration. Faecal recovery of ^{14}C was 90 %, with 3 % of the dose recovered in urine. Mean intestinal absorption was 3.35 % after ingestion of 5 mg ^{14}C-disodium salt/kg bw, and

7.19 % after ingestion of 30 mg ^{14}C-disodium salt/kg bw. The study was pre-GCP but its conduct and reporting appeared to be scientifically reliable.

In another study, the intestinal absorption and urinary excretion of HEDP disodium salt was studied in osteoporotic female patients receiving a long clinical treatment (Heaney and Saville, 1976). Ten individuals were given a daily oral dose of 20 mg disodium salt of HEDP/kg bw/d for 6 months after which absorption and excretion were assessed. Five of the 10 subjects continued the treatment for another 6 months. Urine and faeces samples were collected in three 4-day periods. Urine was collected as 24-hour pooled samples; faeces were collected as 4-day pooled samples following ingestion of 1 g polyethylene glycol marker. Approximately 10 % of the administered dose was absorbed, with an effective retained dose of 1.6 mg HEDP disodium salt/kg bw/d. On average, 19.8 % of the absorbed material was excreted in urine. The study was pre-GCP but its conduct and reporting appears scientifically reliable.

5.2.2 Identification of Critical endpoints

5.2.2.1 Overview on hazard identification

The phosphonic acid compounds ATMP, HEDP, DTPMP and their sodium salts which were evaluated in context of this review can be considered to be of low to moderate acute oral and dermal toxicity. Acute oral LD50 values ranged from 581 mg/kg bw for the disodium salt of HEDP to up to 8610 mg/kg bw for the tetrasodium salt of ATMP. The dermal LD50 values ranged from greater than 860 mg/kg bw for the octasodium salt of DTPMP to greater than 6320 mg/kg bw for the pentasodium salt of ATMP. Only in the case for acute oral toxicity of ATMP, the acid appeared to be slightly more toxic than the sodium salts. In all other instances, there was no apparent difference in acute toxicity between the acid form of the phosphonates and their respective salts.

On the basis of the studies presented, the phosphonic acid compounds ATMP, HEDP, DTPMP and their salts, can generally be considered to be mildly irritating to skin. The PII in most cases did not exceed 3, indicating the compound to be mildly irritating to skin. More severe reactions were observed, when aqueous solutions containing 25 % of ATMP acid or 33 % tetrasodium salt of HEDP were applied to intact rabbit under fully occluded conditions. Longer application time of 24 h caused more irritation then when the acid or salt product was only applied over 4 h were no irritation response was observed in most cases regardless of the strength of the product tested. Applying the neat acid or salt did not seem to produce a consistently greater effect, rather in some cases the neat powder product was less irritating then some tested formulations, indicating reduced potential of the applied powder product for skin reactivity.

The observed eye irritation potential of the phosphonic acid compounds ATMP, HEDP, DTPMP and their salts, ranged from practically non-irritating to severely irritating with irreversible effects. ATMP and DTPMP acids tested as neat products were considered to be moderately irritating to rabbit eyes whereas their salts were considered to be minimally to virtually non-irritating. A 60 % solution of HEDP acid caused severe irritation and persistent effects. The salts of HEDP were, however, milder and considered to be minimally irritating. Generally, the acids were more irritating than the salts and rinsing the animal’s eyes immediately after exposure considerably decreased the symptoms.

The tested phosphonic acid compounds provided a coherent picture in that this compound class should not be considered to be skin sensitizers. None of the tested compounds induced skin sensitization in guinea pigs.

A number of good quality repeated dose toxicity studies were conducted on ATMP, HEDP, DTPMP and/or their salts. In a reliable 2-year chronic rat feeding study, the NOAEL of ATMP acid was established at the highest tested dose level of 500 mg/kg bw/d. The information on the salt of ATMP is less robust, but similarly indicates a low repeated oral toxicity with a NOAEL greater than 600 mg/kg bw/d. On the basis of a good quality 90-day oral feeding study in rats, the NOAEL of the disodium salt of HEDP was established at 41 mg active salt/kg bw/d (males). Anaemia, reduction in red cell parameters and pallor which were observed at higher dose levels, were considered to be related to the perturbation of iron homeostasis as a result of the chelating properties of HEDP. The disodium salt of HEDP was further evaluated in a two year rat study on which basis a NOAEL of 19 mg/kg bw/d was established. At higher doses exposure to the test material induced anaemia and haematological disturbances, likely related due to the iron-chelating properties of HEDP. No repeated dose toxicity studies were identified for DTPMP acid. The sodium salt of DTPMP acid was investigated in several repeated dose toxicity studies. NOAELs were reported to be as low as 4 mg/kg bw/d in a poorly reported subchronic toxicity study for which the reliability could not be assigned and as high as 83 – 92.3 mg/kg bw/d in a reliable high quality 90-day oral feeding study. A NOEL of 20 mg/kg bw/d was established on the basis of a 1-year oral feeding study in rats. Effects observed in this chronic toxicity study included anaemia, the perturbation of haematological parameters and liver effects at the highest dose (*i.e.*, 500 mg/kg bw/d) and a decrease of spleen hemosiderin in males at the mid dose level (*i.e.*, 100 mg/kg bw/d). Similarly to HEDP, the occurrence of anaemia and the observed changes in haematological parameters were considered to be related to the perturbation of iron homeostasis as a result of the chelating properties of DTPMP. However, also for this study, detailed study documentation was not available and the reliability of this study could therefore not be established.

Neither the acid form, nor the salts of ATMP, HEDP and DTPMP are considered to be mutagenic, genotoxic or carcinogenic. Although some conflicting results were observed for HEDP acid in an *in vitro* mouse lymphoma assay, these observations could not be confirmed in a follow-up study. The positive response in the first assay is believed to be artefactual due to excessive pH and possibly also due to the chelation of essential metal ions and/or induction of oxidative species in the presence of S9 mix. Also, the neutralised DTPMP acid showed a positive response in an *in vitro* mouse lymphoma assay in the presence of S9 mix, while testing the salt directly did not provide any evidence of a positive response. While pH effects and a too high osmolarity of the test solution were assessed to be unlikely causes of the observed response, it is believed that similarly to HEDP acid, the strong chelating properties and an interaction with the S9 mix and/or induction of oxidative species in the presence of S9 could have led to the positive response in this assay. The absence of genotoxicity of HEDP and DTPMP acid could be confirmed in further *in vitro* and *in vivo* mutagenicity/genotoxicity tests. Importantly, the acids or salts of ATMP, HEDP and DTPMP did not show any carcinogenic activity when tested in 2-year feeding studies in rodents.

The acids or salts of ATMP, HEDP, and DTPMP have been evaluated for reproductive and developmental toxicity in 2- or 3-generation reproductive toxicity studies in rats and also in some specifically designed fetotoxicity studies. There was no evidence for reproductive or developmental toxicity of ATMP, which led to the establishment of NOAELs for ATMP of greater than 275 mg/kg bw/d for reproductive toxicity and 1000 mg/kg bw/d for fetotoxicity.

For the salt of HEDP, the NOAEL for reproductive toxicity was established at 447 mg/kg bw/d, the highest tested dose. Some minor fetotoxicity indicated by a significant decrease in the number of live pubs born and a non-significant increase in stillborns was observed at this highest dose level, leading to the establishment of a NOAEL of 112 mg/kg bw/d. Also DTPMP did not show reproductive or developmental toxicity when tested in a 2-generation study in rats. On the basis of this study, the NOAEL of DTPMP for reproductive and developmental toxicity was established to be greater than 294 mg/kg bw/d, the highest tested dose. The NOAEL for fetotoxicity was established to be 1000 mg/kg bw/d and that for teratogenicity 2000 mg/kg bw/d on the basis of an additional developmental toxicity study.

5.2.2.2 Rationale for identification of critical endpoints

Dermal exposure is the main exposure route for consumers and subsequently, dermal effects such as skin irritation and sensitization as well as long-term dermal toxicity must be considered for the human health risk assessment. A significant amount of data is available addressing skin irritation and skin sensitization potential of solutions of the acid forms or salts of ATMP, HEDP and DTPMP. Dermal penetration studies in rats have shown that the phosphonates under consideration have only limited potential to penetrate the skin to become systemically available. There are only a few dermal repeated dose toxicity studies available, but by using bridging assumptions, systemic effects after dermal exposure can also be assessed by using the results of oral repeated dose toxicity studies in experimental animals.

5.2.2.3 Adverse effects related to accidental exposure

The acute oral and dermal toxicity of neat ATMP, HEDP, and DTPMP was considered to be moderate to low. The phosphonates can be present in detergent formulations at a maximum of 4%. Generally, accidental oral exposure to a detergent formulation poses a minor risk of aspiration. This is, however, related to the emetic activity and physico-chemical properties of the surfactants present in the product.

The available information suggests that at the levels present, the phosphonates under consideration do not alter the skin or eye irritation profile of the overall detergent formulation. The irritation profile of a detergent product is mainly driven by the type and concentrations of the surfactants present in the formulation. However, generally eye and prolonged skin contact with neat detergent products should be avoided. Other surfactants present in the formulation might contribute to these effects. Therefore, in case of accidental eye contact, immediate rinsing with plenty of water is recommended. This immediate action has been shown in animal experiments to minimize irritation effects.

5.2.3 Determination of NOAEL or quantitative evaluation of data

As discussed before, the available oral and dermal repeated dose toxicity studies demonstrate a moderate level of toxicity of ATMP, HEDP and DTPMP.

The NOAEL of ATMP acid was established to be 500 mg/kg bw/d on the basis of a 2-year rat feeding study. At this dose level, some reduced body weights and changes in organ weights

were observed. However, since these effects were not consistent with time, were minor in extent, were not consistently altered in relation to body weight and not accompanied by any histopathological changes, these findings were considered to be an adaptive response as a result to exposure and not of toxicological significance. On the basis of the 2-year feeding study, the NOEL of ATMP can be considered to be 150 mg/kg bw/d.

HEDP was investigated in a good quality 2-year feeding study. On the basis of this study the NOAEL for HEDP was established to be 19 mg/kg bw/d. At higher dose levels, haematological disturbance indicated by anaemia, reduction red cell parameters and pallor were observed, which were probably due to the chelating properties of HEDP. These were observed in the 2-year feeding study, but also in further subchronic oral toxicity studies in rats and dogs.

The repeated dose toxicity of DTPMP was investigated in subchronic and chronic toxicity studies of varying reliability. In a reliable high quality 90-day oral feeding study the NOAEL for DTPMP was established to be 83 – 92.3 mg/kg bw/d. A NOEL of 20 mg/kg bw/d was established on the basis of a chronic 1-year study for which the reliability could not be established. Effects above the NOAEL/NOEL included haematological disturbances, but also liver alterations indicated by increased relative liver weight. For the purpose of this risk assessment, a conservative approach was taken by considering a NOEL for DTPMP of 20 mg/kg bw/d. Although the reliability could not be established for the underlying study, this NOEL ensures an appropriate level of conservatism as it is based on a chronic toxicity study and it was overall the lowest tested concentrations which unambiguously revealed no effects.

Although some common effects such as increase in relative liver weights and perturbation of haematological parameters (only in the case of HEDP and DTPMP) were observed for the phosphonic acid compounds under consideration, the determined NOAELs/NOELs for the 3 compounds differ significantly. For assessing the risk associated with human exposure to ATMP, HEDP and DTPMP, it is therefore suggested to calculate Margin of Exposures for the 3 phosphonic acid compounds individually. There is, however, no need to distinguish between the acid and salt form of the respective phosphonic acid. The basic justification for this is that in dilute aqueous conditions of defined pH a salt will behave no differently to parent acid. The effect of the counter-ion (usually sodium) is not judged to be significant. The NOAEL values which will be the basis for calculating the MOE’s were derived from sub-chronic and chronic oral feeding studies in rats.

5.3 Risk assessment

5.3.1 Margin of exposure calculation

The Margin of Exposure (MOE) is the ratio of the No Observed Adverse Effect Level (NOAEL) or an appropriate substitute (*e.g.*, NOEL) to the estimated or actual level of human exposure to a substance. NOAELs have been established for the phosphonates based on their different toxicological profiles (Table 24). No distinction was made between the acid of the compound and the salt. However, to ensure conservatism of the assessment, always the lowest NOAEL was chosen.

To allow comparison of the consumer ("systemic") exposure as discussed in Chapter 5.1 with the critical endpoints, the NOAELs determined on the basis of rat feeding studies will be corrected for the systemic availability under the respective study conditions. As discussed in Chapter 5.2.1.8 ('toxicokinetics'), due to their high polarity, charge and complexing power, the phosphonic acid compounds under consideration are only poorly (*i.e.*, 2–10 %) absorbed from the gastrointestinal tract. Thus, to ensure a conservative approach, all NOAELs were corrected by lowest systemic availability which was determined to be 2% for the neutralized form of ATMP acid (Henkel KGaA, 1983b). Table 24 lists in addition to the NOAELs as determined in the animal experiments, the systemic NOAEL for each of the phosphonates.

Table 24: No Observed Adverse Effect Levels of the different phosphonic compounds

Compound	NOAEL[(1)]	$NOAEL_{sys}$[(2)]	Study
ATMP	500 mg/kg bw/d	10 mg/kg bw/d	2-year chronic rat feeding study (Bio/Dynamics Inc., 1979a)
HEDP	19 mg/kg bw/d	0.38 mg/kg bw/d	2-year chronic rat feeding study (Huntingdon Research Centre, 1979)
DTPMP	20 mg/kg bw/d	0.4 mg/kg bw/d	1-year rat feeding study (Procter and Gamble, 1982)

(1) Lowest NOAEL; (2) systemic NOAEL on the basis of an assumed systemic availability of 2 % of orally ingested phosphonate

5.3.1.1 Exposure from the different exposure scenarios

For calculation of the relevant MOEs for all the different exposure scenarios and compounds the following equation was used:

$$\mathbf{MOE_{exposure\ scenario} = NOAEL_{sys} / EXP_{sys}}$$

The results are summarized in Table 25. Since ATMP is only used in hard surface cleaner applications (AISE unpublished data), the MOE for ATMP was only calculated for that category.

Table 25: Margins of Exposure for the different phosphonic acid compounds

Exposure Scenario	(EXP_{sys}) (µg/kg bw/day)	MOE		
		HEDP	**DTPMP**	**ATMP**
Direct contact from hand washing laundry	0.014	27 100	28 600	
Direct skin contact from pre-treatment of laundry	0.42	1 800	1 000	
Direct skin contact from hand dishwashing	0.00045	844 400	888 900	
Direct skin contact from hard surface cleaners	0.004	95 000	100 000	>1 000 000
Direct skin contact from carpet cleaners	0.071	5 400	5 600	
Indirect skin contact from wearing laundered clothe	0.0009	422 200	444 400	
Inhalation of laundry powder dust	0.00015	> 1 000 000	> 1 000 000	
Inhalation of aerosol particles	0.0029	131 000	137 900	
Oral exposure to phosphonates	0.013	28 800	30 300	
Total consumer exposure	**0.53**	**1 200**	**800**	**2 500 000[1)]**

[1)] Total consumer exposure to ATMP amounts to 0.004 µg/kg bw/day

From the three phosphonates assessed in this review, the lowest MOE was calculated for the aggregate consumer exposure to DTPMP to be greater than 800. This provides a substantial safety margin. The MOEs calculated for HEDP and ATMP are significantly higher with 1200 and 2500000 respectively.

However, in reality it can be assumed that the MOE for the use of each of these phosphonates in laundry and cleaning applications is much higher. Summing up all individual consumer exposures to a total consumer exposure can be considered an unrealistic worst-case scenario as it is very unlikely that the same consumer regularly carries out the same tasks at the maximum frequency. Further and more specifically, the lowest MOE for an individual exposure was for DTPMP with 1000 for the pre-treatment of laundry. As elaborated in chapter 5.1.3.3, this exposure estimate can be regarded to be very conservative as consumers typically pre-wet the laundry before applying the detergent for pre-treatment or conduct the

pre-treatment under running tap water. Both practices lead to significant dilution which is not reflected in this exposure estimate.

5.3.1.2 Exposure scenario: oral route from accidental ingestion and accidental eye contact

Accidental ingestion of a few milligrams of phosphonates as a consequence of accidental ingestion of laundry and cleaning products is not expected to result in any significant adverse health effects given the low toxicity profile of laundry and cleaning products in general. This view is supported by available toxicological information from animal studies.

Accidental eye contact with undiluted cleaning products may, depending on the type of product, cause mild to moderate irritation which is fully reversible shortly after the accidental exposure. This response is, however, not related to the phosphonate content in the finished product. At typical product levels between 0.2-1 %, phosphonates are not expected to alter the overall eye irritation profile of detergent formulations. In the case of accidental eye contact, immediate rinsing with plenty of water is recommended. This immediate action has been shown in animal experiments to minimize irritation effects of cleaning products.

5.3.2 Risk characterization

5.3.2.1 Systemic toxicity

Consumers are exposed to phosphonates through their use in laundry and cleaning products. All potential exposure scenarios were identified, quantified and assessed by comparing the estimated systemic exposure values with the systemic NOAEL determined in subchronic and chronic toxicity studies. The lowest MOE for the systemic dose resulting from the total consumer exposure to DTPMP is 800. The MOE calculated for HEDP is 1 200 and that for ATMP is > 1 000 000. These MOE calculations reflect the aggregate of all possible exposure scenarios using mostly worst case assumptions, exposure situations which are very unlikely to occur in real life.

The determined MOEs are certainly large enough to account for the inherent uncertainty and variability of the hazard data on which it is based on. The MOEs are based on worst case exposure assumptions and very conservative, systemic NOAELs.

The available toxicological information indicates that ATMP, HEDP and DTPMP are not mutagenic, genotoxic or carcinogenic. There was no evidence for reproductive and developmental toxicity. The effects observed in repeated dose toxicity studies at high dose levels included anemia and perturbation of hematological parameters, probably due to the iron chelating properties of phosphonates and liver effects indicated by relative liver weight changes which were not accompanied by histopathological findings at post-mortem necropsy.

A large proportion of the total systemic phosphonate exposure results from the percutaneous absorption of ATMP, HEDP and DTPMP in applications involving skin contact. The dermal penetration constant was derived from an *in vivo* rat dermal penetration study with a solution of ^{14}C-labelled ATMP sodium salt. It was assumed that due to their similar chemical structure and physico-chemical characteristics, the percutaneous absorption of the phosphonates under consideration is very similar. Thus, the same dermal penetration constant was used in the

exposure calculations for all three phosphonates. Generally, ionic substances such as salts or organic acids are assumed to penetrate the skin less readily compared to the respective organic acids. It can, however, be assumed that at the pH under in-use conditions, the phosphonates will be present to a large extent in their neutralized forms. Thus, the dermal penetration experiment with the sodium salt of ATMP is assessed to reflect appropriately the in-use exposure scenarios occurring in laundry and cleaning applications. A further level of conservatism is warranted by the fact that rat skin is typically more permeable to chemicals compared to human skin (Schaefer and Redelmeier, 1996; van Ravenzwaay and Leibold, 2004).

In summary, the use of the phosphonic acid compounds ATMP, HEDP and DTPMP in consumer products such as laundry and cleaning detergents does not raise any safety concerns with regard to systemic toxicity.

5.3.2.2 Local toxicity

Neither the acids, nor the salts of ATMP, HEDP or DTPMP are contact sensitizers and their skin irritation potential is concentration dependent. Under normal use conditions with direct skin contact (*e.g.*, in hand laundering or in hand dishwashing) the consumer is exposed to detergent solutions containing 0.001–0.01 % phosphonates. At these concentration levels, phosphonates are not irritating to skin. This has been demonstrated in animal studies. Short-term exposure to neat or concentrated detergent formulations (*e.g.*, pretreatment of clothes) may result in minor signs of superficial irritation, but is easily avoided by rinsing with water. The overall skin irritation profile of cleaning products is driven by other product ingredients such as surfactants or bleach and physico-chemical parameters like pH.

Accidental eye contact with undiluted detergent product may cause mild to moderate irritation which is fully reversible shortly after exposure. This response is, however, not related to the phosphonate content in the finished product. At typical product levels between 0.2–1 %, phosphonates are not expected to alter the overall eye irritation profile of detergent formulations. In the case of accidental eye contact, immediate rinsing with plenty of water is recommended. This immediate action has been shown in animal experiments to minimize irritation effects of cleaning products.

Accidental ingestion of a phosphonate containing detergent product is not expected to result in any significant adverse health effect. This assessment is based on toxicological data demonstrating the low acute oral toxicity of phosphonate containing laundry and cleaning products. National poison control centers have not reported a case of lethal poisoning or severe health effects associated with accidental ingestion of detergents containing phosphonate acid compounds.

5.4 Summary and conclusion

Consumers are exposed to the phosphonic acid compounds ATMP, HEDP and DTPMP through their presence in laundry and cleaning products mainly via the dermal route, but to some extend also via the oral and the inhalatory route. Skin exposure occurs mainly in hand-washed laundry, laundry pre-treatment and hand dishwashing and to a very minor extent also through phosphonate residues in the fabric after the washing cycle and skin contact during hard surface cleaning. Consumers are orally exposed to phosphonates through residues deposited on eating utensils and dishes after hand dishwashing and potentially via the presence at low levels in drinking water. Since phosphonates are also used in spray cleaners, the consumer can also be exposed to phosphonate containing aerosols generated by the sprayer. The consumer aggregate exposure to HEDP and DTPMP has been estimated to be at maximum 0.53 µg/kg bw/day. Total consumer exposure for ATMP, which is only used in hard surface cleaning applications has been estimated to be at maximum 0.004 µg/kg bw/day.

A substantial amount of toxicological data and information *in vivo* and *in vitro* demonstrates that there is no evidence for ATMP, HEDP and DTPMP being genotoxic, mutagenic or carcinogenic. There is some conflicting data with regard to the mutagenicity of DTPMP, but the overall weight of the evidence suggests it is not mutagenic. There wasn't also any evidence of reproductive toxicity or developmental effects in animals. The long-term toxicity of the acid or salt forms of the phosphonates under review was evaluated in several subacute, subchronic and chronic toxicity studies. In the available chronic and subchronic oral toxicity studies, no adverse effects for ATMP, HEDP and DTPMP were observed at dose level of 500, 24 and 20 mg/kg/day respectively. The NOEL of 20 mg/kg bw/d for DTPMP is considered to be very conservative and it is based on a GLP and OECD guideline compliant 1-year feeding study for which, however, no detailed study information was available. Although the reliability of this study could not be established, taking this NOEL for the purpose of this risk assessment ensures an appropriate level of conservatism as it has been established on the basis of a chronic toxicity study and it was overall the lowest tested concentrations which unambiguously revealed no effects. The conservatism of the assessment is underlined by the fact that in context of the OECD HPV programme, the SIDS Initial Assessment Meeting (SIAM 18, 20-23 April 2004) concluded that DTPMP and its salts are of low priority for further work. The underlying SIDS Initial Assessment Profile and Report (SIAP, SIAR) considered the NOAEL for DTPMP to be 83 – 92.5 mg/kg bw/d. This NOAEL was established on the basis of a reliable 90-day oral feeding study.

To allow comparison of the consumer "systemic" exposure, the NOAELs for all three phosphonic acid compounds were corrected by the lowest systemic availability which was determined to be 2 % for the neutralized form of ATMP acid. The systemic NOAELs for ATMP, HEDP and DTPMT were calculated to be 10, 0.38 and 0.4 mg/kg bw/d, respectively. The effects observed at high doses for the acid forms or salts of ATMP, HEDP and DTPMP were confined to anemia and perturbation of hematological parameters, probably due to the iron chelating properties of phosphonates and liver effects indicated by relative liver weight changes which were not accompanied by histopathological findings at post-mortem necropsy.

The comparison of the aggregate exposure and the systemic NOAEL results in MOEs of 800, 1.200 and >1.000.000 for DTPMP, HEDP and ATMP respectively. These margins of exposure are large enough to account for the inherent uncertainty and variability of the hazard database and inter and intra-species extrapolations, which are usually considered by a factor of 100.

Neat phosphonic acid compounds ATMP, HEDP and DTPMP can be moderately to severely irritant to eyes. However, under normal use conditions with direct skin contact (*e.g.*, in hand laundering or in hand dishwashing) the consumer is exposed to detergent solutions containing 0.001–0.01 % phosphonates. At these concentration levels, phosphonates are not irritating to eyes and do not alter the overall eye irritation profile of the cleaning product. Local dermal effects due to direct or indirect skin contact with phosphonate containing solutions in hand-washed laundry or hand dishwashing are not of concern because phosphonates are not contact sensitizers and are not expected to be irritating to the skin at in-use concentrations.

In summary, the human health risk assessment has demonstrated that the use of ATMP, HEDP and DTPMP in household laundry and cleaning detergents is safe and does not cause concern with regard to consumer use.

6. References

ABC (1978). Dynamic Toxicity of Dequest 2060 (AB-78-1384342-2) to Rainbow Trout (*Salmo gairdneri*). Unpublished report for Monsanto Company, St Louis, MO. Solutia report No: AB-78-0379.

ABC Laboratories Inc. (1979a). Dynamic toxicity of Dequest 2000 (AB-79-1384338-1) in Rainbow Trout (*Salmo gairdneri*). Unpublished report for Monsanto Chemical Company, St Louis, MO. Solutia report No: AB-79-0191.

ABC (1979b). Dynamic toxicity of Dequest 2010 (AB-79-1384339-1) to Rainbow trout (*Salmo gairdneri*). Unpublished report for Monsanto Company, St Louis, MO. Solutia report No: AB-79-0192.

ABC Laboratories Inc. (1980a). Early life stage toxicity of Dequest 2000 (AB-79-1384338-2) to Rainbow Trout (*Salmo gairdneri*) in a flow-through system. Unpublished report for Monsanto Chemical Company, St Louis, MO. Solutia report No: AB-80-0192.

ABC (1980b). Study No: 24475. Early life stage toxicity of Dequest 2060 (AB-79-1384342-3) to Rainbow trout (*Salmo gairdneri*) in a flow-through system. Unpublished report number 24475 for Monsanto Company, St Louis, MO. Solutia report No: AB 20020068.

AISE (Association Internationale de la Savonnerie, de la Détergence et des Produits d'Entretien) (2002) Table of Habits and Practices for Consumer Products in Western Europe. Developed within the HERA project in 2002.

AISE (Association Internationale de la Savonnerie, de la Détergence et des Produits d'Entretien) Unpublished Data

Bio/Dynamics Inc. (1976) A four-week dose range finding study of CP 42902 in rats. Unpublished report for Monsanto, Project number BDN-75-117.

Bio/Dynamics Inc. (1979a) A twenty-four month oral toxicity/carcinogenicity study of CP 42902 in rats. Unpublished report for Monsanto, Project number BDN-75-118.

Bio/Dynamics Inc. (1979b) A three-generation reproduction study of CP 42902 in rats. Unpublished report for Monsanto, Project number BDN-76-118.

Bio/Dynamics Inc. (1979c) A one generation reproduction study of CP 66257 in rats. Unpublished report for Monsanto, Project number BDN-77-163.

Bio/Dynamics Inc. (1979d) A segment II teratology study of FA 42902 in rats. Unpublished report for Monsanto, Project number BD-78-54.

Bio/Dynamics Inc. (1980) A segment II teratology study of FA 42902 in mice. Unpublished report for Monsanto, Project number BD-79-179.

Bio/Dynamics Inc. (1985a) Acute oral toxicity study in rats. Test material Dequest 2016. Unpublished report for Monsanto, Project number BD-85-028.

Bio/Dynamics Inc. (1985b) Acute dermal toxicity study in rabbits. Test material Dequest 2016. Unpublished report for Monsanto, Project number BD-85-028.

Bio/Dynamics Inc. (1985c) Primary dermal irritation study in rabbits (4- and 24-hour exposure). Test material Dequest 2016. Unpublished report for Monsanto, Project number BD-85-028.

Bio/Dynamics Inc. (1985d) Eye irritation study in rabbits. Test material Dequest 2016. Unpublished report for Monsanto, Project number BD-85-028.

Bundesinstitut für Gesundheitlichen Verbracherschutz und Veterinarmedizin (BgVV) (1999) Ärtzliche Mitteilungen bei Vergiftungen. ISBN 3-931675-59-9.

Cegarra J., Gacén J., Cayuela D. and Riva M.C. (1994) Comparison of stabilisers for wool bleaching with hydrogen peroxide, JSDC, 110, 308-310

Central Toxicology Laboratory (1997) Dequest 2066A: L5178Y TK +/- mouse lymphoma mutation assay. Unpublished report for Monsanto, Project number XX1999030.

Central Toxicology Laboratory (1998) Dequest 2066A: 90 day dietary toxicity study in rats. Unpublished report for Monsanto, Project number CTL/P/5888.

Chemstar PAC (2003) Draft SIARs for ATMP, HEDP and DTPMP; draft SIDS packages for ATMP, ATMP sodium salts, HEDP, HEDP sodium salts, DTPMP and DTPMP sodium salts, submitted for review to the UK Environment Agency

Covance Laboratories (1998a) Mutagenicity test on Dequest 2006 Deflocculent and Sequestrant measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells. Unpublished report for Solutia, Project number HL 1998046.

Covance Laboratories (1998b) Mutagenicity test on Dequest 2006 Deflocculant and Sequestrant in the in vivo mouse micronucleus assay. Unpublished report for Solutia, Project number HK 1998047.

Davenport R. E. (2000) CEH Product Review, Chelating Agents, Chemical Economics Handbook, SRI International

DTI (1988) Home accident surveillance system including leisure activities. 22nd Annual Report, 1998 Data. Department of Trade and Industry, UK.

Draize J.H., Woodward G. and Calvery H.O. (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membrane. J. Pharmacol. Exptl. Therap. 82, 377-390.

ECB (European Chemicals Bureau) (2000) Iuclid CD-ROM, Year 2000 Edition

EG&G (1976a). Acute toxicity of FA-66257 (BN-76-94) to Bluegill (*Lepomis macrochirus*), Channel catfish (*Ictalurus punctatus*), Rainbow trout (*Salmo gairdneri*) and Midge (*Chironomus tentans*) larvae. Unpublished report for Monsanto Company, St Louis, MO. Solutia Report No: BN-76-0094

EG&G Bionomics (1976b). Accumulation and Elimination of 14C-residues by Carp (Cyprinus carpio) exposed to 14C-CP-42902. Unpublished report for Monsanto Company, St Louis, MO. Solutia Report No: BN-76-0285.

EG&G Bionomics (1976c), Accumulation and Elimination of 14C-Residues by Carp (Cyprinus carpio) exposed to 14C-CP-65184, EG&G Bionomics, Wareham, Massachusetts, September 1976, prepared by Stephen Ellis. Solutia report BN-76-0286

EG&G (1977a). Acute toxicity of CP 42902 to eastern oysters (*Crassostrea virginica*). Unpublished report for Monsanto Company, St Louis, MO. Solutia Report No: BN-76-0090C.

EG&G (1977b). Acute toxicity of CP 65184 to eastern oysters (*Crassostrea virginica*). Unpublished report for Monsanto Company, St Louis, MO. Solutia report No: BN 76-0091C.

EG&G (1977c). Acute toxicity of FA-66257 to oysters (*Crassostrea virginica*). Unpublished report for Monsanto Company, St Louis, MO. Solutia report No: BN 76-0094C.

ESIS (2003), Internet website of the ECB (European Chemicals Bureau)

Fischer (1991) Sorption of chelating agents (HEDP and NTA) onto mineral phases and sediments in aquatic model systems. Part I: sorption onto clay minerals. Chemosphere, **22**, 15

Fischer (1992) Sorption of chelating agents (HEDP and NTA) onto mineral phases and sediments in aquatic model systems. Part II: sorption onto sediments and sewage sludges.Chemosphere, **24**, 51

Fischer (1993) Distribution and elimination of HEDP in aquatic test systems.Water Research, **27**, 485

Gledhill W.E. and Feijtel T.C.J. (1992) "Environmental Properties and Safety Assessment of Organic Phosphonates Used for Detergents and Water Treatment Applications", in The Handbook of Environmental Chemistry, Volume 3 Part F, Ed. O. Hutzinger, © Springer-Verlag Berlin Heidelberg 1992, ISBN 3-540-53797-X

Gloxhuber C. Unpublished data from Henkel KGaA, Archive number 690076.

Grohmann A. and Horstmann B. (1988) UBA research report 102 063 22, UBA FB 89-018 “Investigation into the biodegradability of phosphonates”. Vom Wasser 70:163

Hanstveit A.O. and Oldersma H. (1996) Effect of diethylene triamine penta(methylenephosphonic acid) on the growth of green algae Selenastrum capricornutum in relation to the free ion concentration in algal growth medium with increased iron, cobalt, copper and zinc concentrations (OECD Guideline No. 201). TNO report V96.099, March 14, 1996.

Hazleton Laboratories Europe Ltd. (1979) Acute toxicity studies. Test article: sodium salt of diethylene triamine pentamethylenephosphonic acid: SPE 7910. Unpublished report.

Heaney R.P. and Saville M.D. (1976) Etridonate disodium in postmenopausal osteoporosis. Clin. Pharmacol. Therap. 20, 593-604.

Henkel (1972). Henkel KGaA, Biological Research and Product Safety/Ecology: unpublished results (Ttest substance No. 2387).

Henkel KGaA (1975) Unpublished data, Archive number TBD 750015.

Henkel KGaA (1982) Konzentrat SP Z1/03 A (CA2) Prüfung auf allergieausloesende Eigenschaften am Meerschweinchen Abschlussbericht. Unpublished report.

Henkel KGaA (1983). Henkel KGaA, Biological Research and Product Safety/Ecology: unpublished results (Test substance Nos. 6985, 6986)

Henkel KGaA (1983a) Unpublished data, Archive number 830008

Henkel KGaA (1983b) Unpublished data, Archive number 830091.

Henkel KGaA (1984a). Henkel KGaA, Biological Research and Product Safety/Ecology: unpublished results; internal report 1984/2247

Henkel KGaA (1984b) Aminotris (methylen-phosphonsaeure) (CA 3) Prüfung auf allergieausloesende Eigenschaften am Meerschweinchen Abschlussbericht. Unpublished report.

Henkel KGaA (a). Unpublished data, Archive number TBD 690076.

Henkel KGaA (b). Unpublished data, Archive number TBD 740082.

Henkel KGaA (c). Unpublished data, Archive number R 9500491.

Henkel KGaA (d). Unpublished data, Archive number TBD 770054.

Henkel KGaA (e). Unpublished data, Archive number TBD 820193.

Henkel KGaA (f). Unpublished data, Archive number TBD EX 00046.

Henkel KGaA (g). Unpublished archive data TBD 79001, TBD EX 00046, TBD 790021 quoted in ECB IULCID 2000 for Cas No. 7414-83-7.

Henkel KGaA (h). Unpublished archive data TBD 790050, TBD EX 00046 quoted in ECB IUCLID 2000 for Cas No. 7414-83-7.

HERA (2002) Volume data obtained from formulator companies

HERA (2003) Volume data obtained from formulator companies, and extrapolation to 100% market

Hotz K.J., Warren J.A., Kinnett M.L. and Wilson A.G.E. (1995) Study of the pharmacokinetics of absorption, tissue distribution and excretion of ATMP in Sprague-Dawley rats. Unpublished report for Ceregen, Project number MSL 14475.

Huntingdon Research Centre (1977) Potential tumorigenicity and toxicity to rats in repeated dietary administration for 104 weeks. Unpublished report for Henkel KGaA, Project number HKL/11-G/76894.

Huntingdon Research Centre (1979) Potential tumorigenicity and toxicity to rats in repeated dietary administration for 104 weeks. Unpublished report for Henkel KGaA, Project number HKL/11-G/7923.

Industrial Biotest Labs Inc. (1975) 90-day sub-acute oral toxicity study with Dequest 2010 in Beagle dogs. Unpublished report for Monsanto, Project number BTL-74-21.

Industrial Biotest Labs Inc. (1979) 90-day sub-acute oral toxicity study with Dequest 2010 in albino rats. Unpublished report for Monsanto, Project number BTL-74-20.

IUCLID (2000a) CD ROM CAS No: 6419-19-8. Henkel KgaA, unpublished results (Registry No. 2387).

IUCLID (2000b) CD ROM CAS No: 6419-19-8. Private communication to Monsanto.

Japan Oilstuff Inspectors Corporation (2001) Bacterial reverse mutation study of Dequest 2066. Unpublished report for Solutia, Study number B001156.

Jaworska J., Van Genderen-Takken H., Hanstveit A., Van de Plassche E and Feijtel T. (2002) “Environmental risk assessment of phosphonates, used in domestic alundry and cleaning agents in the Netherlands", Chemosphere, **47**, 655

Kastner, W. von and Gode, P. (1983). Method und Ergebnisse bei der toxicologischen und okologischen Prufung von Wasserbehandlungsmitteln. Z. Wasser Abwasser Forsch. 16 (2) 39-47.

Kay J.H. and Calandra J.C. (1962) Interpretation of eye irritation tests. J. Soc. Cosmet. Chem. 13, 281-289.

Landner L. and Walterson E. (1993) Phosphonates used for detergent applications. An environmental risk assessment for Sweden, Swedish Environmental Research Group, Stockholm

Larsson A. and Rohlin M. (1980) *In vivo* distribution of 14C-labeled ethylene-1-hydroxy-1,1-diphosphonate in normal and treated young rats. An autoradiographic and ultrastructural study. Toxicol. Appl. Pharmacol. 52, 391-399.

Litton Bionetics (1978) Mutagenicity evaluation of BIO-77-401 FA 65184 in the mouse lymphoma forward mutation assay. Unpublished report for Monsanto, Project number BIO-77-401.

Manley A. (1981) A toxicological investigation on a series of Briquest organophosponate compounds. M Sc thesis, University of Birmingham, UK

Michael P.R., Kaley R.G. (1979) Octanol/Water partition coefficients of Dequest phosphonates. Solutia report MO20020294

Michael P.R., Litschgi W.J., Yepez H., Kaeble E.F. and Kaley R.G. (1980) Sediment/Water partition Coefficients of Dequest Phosphonates. Solutia report MO20020301 (ES-79-SS-23)

Michael W.R., King W.R. and Wakim J.M. (1972) Metabolism of disodium ethane-1-hydroxy-1-disphosphonate (disodium etidronate) in the rat, rabbit, dog and monkey. Toxicol. Appl. Pharmacol. 21, 503-515.

Microbiologicals Associates (1983) L5178Y TK+/- mouse lymphoma mutagenesis assay utilizing a 0, 2 and 6 day expression period. Unpublished report for Monsanto, Project number MR-83-133.

Monkkonen J., Koponen H.M. and Ylitalo P. (1989) Comparison of the distribution of three bisphosphonates in mice. Pharmacol. Toxicol. 65, 294-298.

Monsanto Research Corporation (1972). Job Number: 03-000-760, 117031-K3. Project No: MO-76-347. Dequest - Algae Part II. Unpublished progress report for Monsanto Company, St Louis, MO.

Monsanto Research Corporation (1976). Job No: 45-000-760.40-6956000. Acute and chronic toxicity tests on *Daphnia* with DEQUEST Phosphonates. Unpublished report for Monsanto Company, St Louis, MO. Solutia report No: MO-77-0468-0469.

Monsanto (1977) Mutagenicity plate assay. Dequest 2010. Unpublished data, reported as internal memo.

Monsanto (1979a) Acute oral toxicity. Unpublished report, Project number ML-79-026.

Monsanto (1979b) Acute dermal toxicity. Unpublished report, Project number ML-79-0095.

Monsanto (1979c) Primary skin irritation. Unpublished report, Project number ML-79-026.

Monsanto (1979d) Acute eye irritation. Unpublished report, Project number ML-79-026.

Monsanto (1981a). Acute toxicity of Dequest 2000, 2010, 2041, 2051 and 2060 to *Daphnia magna*. Unpublished report for Monsanto Company, St Louis, MO. Solutia report No: MO-81-SS13.

Monsanto (1981b) Salmonella mutagenicity assay of Dequest 2000, CP 42902. Unpublished report for Monsanto, Project number DA-81-234.

Monsanto (1981c) Salmonella mutagenicity assay of Dequest 2060, CP 66291. Unpublished report, Project number DA-81-235.

Monsanto (1982) Teratology study of Dequest 2061 phosphonate (neutral sodium salt) in the Sprague-Dawley rat. Unpublished report, Project number ML-80-084.

Monsanto (1983) In vivo bone marrow chromosome aberration study in rats. Unpublished report, Project number HL-83-197.

Monsanto Research Corporation (1992). Project No: 43-000-755.10-168. Determination of toxic and/or lethal effects of phosphonates on *Selenastrum capricornutum* as influenced by media, pH and hardness. Unpublished report for Monsanto Company, St Louis, MO. Solutia report No: MO ESC9271.

Muller G, Steber J, Waldhoff H (1984) The effect of hydroxyethane diphosphonic acid on phosphate eluminations with FeCl3 and remobilization of heavy metals. Vom Wasser 63:63

Nixon G.A., Buehler E.V. and Newmann E.A. (1972) Preliminary safety assessment of disodium etidronate as an additive to experimental oral hygiene products. Toxicology and Applied Pharmacology 22, 661-671.

Nolen G.A. and Buehler E.V. (1971) The effects of disodiumetidronate on the reproductive functions and embryology of albino rats and New Zealand rabbits. Toxicology and Applied Pharmacology 18, 548-561.

Nowack B. (1997) Determination of phosphonates in natural waters by ion-pair high performance liquid chromatography. J. Chrom. A **773**, 139

Nowack B. (1998) Behavior of phosphonates in wastewater treatment plants of Switzerland., Water Research, 32, 1271-1279

Nowack B. and Saladin R. (2000) Phosphonate removal during water treatment by adsorption onto activated sludge and humic acids. 220th ACS national Meeting, Washington, DC, August 20-24, 2000; ACS symposium papers, Preprints of extended abstracts, **40**, 622

Nowack B. and Stone A.T. (2000) Degradation of nitrilotris(methylenephosphonic acid) and related (amino)phosphonate chelating agents in the presence of manganese and molecular oxygen. Environ. Sci. Technol., **34**, 4759

Nowack B. (2002) Aminopolyphosphonate removal during wastewater treatment. Water Res., **36**, 4636

OECD (1993) SIAR CAS No 6419-19-8

OECD (2000) Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, OECD SERIES ON TESTING AND ASSESSMENT Number 23

Owens J.L., Davis J.L., Guerry, D., Litschgi W.L., Yepez, H., Leahym K.M., Kaeble E.F. and Kaley R.G. II, (1980a) "Metals remobilisation by Dequest phosphonates", Monsanto unpublished report ES-80-SS-2 (MO20020301)

Owens J.L., Davis J.L. (1980b), "Determination of the stability constants of metal complexes of five phosphonic acids", Monsanto unpublished report ES-80-SS-22 (MO20020316)

Pharmakon Research International (1984) CHO/HGPRT mammalian cell forward gene mutation assay. Unpublished report for Monsanto, Project number PK-83-203.

Popov K., Rönkkömäki H. and. Lajunen L.H.J. (2001) Critical Evaluation Of Stability Constants Of Phosphonic Acids, Pure Appl. Chem., Vol. 73, No. 10, pp. 1641–1677, 2001.

Procter and Gamble (1976) Unpublished data, Report number ECM BTS 162, E-7006.

Procter and Gamble (1978) Unpublished data, Report number ECM BTS 476, E-8218, MVL YE 205.

Procter and Gamble (1982) Unpublished report: ECM BTS 481, E-8225, 81/PGN 372/142, 82/PGN 372/450 quoted in ECB IUCLID 2000 for Cas No. 22042-96-2.

Procter and Gamble (1985) Unpublished report: ECM BTS 181, E-7072, 77/PGN 223/410, 78 223/291, Schenk, 78 quoted in ECB IUCLID 2000 for Cas No. 22042-96-2.

Procter and Gamble (1987) Unpublished report ECM BTS 482, E-8225, 82/PGN 373/470, 83/PGN 373/255 quoted in ECB IUCLID 2000.

Procter and Gamble (1996) Unpublished data.

Recker R.R. and Saville P.D. (1973) Intestinal absorption of disodium ethane-1-hydroxy-1,1-diphosphonate (disodiumetidronate) using a deconvolution technique. Toxicol. Appl. Pharmacol. 24, 580-589.

Saeger, V.W., Tucker E.S., Frazier T. and Mieure J.P. (1977) Biodegradation of Dequest 2001 and 2011 in a soil environment. Monsanto unpublished report MO770622

Saeger V.W., Tucker E.S., Frazier T., Renaudette W.J., Yepez H. and Mieure J.P (1978) Environmental Fate testing of Dequest Phosphonates. Monsanto unpublished report AC-77-SS-13

Saeger V.W., (undated, believed to be 1979) Photochemical transformation of Dequest phosphonates in aqueous solution. Monsanto unpublished report ES-79-SS-21. Unpublished report from Monsanto Company, St Louis, MO

Saeger V.W., Busiere D, Linck C., Calvert C. and Gledhill W.E. (1979) Environmental Fate of Dequest Phosphonates in Natural Water Microcosms. Monsanto unpublished report ES-79-SS-20

Safepharm Laboratories Ltd. (1982a) An assessment of the acute oral toxicity of Briquest 301-32S to the rat. Unpublished report for Albright & Wilson Ltd., Experiment number 818/8208.

Safepharm Laboratories Ltd. (1982b) An assessment of the acute oral toxicity of Briquest 301-32S to the rat. Unpublished report for Albright & Wilson Ltd., Experiment number 91/8208.

Safepharm Laboratories Ltd. (1982c) An assessment of the acute oral toxicity of Briquest 543-33S to the rat. Unpublished report for Albright & Wilson Ltd., Experiment number 557/8208.

Safepharm Laboratories Ltd. (1982d) An assessment of acute oral toxicity of Briquest 543-33S to the rat. Unpublished report for Albright & Wilson Ltd., Experiment number 92/8208.

Safepharm Laboratories Ltd. (1982e) An assessment of the acute percutaneous toxicity of Briquest 301-32S to the rat. Unpublished report for Albright & Wilson Ltd., Experiment number 57/8208.

Safepharm Laboratories Ltd. (1982f) An assessment of the acute percutaneous toxicity of Briquest 543-33S to the rat. Unpublished report for Albright & Wilson Ltd., Experiment number 58/8208.

Safepharm Laboratories Ltd. (1982g) OECD skin irritation test: Determination of the degree of primary cutaneous irritation caused by Briquest 301-50A in the rabbit. Unpublished report for Albright & Wilson Ltd., Experiment number 724/8207.

Safepharm Laboratories Ltd. (1982h) OECD skin irritation test: Determination of the degree of primary cutaneous irritation cuased by Briquest 301-32S in the rabbit. Unpublished report for Albright & Wilson Ltd., Experiment number 723/8207.

Safepharm Laboratories Ltd. (1982i) OECD skin irritation test: Determination of the degree of primary cutaneous irritation caused by Briquest 543-50A in the rabbit. Unpublished report for Albright & Wilson Ltd., Experiment number 726/8207.

Safepharm Laboratories Ltd. (1982j) OECD skin irritation test: Determination of the degree of primary cutaneous irritation caused by Briquest 543-33S in the rabbit. Unpublished report, Experiment number 725/8207.

Safepharm Laboratories Ltd. (1982k) OECD eye irritation test: Determination of the degree of ocular irritation caused by Briquest 301-32S in the rabbit. Unpublished report for Albright & Wilson Ltd., Experiment number 678/8208.

Safepharm Laboratories Ltd. (1982l) OECD eye irritation test: Determination of the degree of ocular irritation caused by Briquest 543-33S in the rabbit. Unpublished report for Albright & Wilson Ltd., Experiment number 681/8208.

Safepharm Laboratories Ltd. (1982m) Ninety day oral toxicity study in the rat with dietary administration of Briquest 301-32S, Briquest 422-24S, Briquest 462-25S and Briquest 543-33S. Unpublished report for Albright & Wilson Ltd., Report number 625/8208.

SafePharm Laboratories report 71/75 (1992) J.W. Handley and C. Mead.

Safepharm Laboratories Ltd. (1995a) Acute dermal irritation test in the rabbit. Unpublished report for Albright & Wilson Ltd., Project number 71/355.

Safepharm Laboratories Ltd. (1995b) Acute eye irritation test in the rabbit. Unpublished report for Albright & Wilson Ltd., Project number 071/420.

Schaefer H. and Redelmeier T.E. (1996) Skin barrier: Principles of percutaneous absorption. S. Karger AG, P.O. Box, CH-4009 Basel (Switserland), ISBN 3-8055-6326-4.

Schmitz J. (1973) Tenside Detergents 10, 11-13.

Schowanek D. (1990) Degradation and Microbial Metabolism of Phosphonates, Ph.D Thesis, University of Ghent, Belgium

Schowanek D., McAvoy D., Versteeg D. and Hanstveit A.O. (1996) Effects of nutrient trace metal speciation on algal growth in the presence of the chelator [S,S]-EDDS. Aq. Toxicol., 36 pp 253 - 275; and references cited therein

Solutia (undated), "Dequest. Ingredients for the detergent industry", Technical Brochure

Springborn Laboratories Limited (1990). SLI Study No: 252-0690-6132-630. (ESI-50) - Sub-acute toxicity to earthworms (*Eisenia foetida*). Unpublished report for Mosanto Company, St Louis, MO. Solutia report No: SB-90-9519.

SRI International (1980a). Project No. 1741. Dequest 2000: 14-day algal bottle assay. unpublished bioassay data summary for Monsanto Company, St Louis, MO. Solutia report No: SR-81-247.

SRI International (1980b). Project No: 1741. Unpublished bioassay data summary for 14-day algal bottle assay with *Selenastrum capricornutum* on Dequest 2010 for Monsanto Company, St Louis, MO. Solutia report No: SR-81-0248.

SRI International (1980c). Project No. 1741.Unpublished bioassay data summary for 14-day algal bottle assay with *Selenastrum capricornutum* on Dequest 2060. Unpublished data summary for Monsanto Company, St Louis, MO. Solutia report No: SR-81-0251.

SRI International (1982a) An evaluation of mutagenic potential of 64-191-98 employing for the L5178Y TK+/- mouse lymphoma assay. Unpublished report for Monsanto, Project number SR-81-019.

SRI International (1982b) An evaluation of mutagenic potential of 158-27-08 employing the L5178Y TK +/- mouse lymphoma assay. Unpublished report for Monsanto, Project number SR-81-021.

SRI International (1983) Investigation of the stability of trifluorthymidine restistant colonies induced by compound L58-276-08 with metabolic activation. Unpublished report for Monsanto, Project number SR-82-249.

SRI International (1988) Mouse lymphoma cell mutagenesis assay (TK+/- and TK -/-) with neutralized Dequest 2000 using an exogenous activation system. Unpublished report for Monsanto, Project number SR-85-373.

Steber J. and Wierich P. (1986) Properties of hydroxyethane diphosphonate affecting its environmental fate: degradability, sludge adsorption, mobility in soils, and bioaccumulation., Chemosphere, **15**, 929

Steber J. and Wierich P. (1987) Properties of aminotris(methylenephosphonate) affecting its environmental fate: degradability, sludge adsorption, mobility in soils, and bioaccumulation. Chemosphere, **16**, 1323

Technical Guidance Document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances (1996). Ed. EC Commission, Luxemburg.

TNO (1997) Study No: IMW-95-0001-02. A 10 day acute toxicity test with Dequest 2066 and the sediment reworking species Corophium sp. (Amphipoda, Crustacea: proposed ISO/PARCOM method). Unpublished study for Monsanto Europe S.A., Lovain-la-Neuve, Belgium. TNO Report No: V95.754. Solutia report No: TN19970031.

Unilever Unpublished data, Report number SSM79.75.

Van de Plassche *et al.* (1998). Moret Ernst & Young Management Consultants, Second draft, Rep. No. 601503 013, p. 1-64.

Van Ravenzwaay, B. and Leibold, E. (2004) The significance of *in vitro* rat skin absorption studies to human risk assessment. Toxicology in vitro, 18:219-225.

Vermeire T.G. *et al.* (1993) Estimation of consumer exposure to chemicals: Application of simple models. Science Total. Environ. 136, 155-176.

Whittaker P. *et al.* (2001) Genotoxicity of iron chelators in L5178Y mouse lymphoma cells. Environ. Mol Mutagen. 38(4), 347-356.

Wildlife International Limited (1978a) Study No: 139-149. Acute oral LD50 - Mallard Duck. Dequest 2000 22.5% active acid. Unpublished report for Monsanto Company, St Louis, MO. Solutia report No: WL-78-0036.

Wildlife International Limited (1978b) Study No: 139-144. Acute oral LD50 - Bobwhite Quail. Dequest 2000 22.5% active acid. Unpublished final report for Monsanto Company. Solutia report No: WL-78-0031

Wildlife International Limited (1978c) Study No: 139-150. Acute oral LD50 - Mallard Duck DEQUEST 2010 11.3% active acid. Unpublished report for Monsanto Company, St Louis, MO. Solutia report No: WL-78-0037.

Wildlife International Limited (1978d) Project No: 139-145. Acute oral LD50 - Bobwhite Quail DEQUEST 2010 11.3% active acid. Unpublished report for Monsanto Company, St Louis, MO. Solutia report No: WL-78-0032.

Wildlife International Limited (1978e) Study No: 139-153. Acute oral LD50 - Mallard Duck Dequest 2060 18.1% active acid. Unpublished report for Monsanto Company, St Louis, MO. Project No. WL-78-40.

Wildlife International Limited (1978f) Study No: 139-148. Acute oral LD50 - Bobwhite Quail Dequest 2060 18.1% active acid. Final report for Monsanto Company, St Louis, MO.Project No: WL-78-35.

W/M (1997), "Phosphonates in domestic and cleaning agents. Environmental fate, behaviour, exotoxicology and environmental risk assessment", Nota 4, December 1997.

Younger Laboratories (1962) Certificate of analysis. Toxicological investigation of ID-2006. Unpublished report for Monsanto, Project number Y-62-105.

Younger Laboratories (1965a) Toxicological investigation of Dequest 2010. Unpublished report for Monsanto, Project number Y-65-74.

Younger Laboratories (1965b) Certificate of analysis. Toxicological investigation of Dequest 2016. Unpublished report for Monsanto, Report number Y-65-75.

Younger Laboratories (1967) Certificate of analysis. Toxicological investigation of Aminotri (methylphosphonic anhydride) SR450. (Handwritten anotation: "Dequest 2000"). Unpublished report for Monsanto, Project number Y-66-199.

Younger Laboratories (1971a) Toxicological investigation of diethylenetriamine penta(methylene phosphonic acid). Unpublished report for Monsanto, Project number Y-71-73.

Younger Laboratories (1971b) Toxicological investigation of Dequest 2060. Unpublished report for Monsanto, Project number Y-71-73.

Younger Laboratories (1977) Toxicity studies on FA-65184: Dequest 2010. Unpublished report for Monsanto, Project number Y-77-297.

Zeneca (1995). Study Reference No: AB0686/C. Briquest 301-50A: Toxicity to the marine amphipod, *Corophium volutator*. Unpublished summary report for Albright & Wilson Limited, Warley, UK. BEL Ref No: BLS 1971/B.

ZVEI and IKW (1999) Untersuchung und Bewertung der Hautverträglichkeit moderner Haushaltswaschverfahren. Industrieverband Körperpflege- und Waschmittel e.V. und Zentralverband der Elektro- und Elektronikindustrie, Frankfurt/Main.

7. Contributors to the report

This environmental part of this risk assessment was developed by experts from the following companies: Henkel, Rhodia and Solutia (Lead). Additional input was given by the HERA Environmental Task Force.

The human health part was developed by THE WEINBERG GROUP LLC on behalf of Solutia (Lead), Rhodia, Unilever and P&G. Additional input was given by the HERA Human Health Task Force.

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APPENDIX A

Substances covered under the HERA Risk Assessment on Phosphonates

The substances will be grouped by acid and treated as a category, unless there is evidence that there is evidence that a salt would have different properties for a given end-point than the acid (e.g. acute toxicity and irritation). Data will be reported as “active acid”.

The reasons for this are:

1) phosphonates are multifunctional acids, and will form a series of salts when the acid is being neutralised. Each of the salt (e.g. mono-, di-, tri-, tetra-, etc.) may have its own CAS number. In addition there are CAS numbers covering the salts in less specified way (e.g. x sodium salt).

2) Phosphonates are supplied either as the acid or as salts, often a sodium salt, but other salts are in use. Different suppliers do not always use the same CAS number for the same salt. Some may use a specific entry, some a general entry.

3) There are products with specific CAS numbers which are only provided to a single formulator. This may raise a issue of confidentiality, both for the producer and for the formulator.

In addition, the active phosphonate concentration of these products is often expressed as "active acid", but sometimes also in other ways. The proposal is to use "active acid" as the measure for expressing the amount of phosphonate.

CAS No	Common chemical name	AISE Applications	Other applications
ATMP and salts*		Hard surface cleaners	Water treatment scale inhibition
6419-19-8	Amino-tris(methylene phosphonic acid)		
See Annex	Sodium salts of ATMP		
See Annex	Other salts if used in the detergent industry		

HEDP and salts		Hard surface cleaners, laundry detergents, dish wash products	Water treatment scale inhibition Boiler water treatment
2809-21-4	Phosphonic acid, (1-hydroxyethylidene)di-		
See Annex	Sodium salts of HEDP		
See Annex	Other salts if used in the detergent industry		
DTPMP and salts		Laundry detergents, I&I cleaners	Water treatment scale inhibition Hydrogen peroxide stabilisation
15827-60-8	Diethylenetriamine penta(methylenephosphonic acid)		
See Annex	Sodium salts of DTPMP		
See Annex	Other salts if used in the detergent industry		

* the list of CAS numbers given is limited to the acid. This CAS number will be used to represent the whole group of an acid and its salts. CAS numbers for sodium, potassium and calcium salts are given in the annex.

APPENDIX B

Phosphonate CAS numbers on worldwide inventories

The following tables are extracted from a regulatory database and lists the CAS numbers identified for for sodium, potassium and calcium salt of the four major phosphonates used in the detergent industry. CAS numbers are reported if they are listed on any of the regulaotry substances inventories such as EINECS, TSCA and DSL.

Acid:	ATMP (CAS No 6419-19-8)
CAS No	**Name**
2235-43-0	Pentasodium aminotris(methylphosphonate)
3998-50-3	Dipotassium tetrahydrogen [nitrilotris(methylene)]trisphosphonate
4105-01-5	Disodium tetrahydrogen [nitrilotris(methylene)]trisphosphonate
6419-19-8	**Phosphonic acid, [nitrilotris(methylene)]tris-**
7611-50-9	Trisodium trihydrogen [nitrilotris(methylene)]trisphosphonate
15505-05-2	Hexasodium [nitrilotris(methylene)]trisphosphonate
20592-85-2	[Nitrilotris(methylene)]triphosphonic acid sodium salt
27794-93-0	Phosphonic acid, [nitrilotris(methylene)]tris-, potassium salt
40588-62-3	Hexapotassium [nitrilotris(methylene)]trisphosphonate
94021-23-5	Phosphoric acid, [nitrilotris(methylene)]tris-, tetrasodium salt
94021-24-6	Tripotassium trihydrogen [nitrilotris(methylene)]trisphosphonate
94021-25-7	Tetrapotassium dihydrogen [nitrilotris(methylene)]trisphosphonate
94021-26-8	Pentapotassium hydrogen [nitrilotris(methylene)]trisphosphonate

Phosphonate CAS numbers on worldwide inventories (cont.)

Acid:	HEDP (CAS No 2809-21-4)
CAS No	**Name**
2666-14-0	Trisodium hydrogen (1-hydroxyethylidene)bisphosphonate
2809-21-4	**(1-Hydroxyethylidene)bisphosphonic acid; Etidronic acid**
3794-83-0	(1-Hydroxyethylidene)bisphosphonic acid tetrasodium salt; Tetrasodium etidronate
7414-83-7	(1-Hydroxyethylidene)bisphosphonic acid disodium salt; Disodium etidronate
14860-53-8	Tetrapotassium (1-hydroxyethylidene)bisphosphonate
21089-06-5	Dipotassium dihydrogen (1-hydroxyethylidene)bisphosphonate
29329-71-3	(1-Hydroxyethylidene)bisphosphonic acid sodium salt
34318-59-7	Phosphonic acid, (1-hydroxyethylidene)bis-, calcium salt (1:1)
36669-85-9	Phosphonic acid, (1-hydroxyethylidene)bis-, calcium salt, dihydr
60376-08-1	Tripotassium hydrogen (1-hydroxyethylidene)bisphosphonate
67953-76-8	(1-Hydroxyethylidene)bisphosphonic acid, potassium salt

Phosphonate CAS numbers on worldwide inventories (cont.)

Acid:	DTPMP (CAS No 15827-60-8)
CAS No	**Name**
15827-60-8	**Phosphonic acid, [[(phosphonomethyl) imino] bis[2,1-ethanediylnitrilobis(methylene)]] tetrakis-**
22042-96-2	Phosphonic acid, [[(phosphonomethyl) imino] bis[2,1-ethanediylnitrilobis(methylene)]] tetrakis-, sodium salt
61792-09-4	Pentasodium pentahydrogen [[(phosphonatomethyl) imino] bis[ethane-2,1-diylnitrilobis(methylene)]] tetrakisphosphonate
68155-78-2	Heptasodium trihydrogen [[bis[2-[bis(phosphonatomethyl) amino] ethyl] amino] methyl] phosphonate
68400-03-3	Pentapotassium pentahydrogen [[bis[2-[bis(phosphonatomethyl) amino] ethyl] amino] methyl] phosphonate
84852-49-3	[[(Phosphonomethyl) imino] bis[ethylenenitrilobis(methylene)]] tetrakisphosphonic acid, potassium salt
93841-74-8	Hexasodium tetrahydrogen [[(phosphonatomethyl) imino] bis[ethane-2,1-diylnitrilobis(methylene)]] tetrakisphosphonate
93841-75-9	Nonasodium hydrogen [[(phosphonatomethyl) imino] bis[ethane-2,1-diylnitrilobis(methylene)]] tetrakisphosphonate
93841-76-0	Decasodium [[(phosphonatomethyl) imino] bis[ethane-2,1-diylnitrilobis(methylene)]] tetrakisphosphonate
93841-77-1	Hexapotassium tetrahydrogen [[(phosphonatomethyl) imino] bis[ethane-2,1-diylnitrilobis(methylene)]] tetrakisphosphonate
93919-68-7	Nonapotassium hydrogen [[(phosphonatomethyl) imino] bis[ethane-2,1-diylnitrilobis(methylene)]] tetrakisphosphonate
93919-69-8	Decapotassium [[(phosphonatomethyl) imino] bis[ethane-2,1-diylnitrilobis(methylene)]] tetrakisphosphonate