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DHTDMAC:- Aquatic and Terrestrial Hazard Assessment CAS No. 61789-80-8

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AQUATIC AND TERRESTRIAL HAZARD ASSESSMENT

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SECTION 1. SUMMARY AND CONCLUSIONS

Dihydrogenatedtallow dimethyl ammonium chloride (DHTDMAC) is almost exclusively used as a fabric softener in the household laundry rinsing process. Consequently the chemical is widely dispersed and may reach the aquatic and terrestrial environment following sewage treatment. This report refers to data related to the time period when the highest quantities (approx. 50,000T/year) of DHTDMAC were consumed in Europe. Since 1990 changes in the fabric softener formulations on the European market have resulted in a 80-90% decrease of consumption.

Due to its physical and chemical properties DHTDMAC adsorbs strongly onto surfaces and easily forms complexes with anionics such as alkylsuphonates or natural humic acids.

In standard laboratory tests, DHTDMAC is not readily biodegradable but in the presence of adapted biomass it shows total mineralization in up to 200 days.

In sewage treatment an average of 95% of DHTDMAC is removed from waste water. A large part of this removal is due to adsorption on sludge solids. Mass balance studies suggest that primary biodegradation in the biological step of sewage treatment may be significant.

In river water systems 70% of primary biodegradation was observed after 40 days. This result is consistent with mineralization studies performed without and with sediments showing respectively 10 and up to 65% conversion of ^{14}C α-alkylcarbon into $^{14}CO_2$.

Studies performed in soil indicated that 50-60% mineralization occurs in 120-430 days.

The highest environmental concentrations were found in waste water. The mean concentrations are normally around 1mg/l; exceptionally they may reach 4mg/l. Concentrations in effluents are generally in the order of 0.05mg/l.

The concentrations measured in rivers in different countries vary between 0.002 and 0.04mg/l depending on the sites, river size etc. In some low velocity systems e.g. canals and polders substantially polluted by other chemicals, concentrations of DHTDMAC up to 0.1mg/l have been measured.

The laboratory results on aquatic toxicity of DHTDMAC are highly dependent on the test conditions, sample preparation and presence of impurities.

The chemical appears to be very toxic especially to algae when tested only in laboratory water, whereas in natural waters, effects may be observed only at concentration 2-3 orders of magnitude higher. The lowest NOEC in laboratory water was observed with *Selenastrum capricornutum* (0,006mg/l). In treated sewage effluent diluted in river water it was 20.3mg/l. In the same effluent diluted river water the NOEC for the most sensitive species *Ceriodaphnia dubia* was 4.53mg/l.

Hazard assessment of chemicals of this type present particular difficulties because of their physico-chemical properties (insolubility/adsorption/complexation) which determine their bioavailability and thus the toxic effects. Hence methods have to be adopted which take proper account of the factors applying in practical situations. For DHTDMAC such approaches lead to PNEC/PEC ratios in the range of 8-450 using conservative approaches.

Terrestrial organisms, higher plants and earthworms exposed to DHTDMAC in sludge amended soil, even at levels grossly in excess of those expected during normal practice, do not exhibit adverse effects in toxicity tests. There was no evidence of bioaccumulation in toxicity tests to earthworms or of impairment of the general metabolism of soil as a result of contamination with DHTDMAC.

Based on the above considerations, it can be concluded that the environmental concentration of DHTDMAC discussed do not pose a hazard to aquatic and terrestrial systems.

SECTION 2. CHEMISTRY, PHYSICO-CHEMICAL PROPERTIES AND USES

2.1. INTRODUCTION

Dihydrogenatedtallow dimethyl ammonium chloride (DHTDMAC), also called Dihardenedtallow dimethyl ammonium chloride, is a commercial dialkyl dimethyl ammonium chloride in which the alkyl groups are derived from hardened tallow fatty acids. The product contains a mixture of dialkyl dimethyl quaternary ammonium compounds, with carbon chain lengths varying from C_{14} to C_{18}, the C_{16} and C_{18} being the most abundant. The alkyl chains are saturated, that is, they contain no chemical double bonds.

CAS No. 61789-80-8

CAS Name: Quaternary ammonium compounds, bis (hydrogenated tallow alkyl) dimethyl chlorides.

EINECS No. 2630902

Other acronyms often wrongly used for DHTDMAC are:

- DTDMAC (Ditallow dimethyl ammonium chloride); a mixture of quaternary ammonium compounds with the same chain length distribution as DHTDMAC but with some unsaturated bonds in the alkyl chains.

- DSDMAC (Distearyl dimethyl ammonium chloride) and DODMAC (Dioctadecyl dimethyl ammonium chloride; two names for a quaternary dialkyl dimethyl ammonium compound in which the alkyl chains are saturated C_{18} only. This substance is the major component of DHTDMAC.

These compounds are commercially available and have slightly different properties from each other and from DHTDMAC.

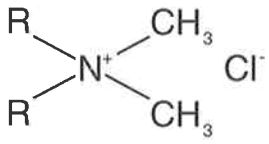
DHTDMAC contains two hydrophobic alkyl chains and a hydrophillic, positively charged, quaternary nitrogen; it has strong surface active properties. It has a low true aqueous solubility but is easily dispersed in water where it forms lamellar structures. These structures can be dispersed as single bilayer spherical vesicles or multilamellar liposomes. DHTDMAC adsorbs strongly to surfaces making them more hydrophobic. This is the basis for the technical applications of DHTDMAC of which rinse-added fabric softener has been traditionally the largest used sector.

2.1.1. Production Process

The product is manufactured from tallow fatty acids, which are reacted with ammonia and dehydrated to form an intermediate fatty acid nitrile. This is catalytically reduced with hydrogen to form a secondary amine, splitting off ammonia and saturating the double bonds in the alkyl chains. The secondary amine is methylated with formaldehyde under reductive conditions and then quarternized with methylchloride.

2.1.2. Chemical Structure and Composition

The structural formula is:



in which:

R = alkyl

The alkyl chain length distribution is:

C_{12}	max 2%
C_{14}	1-5%
C_{16}	25-35%
C_{18}	60-70%
C_{20}	max 2%

The average molecular weight ranges from 567 to 573.

The impurities in standard European products are:

- dihydrogenatedtallow methylamine and trihydrogenated-tallow amine together with the corresponding hydrochlorides, less than 2%;
- monohydrogenatedtallow trimethyl ammonium chloride, less than 4% of total solids;
- trihydrogenatedtallow methyl ammonium chloride, less than 4% of total solids.

2.1.3. Commercial DHTDMAC

Commercial quality DHTDMAC sold in Europe normally consists of 75-78% active substance and 10-15% isopropanol, the balance being water. It contains 0.1-0.3% sodium chloride. Its melting point is typically between 30 and 45°C.

2.2. PHYSICO-CHEMICAL PROPERTIES

Table 1 Physical Properties

	DHTDMAC, commercial grade	DHTDMAC, 100% active	DODMAC, DSDMAC, 100% active
CAS number	61789-80-8	61789-80-8	107-64-2
Mol. weight	565-570	565-573	586.48
Empirical formula			$C_{38}H_{80}HCl$
Active content, %	75-78	100	100
Iodine Colour No. (50 C°)	max 4		
Density, g/cm^3	0.86 (50)		0.84 (88)
Melting point, °C	30-45	50-60	72-122
Thermal stability, decomposes at, °C	~135	~135	135
pH value (10 g/l)	4-6		
Solubility at 25 °C,			
- water	<1µg/l	<1µg/l	<1 pg/l
- isopropanol	≥5%		
- ethanol	≥5%		
- acetone	slightly		
- chloroform	≥5%		
Adsorption, K_d l/kg	85,000		12,489 3,833 10,775

2.2.1. Solubility

Because commercial DHTDMAC is a mixture of chain length homologues with extremely low aqueous solubility, the determination of its true solubility is very difficult. This explains apparently contradictory results published in the scientific literature. Kunieda and Shinoda (1978) suggested that the aqueous solubility of pure DODMAC is about 3mg/l. Later, Evans and Needham (1987) estimated the solubility of a polar lipid of similar chain length

to be less than $5.7x10^{-10}$mg/l from observations made on a single vesicle in a mixture of known composition. Based on thermodynamical considerations, Laughlin *et al* (1990) concluded that the solubility of DODMAC is expected to be similar to that of this polar lipid (Laughlin *et al*, 1990). The true water solubility of DODMAC is therefore extremely low.

Aqueous solubility is affected by purity. Additionally, the shorter chain length homologues in commercial DHTDMAC are more soluble than DODMAC. Since DHTDMAC contains about 0.95 molar fraction units of two C_{16}-alkyl chains and higher homologues, this is not expected to have a significant effect on the solubility of commercial DHTDMAC. Therefore, for practical purposes, the "true" solubility of DHTDMAC can be safely considered not to exceed 10^{-3}mg/l.

2.2.2. Colloidal Properties

The colloidal properties of various dialkyl dimethyl ammonium compounds have been studied by Fontell *et al* (1986), Dubois and Zemb (1991) and Laughlin *et al* (1991). They showed that long chain dialkyl dimethyl ammonium salts exist in lamellar structures either crystalline or as liquid crystals, depending on temperature. These compounds form dispersions in water containing either unilamellar or mutilamellar particles such as vesicles. The size of the dispersed particles depends on the temperature, the sheer forces applied (i.e. the amount of energy put into the system) when making the dispersion and the presence of dispersants. High levels of energy (e.g. ultrasonification) produce small particle sizes.

The Krafft temperature is the temperature below which colloidal particles crystalline molecular aggregates and above which they are liquid crystals (Lindmann and Wennerstroem, 1980). The Krafft temperature of DODMAC has been determined by Laughlin *et al* (1990) to be 47.5°C. Dubois and Zemb (1991) also determined the Krafft temperature of Dialkyl dimethyl ammonium chlorides by measuring the melting point of the carbon chains in a dispersion using differential scanning calorimetry. They found 22°C for Didocecyl dimethyl ammonium chloride (DDdDMAC) and 37°C for Dihexadecyl dimethyl ammonium chloride (DHDMAC). Determination of the melting point of the carbon chains in a dispersion of DHTDMAC gave about 33-35°C (Berol Nobel, 1990).

Colloidal surfactant particles are crystalline at temperatures below the Krafft temperature. Therefore, the molecular movements in a system below its Krafft temperature are slow and the time needed to reach any physical-chemical equilibrium can be of the order of months. The actual time needed depends on factors like the size of the colloidal particles, the concentration of solid particles and the agitation in the system. Since the Krafft point

of DHTDMAC lies above room temperature, physical-chemical equilibria with DHTDMAC will be slow to reach. This explains why several authors regard the adsorption of DHTDMAC to particles to be essentially irreversible (see section 2.2.3) (Neufarth *et al*, 1978).

DHTDMAC can also form mixed aggregates with other surfactants. This is shown by the phase diagram of Sodium dodecyl sulphonate (SDS)/Didodecyl dimethyl ammonium bromide (DDdDMAB)/H_2O which shows the existence of an isotropic solution containing about 4% of DDdDMAB. DHTDMAC also can form mixed aggregates with monoalkyl trimethyl ammonium compounds (Lindman, 1992).

2.2.3. Adsorption and Desorption

Cationic surfactants, and especially the dialkyl dimethyl ammonium compounds, adsorb strongly onto surfaces. This has been studied by several authors using model systems. The adsorption of DHTDMAC in natural systems is more complex than in the models and depends on many factors such as:

- size of DHTDMAC aggregates,
- existence of mixed aggregates where DHTDMAC is associated with other surfactants,
- competitive adsorption with other surfactants,
- type of suspended particles present,
- concentration of particles,
- amount of surfactants in relation to particles,
- temperature.

Larson and Vashon (1983) studied structure/activity relationships for adsorption and biodegradation of a series of long chain quaternary ammonium compounds using radiolabelled DODMAC, hexadecyl trimethyl ammonium bromide (HTMAB) and octadecyl trimethyl ammonium chloride (OTMAC) and various sediments collected from the Ohio River and Rapid Creek together with a standard EPA sediment (EPA 18), using the method described by Games *et al* (1982).

The characteristics of the sediments which may influence adsorption were as follows:

Sediment	% Organic carbon	% Sand	% Clay	% Silt	CEC meq/100g	pH
EPA 18	0.7	34.6	39.5	25.8	15.4	7.8
Ohio River	2.0	28.0	39.7	32.4	18.4	7.1
Rapid Creek	3.5	0.2	2.4	97.4	15.7	

The adsorption results showed that the substances were extensively bound to all of the sediments:

SEDIMENT	COMPOUND	K_d (l/kg)
EPA 18	DODMAC	12,489
Ohio River	DODMAC	3,833
Rapid Creek	DODMAC	10,775

sediment water partitioning coefficient $K_d = C_{solids(mg/kg)} / C_{solution(mg/l)}$

Desorption experiments indicated that all compounds tested bind strongly to both organic and inorganic particulate matter (Larson and Vashon, 1983).

In another experiment DODMAC did not desorb from a 1g/l concentration dispersion of laponite (a synthetic sodium hectorite) in the presence of up to 5g/l sodium dodecyl sulphate (Capovilla *et al*, 1991).

Van Leeuwen *et al* (1990) calculated the K_d for DHTDMAC on suspended solids from data generated on samples from German rivers published by Klotz (1987a) on DHTDMAC and found it to be 8.5×10^4l/kg.

Neufahrt *et al* (1978) studied the adsorption of radiolabelled distearyldimethyl ammonium bromide (DSDMAB) on activated sludge. More than 50% was adsorbed within 20 minutes. Equilibrium was not even reached after 280 minutes when more than 90% had been adsorbed. The initial rate of DHTDMAC adsorption to particles is high but equilibrium is reached slowly. Additionally, the desorption process is extremely slow. Their observations are in agreement with the observations described in section 2.2.2 that the Krafft temperature of DHTDMAC is higher than room temperature, causing physical-chemical equilibria to be reached very slowly.

Therefore, for practical purposes adsorbed DHTDMAC can be considered as permanently bound to particles. This creates difficulties for the analysis of DHTDMAC in environmental samples. It requires for example very powerful extraction techniques to detect the adsorbed DHTDMAC.

2.2.4. Complexing with Anionics

DHTDMAC forms complexes with anionic compounds such as anionic surfactants e.g. linear alkyl benzene sulphonate (LAS) etc, and humic acids. The complexes are neutral and have a low solubility in water. Capovilla *et al* (1991) studied the complexing of DODMAC with sodium dodecyl sulphate. They found that the addition of sodium dodecyl sulphate to DODMAC mixed in water led to the formation of insoluble complexes. Unlike complexes with monoalkyl ammonium compounds, these complexes did not dissolve in the form of mixed micelles.

Buecking *et al* (1978) showed that DHTDMAC does not adsorb onto siliceous materials in the presence of a large excess of anionic or nonionic surfactant.

2.3. USES

About 90% of the total production of DHTDMAC is used as the active component in liquid formulations of fabric softeners designed to be added to the last rinse of a machine wash cycle. Due to market changes the quantity of DHTDMAC used in Europe as fabric softener has decreased by 80%-90% in the last 3 years. Rinse-added fabric softeners come in dilute and concentrated forms containing various levels of DHTDMAC. They are manufactured by dispersing DHTDMAC in hot water together with a dispersant and adding colour and perfume. Active content in normal fabric softeners is in the range of 4-6% and in concentrates 10-22%. Nonionic surfactants (ethoxylated alcohols) can be used as dispersing agents.

On dilution in the rinse water, more than 95% of the DHTDMAC adsorbs uniformly onto cloth. This is what gives the feeling of softness and may also improve the drying of the clothes and decrease wear. The adsorbed DHTDMAC remains on cloth until the next wash when the detergent used in the wash strips DHTDMAC from the cloth. Thus the DHTDMAC used will eventually end up in waste water, always associated with detergent surfactants by forming ion pairs with anionics surfactants during the wash (see section 2.2.4).

DHTDMAC is also used as conditioning agent in personal care products such as shampoos and hair conditioners and as emulsifier in lotions. The largest industrial application is in the production of organo-clays. These are used in the oil-industry as drilling muds and by the paint industry as rheological additives. Another industrial application is in sugar refining.

2.4. CONCLUSION

The aqueous solubility of DHTDMAC is very low (less than 10^{-3}mg/l). Above the solubility limit, it forms colloidal particles which are crystalline at room temperature and in the form of liquid crystals above the Krafft point (35°C). The size of the colloidal particles depends on the dispersion conditions.

DHTDMAC adsorbs strongly onto mineral and organic surfaces. Therefore, almost all of the DHTDMAC present in a natural system is adsorbed on particulate matter. Desorption is extremely slow being below the Krafft temperature. In summary DHTDMAC is easily dispersed in aqueous solutions which then may be dosed to standard laboratory tests. The interpretation of these tests will require caution since in realistic conditions, adsorption or chemical complexation of DHTDMAC will vary and may reduce substantially the bioavailability (see section 5). The concentration of 'free' DHTDMAC may be reduced by several orders of magnitude.

SECTION 3. ANALYTICAL METHODS

3.1. INTRODUCTION

Until the middle of the seventies DHTDMAC was analyzed using spectrophotometric methods such as the Disulfine Blue Active Substance (DBAS method), which measured the intensity of the blue complex formed between the cationic compound and the anionic Disulfine Blue dye. Another reagent used for colorimetric determination of DHTDMAC is the Dragendorff reagent. The disadvantage of these colorimetric methods is that they determine most of the synthetic and natural long chain quaternary compounds and amines which may be present in the sample. Therefore these methods are not specific for DHTDMAC and cannot be used to determine the exact concentration of DHTDMAC in environmental samples.

Analytical developments of the last fifteen years have resulted in chromatographic methods being used in combination with colorimetric determinations (Osburn, 1982) and more recently techniques employing High Performance Liquid Chromatography (HPLC) coupled with conductimetric detection or post column ion-pair extraction detection. These analytical methods are reliable and specific for DHTDMAC. Reviews of available analytical methods for cationic surfactants have been published by Kupfer (1982), Klotz (1987a, 1990a) and Matthijs and Hennes (1991).

The physico-chemical properties of DHTDMAC (water insolubility, strong adsorption onto solids and colloidal properties) make it necessary to take special care during sample collection, sample preparation and clean up.

3.1.1. Disulfine Blue Active Substance (DBAS)

Cationic surfactants are determined spectrophotometrically as coloured complexes with 9,10-Dimethoxyanthracene-2-sulphonate: Disulfine-blue. The method was developed to follow the primary biodegradation of cationic surfactants (Kupfer, 1976). However, disulfine blue forms complexes with many natural organic materials as well as with quaternary compounds and fatty amines (Klotz, 1984). Due to this non-specificity, the concentration of DHTDMAC in environmental samples are often significantly overestimated (Wee, 1984; Waters *et al*, 1992). Osburn (1982) improved the procedure by introducing further clean up steps and a thin layer chromatographic separation which then enabled semi-quantitative determinations of DHTDMAC in environmental samples to be made (see below).

3.1.2. Thin Layer Chromatography

The determination of cationic surfactants by means of thin layer chromatography (TLC) was first introduced by Michelsen (1978) and further developed by Osburn (1982). The use of a chromatographic separation step made it possible to determine DHTDMAC specifically. The quantification and sensitivity depends on the derivatisation procedure and detection principle used. A procedure using Dragendorff-derivatisation and UV detection has been published by Michelsen (1978) and others using Primulin "derivatisation" with fluorescence detection reported by Hohm (1990) and Klotz (1990b). The elution of DHTDMAC spots from the TLC layer permits further examination of the reported material by means of mass spectrometry (MS) and infrared spectroscopy (IR).

3.1.3. Mass Spectrometry (MS)

MS methods have not found wide application. Field Desorption Mass Spectrometry (FDMS) (Levsen and Schneider, 1987) and Fast Atom Bombardment Mass Spectrometry (FAB-MS) (Simms *et al*, 1988) have been mentioned as possible tools.

3.1.4. High Performance Liquid Chromatography (HPLC)

The selective and specific determination of DHTDMAC by means of HPLC with conductivity detection was introduced by Wee and Kennedy (1982) and Wee (1984). This technique is characterized by ion chromatography in a non-aqueous medium without ion-pairing and in the absence of a suppressor column. This is possible because the long chain quaternary compounds are soluble and ionized in organic solvents. Some separation principles of this system have been described by Klotz (1990a). Besides the most widely used conductivity detection, only the on-line post column ion-pair extraction detector is of importance (Brinkman *et al*, 1987; Schoester and Kloster, 1991). Compared with the conductivity detection, this technique has the advantage of allowing the use of gradient elution, which extends the possibilities to resolve DHTDMAC from other cationic substances and from interferences in complex matrices.

Further work has resulted in an analytical procedure combining the concentration/clean-up steps outlined by Osburn (1982) with the HPLC techniques described by Wee and Kennedy (1982) and Wee (1984). This procedure has been validated for analysis of DHTDMAC in different environmental matrices such as sludges, sediments, soils and aqueous samples in an interlaboratory exercise (Waters *et al*, 1992). This optimised procedure gives accurate and reproducible results for environmental samples and high recoveries of standard additions, generally > 90%. The detection limits for DHTDMAC in

environmental liquid and solid samples are estimated to be 2.5µg/l and 0.5mg/kg respectively.

3.1.5. Sample Preparation

The accurate determination of DHTDMAC at low concentrations in environmental samples is influenced by its low solubility in water and its tendency to adsorb strongly onto solids. Special precautions, as described by Waters *et al* (1992), have to be taken to avoid losses due to adsorption onto the surface of sample vessels used and to ensure that homogeneous samples are taken from samples containing both water phase and suspended solids.

3.1.6. Dissolved/Adsorbed DHTDMAC

In an environmental aqueous sample containing suspended solids, the total amount of DHTDMAC can be determined using the procedure discussed above. A complete redispersion of suspended solids in the environmental sample taken is important to ensure analysis of a homogeneous sample. Separating the suspended solids by centrifugation and analyzing the aqueous phase for DHTDMAC will give the "dissolved" DHTDMAC. The adsorbed part is calculated as the difference between total and dissolved. The accuracy of this method depends on the efficiency of isolating the suspended solids and colloidal particles from the solution. Difficulties in achieving a good separation can result in values of "dissolved" DHTDMAC being above the limit of solubility.

SECTION 4. ENVIRONMENTAL FATE

Since DHTDMAC is largely used in fabric softeners added in the rinse most is discharged into municipal sewers. The fate of DHTDMAC is discussed here in sections covering the laboratory investigations on biodegradation in the aquatic environment, the behaviour in municipal waste water treatment with an emphasis on activated sludge treatment, the fate in sludge, soils and sediments, the abiotic degradation, and the theory of the biodegradation pathway of quaternary alkyl ammonium compounds.

Section 7 will deal with the concentrations expected and encountered in waste water as well as in the environment at large.

4.1. LABORATORY TESTS ON THE BIODEGRADATION OF DHTDMAC

This section focuses on the inherent biodegradation properties of DHTDMAC. Biodegradation in sewage treatment is described in section 4.2.2.

4.1.1. Ready Biodegradability Tests

The laboratory results of low biomass biodegradation tests with DHTDMAC are summarised in Table 2.

Studies performed by Schoeberl *et al* (1988) without adaptation showed insignificant biodegradation. Baleux and Caumette (1977), studied the biodegradation of a number of different cationic surfactants using methods developed in their laboratory. They kept unadapted waste water containing DHTDMAC in a closed flask and monitored the substance by a colorimetric method. No primary degradation of DHTDMAC in this system had occurred after 28 days.

Larson and Vashon (1983), reported biodegradation screening tests carried out with pure DSDMAC. The inoculum for these tests had been subjected to an adaptation phase of a few days in a semi-continuous activated sludge test (SCAS) system at 20mg DSDMAC/l. They ran the screening tests with 1% by volume of sludge inoculum and found little degradation of 10mg/l and 20mg/l test substance (less than 5% CO_2 production after 33 days). This result suggests that a few days of adaptation is not enough to increase significantly the mineralization of DHTDMAC.

Van Ginkel and Stroo (1991) showed that the adsorption of test substance on silica gel does not influence biodegradation, at least up to 84 days. The test bottle without silica gel

Table 2 Summary table of various ready biodegradability tests on DHTDMAC

Test	Material	Conc. (mg/l)	Adaptated biomass	Duration (days)	Results (%)	Reference
CO_2	DSDMAC	? 10-20	No Yes	28 33	0 <5	Schoeberl *et al*, (1988) Larson and Vashon, (1983)
	DHTDMAC	5 16.8	No No No	49 26	2.8 4.8 2.3	Procter and Gamble, (1974-1986) Procter and Gamble, (1974-1986) Procter and Gamble, (1974-1986)
BOD	DHTMAC	5-20	No	5-20	negligible	Procter and Gamble, (1974-1986)
	DSDMAC	2 2 ? 1	No No No Yes	84 287 30 20	3 38 5 36	van Ginkel and Stroo, (1991) van Ginkel and Stroo, (1991) Schoeberl *et al*, (1988) Clancy and Tanner, (1991)
	DTDMAC	1	Yes	20	26	Clancy and Tanner, (1991)
	DHTDMAC	20 1 1 0.8 0.5 0.4	No Yes No No No No	28 20 20 20 20 20	0 19 8 12 17 35	Baleux and Caumette, (1977) Clancy and Tanner, (1991) Clancy and Tanner, (1991) Clancy and Tanner, (1991) Clancy and Tanner, (1991) Clancy and Tanner, (1991)
	DHTDMAA	1 1	Yes Yes	180 196	81 119	van Ginkel and Stroo, (1990) van Ginkel and Stroo, (1990)

was kept for 287 days and eventually yielded a 38% level of biodegradation. At that point, the biodegradation curve had not reached a plateau, suggesting that biodegradation was likely to continue. The time lag shown in this test also suggests that a long adaptation is needed before the biodegradation of DHTDMAC can become significant.

The modified closed bottle test reported by Clancy and Tanner (1991) on adapted inoculum (Polyseed, a mixture of 12 soil bacteria) showed up to 36% biodegradation after 20 days, confirming the importance of adaptation in the biodegradation of DHTDMAC.

BOD tests were performed by van Ginkel and Stroo (1990) with inoculum from sludge that had been adapted for several weeks to dihydogenatedtallow dimethyl ammonium acetate (DHTDMAA) in a semi-continuous activated sludge (SCAS) test. Dissolved oxygen was measured in 2 closed bottle tests using sludge taken from the SCAS unit at day 50 and day 84. The results showed a virtually complete (81% and 119%) degradation of DHTDMAC in less than 200 days. This compares with 38% degradation after 287 days in a non adapted system run in the same study. Although it is unclear whether adaptation

was greater at day 84 than at day 50. This test does demonstrate that DHTDMAC can mineralize completely with adequately adapted biomass.

These results of biodegradability tests with low biomass demonstrate a slow but extensive biodegradation of DHTDMAC when the inoculum is adapted. Given enough time, high levels of mineralization can be achieved. It is evident that with non adapted low levels of biomass, DHTDMAC biodegrades very slowly, if at all. Therefore, it cannot be considered as "readily biodegradable" according to the EC (1991). However, essentially complete biodegradation has been shown in approximately 200 days with adapted sludge.

4.1.2. Semi-Continuous Activated Sludge Tests (SCAS)

A SCAS test was performed on radiolabelled DSDMAC and DHTDMAC at 0.5mg/l. Using a 7 day adaptation period, 80 to 98% of the test substance remained on the sludge and no production of $^{14}CO_2$ could be detected (Hopping, 1975).

Van Ginkel and Stroo (1991) tested a concentration of 20mg/l total organic carbon (TOC) in the form of the ditallow dimethyl ammonium acetate. This test was run for 90 days and was followed by TOC measurements. In parallel to this SCAS, two closed bottle tests were performed on the same test material using the adapted sludge from the SCAS (see section 4.1.1). The SCAS test demonstrated 100% removal of the test substance over 84 days confirming the high degree of removal of DHTDMAC from waste water.

In Zahn-Wellens tests (OECD 302 B) dissolved organic carbon (DOC) removal was reported to be more than 80% after 3 hours and 100% after 1 day in a first test. In a second test, DOC removal was more than 50% after 3 hours, 80% after 5 days and 93% after 15 days (Hoechst, 1991). This second test indicates the absence of soluble biodegradation intermediates in the effluents of biological systems treating sewage containing DHTDMAC.

These tests confirm the high degree of removal of DHTDMAC from waste water and the absence of soluble biodegraded intermediates.

4.1.3. Batch Activated Sludge Tests

The data from these tests are summarised in Table 3.

Brown (1975), ran batch activated sludge tests on radiolabelled DSDMAC and DHTDMAC at 0.5mg/l. The positions of the ^{14}C label were methyl for DHTDMAC and alpha alkyl for DSDMAC. The tests were under three conditions: the quaternary ammonium compound

Table 3 Summary of the Batch Activated Sludge tests run on DHTDMAC

Material	Duration (days)	Conc. (mg/l)	MLSS (g/l)	Label	Prim. biod. (%)	CO_2 (%)	Reference
DHTDMAC	240	0.5	-	Me	-	89.8	Brown, 1975
DSDMAC	240	0.5	-	Alpha	-	31.7	
DHTDMAC	120	0.5[1]	-	Me	-	75.9	
DSDMAC	120	0.5[1]	-	Alpha	-	10.8	
DHTDMAC	240	0.5[2]	-	Me	-	73.6	
DSDMAC	240	0.5[2]	-	Alpha	-	67.7	
DHTDMAC	240	0.5	A.B.[3]	Me	-	74.0	
DSDMAC	240	0.5	A.B.[3]	Alpha	-	60.2	
DHTDMAC	240	0.5[2]	A.B.[3]	Me	-	73.6	
DADMAC	240	0.5[2]	A.B.[3]	Alpha	-	65.6	
DSDMAC	34	2	1	Alpha	50	53.1	Holman, 1978
DSDMAC	39	2.1	1.7-6.7	Alpha	61	31[4]	Sullivan, 1983
DSDMAC	39	2.1	1.7-6.7	Methyl	72	40[4]	
DSDMAC	39	2.1	1.7-6.7	Uniform	59	22[4]	
DSDMAC	39	2.1	1.1-2.0	Methyl	77	53[4]	
DSDMAC	39	2.1	1.1-2.0	Uniform	81	31[4]	

1 With 0.29 mg/l LAS
2 With 5 mg/l LAS
3 Adapted biomass
4 The capture of CO_2 was imperfect

alone, with a stoichiometric quantity of LAS (0.29mg/l) and with an excess of LAS (5mg/l). The tests with a stoichiometric dosing of LAS were terminated at 120 days. The other tests ran for 240 days. Mineralization was slow but reached higher levels with the methyl label (levels up to 90%) than with the alpha alkyl label. Adaptation of the biomass improved only the mineralization of alpha alkyl labelled DSDMAC tested alone (from 31.7% to 60.2% $^{14}CO_2$ production after 240 days). The presence of LAS did not affect the mineralization of DHTDMAC.

A batch activated sludge test carried out by Holman (1978) showed that alpha alkyl ^{14}C radiolabelled DSDMAC degraded to a significant extent. The test was run in unadapted domestic activated sludge (1,000mg mixed liquor suspended solids/l) for 34 days. The nominal test concentration was 2mg/l. Synthetic sewage was regularly added to simulate the organic loading of a real activated sludge plant. 53.1% CO_2 production was observed at the end of the test and no radiolabelled intermediate was detected by a combination of DBAS, radioactivity counting and TLC analytical techniques, suggesting that primary biodegradation is the limiting factor in the ultimate biodegradation of DHTDMAC.

Sullivan (1983) ran five tests using 2.1mg DSDMAC/l radiolabelled in three different positions: methyl, alpha alkyl and uniformly alkyl and in association with a 2:1 molar

excess of LAS. The units were run in batch mode but fed synthetic sewage daily to simulate the organic loadings found in conventional (0.07 - 0.31g BOD/g MLVSS.day) and extended aeration (0.04 - 0.10g BOD/g MLVSS.day) treatment. Each type of radiolabel was run in the conventional loading mode but only the methyl and uniform labels were run under the extended aeration mode. The levels of suspended solids increased by a factor of almost 5 in the conventional loaded units but by only a factor of 2 in the extended aeration type loading. Primary biodegradation appeared to be more extensive than mineralization but it was noted that the CO_2 traps did not capture the gas optimally. Because of the radiolabel, most of the remaining DSDMAC could be detected associated with the biomass (adsorbed or incorporated). The fraction associated with the biomass decreased from about 50% at the beginning of the test to about 30% after 39 days, also suggesting a degree of adaptation.

These results indicate that true biodegradation takes place in activated sludge systems. The level of biodegradation will depend on biomass adaptation and operating conditions.

4.1.4. River Water Die-Away Tests

Larson and Vashon (1983), ran tests in two types of river water taken downstream from discharges from activated sludge waste water treatment plants. They used alkyl radiolabelled DSDMAC at 0.05 and 0.50mg/l. Tests were run with and without river sediments. Without sediments (less than 25mg/l) degradation was slow and reached about 10% with 0.05mg/l test substance and 20% with 0.5mg/l test substance after 63 days. In the presence of 5,000mg adapted sediments/l, biodegradation was much faster and the production of $^{14}CO_2$ exceeded 65% after 9 weeks. In both cases, the biodegradation kinetics were pseudo first order. The estimated mineralization half-life for DSDMAC in the test with 5,000mg sediments/l was 4.9 days.

Larson (1983) also gave the results from a similar test in river water containing about 50mg/l river sediments. The level of biodegradation appeared to be intermediate between a test run without sediments and a test run with 5g sediments/l, adding support to the theory that adapted sediments enhance the biodegradation of quaternary ammonium compounds.

Schneider and Levsen (1987) ran a river water die-away test with DHTDMAC and monitored the test by field desorption mass spectrometry. They observed 70% primary biodegradation in 40 days with an initial concentration of 8.25mg/l. The 30% remaining did not degrade in up to 70 days. In a second test at 0.5mg/l initial concentration, 75% primary biodegradation was observed in 40 days and the remaining 25% remained until

the end of the test, at day 55. The compounds monitored were Dicetyl dimethyl ammonium chloride, Cetyl stearyl dimethyl ammonium chloride and Distearyl dimethyl ammonium chloride.

The results of biodegradation in river water die-away tests are summarised in Table 4.

Table 4 Biodegradation results in river water die-away tests

Material	Duration (days)	Conc. (mg/l)	End point	biodegradation (%)	Reference
DSDMAC	63	0.05	$^{14}CO_2$	10	Larson and Vashon (1983)
DSDMAC	63	0.50	$^{14}CO_2$	20	
DSDMAC	63	0.05	$^{14}CO_2$	67[1]	
DSDMAC	63	0.50	$^{14}CO_2$	67[1]	
DSDMAC	33	0.50	$^{14}CO_2$	43[2]	Larson (1983)
DTDMAC	70	8.25	Mass spec.	70	Schneider and Levsen (1987)
DTDMAC	55	0.5	Mass spec.	75	

1 Test run with 5g/l river sediments
2 Test run with 50mg/l river sediments

Thus, in river water die-away tests, significant levels of biodegradation may be achieved. Factors like biomass adaptation, presence of suspended solids and dosing of the test substance influenced the test results. Overall, the biodegradation in this type of system may be significant when measured in conditions of adaptation but the rates appear generally slow.

4.1.5. Conclusions

DHTDMAC is not readily biodegradable as defined by the EC. However, DHTDMAC is amenable to complete mineralization. Biodegradation of DHTDMAC in batch conditions (Sturm test, batch activated sludge test, river water die-away) appears to be slow but adaptation of the microbial biomass greatly increases the biodegradation rate. The presence of sediments to which DHTDMAC can adsorb does not hinder biodegradation and may even stimulate it. SCAS tests confirm the DHTDMAC can be removed from waste water.

4.2. FATE OF DHTDMAC IN WASTE WATER TREATMENT

In a vast majority of cases, municipal sewage consists of primary treatment (settling), and of a secondary treatment (biological step). In this biological step (activated sludge or tricking

filters systems) bacterial mass is maintained to biodegrade organic materials. Therefore, in practice, removal in a plant is by a combination of the primary and secondary removals. This primary stage is considered first and then data available on the combined primary/secondary treatment are discussed. The available information is summarised in Figure 1. (see page 27)

4.2.1. Primary Treatment

Primary treatment is the elimination of the settleable fraction of waste water. This is mainly grit removal, sometimes grease removal by flotation and primary settling. Primary settling is carried out in large basins with water retention times generally in the range of 1.5 to 2.5h (Metcalf and Eddy, 1979). Because DHTDMAC adsorbs readily to particles, a significant fraction is expected to be removed by association with the settleable solids. It does not provide good conditions for biodegradation because of the lack of active biomass, and low levels of dissolved oxygen.

Only few data are available on the removal of DHTDMAC from waste water in primary settling. Monitoring studies in two German waste water treatment plants (Matthijs *et al*, 1992) showed a removal of 38.7% and 23.0% at two different periods in one plant and 50.5% in the second plant. In all cases, essentially all of the DHTDMAC removed was found in the sludge.

Another study (Topping and Waters, 1982) monitored DHTDMAC in one British and one German waste water treatment plant. Removal of DHTDMAC was about 40% in the British plant and about 20% in the German plant. The British raw sewage contained a higher level of suspended solids and the German plant was acknowledged to receive a particularly weak sewage. The waste activated sludge was returned to the primary settler in the British plant; it is unclear whether this was the case in the German plant.

Thus, primary removal of DHTDMAC appears to be linked to the amount of solids eliminated in the primary stage and no degradation of DHTDMAC occurs. Removal varies from 25% to 50% according to the conditions and what is eliminated from the waste water flow is found in the sludge.

4.2.2. Secondary Treatment (Activated Sludge)

Many authors have published data on the secondary removal of DHTDMAC. Laboratory simulation tests can reproduce the secondary stage of activated sludge treatment; these are called continuous activated sludge (CAS) tests, the results of which give a good

indication of what happens in a waste water treatment plant. To understand the data it is necessary to recognise that there are three main ways to monitor a continuous activated sludge test:

- **Removal of parent material:** this is determined by measuring concentrations of pollutant in influent and effluent waters using a specific analytical technique. This measures combined removal by adsorption and primary biodegradation. If the sludge is analyzed and all flow rates are known, it is possible to follow the fluxes of parent material and therefore distinguish adsorption from primary degradation. Determination of removal provides no indication of the extent of mineralization, only of primary biodegradation.

- **Removal of DOC:** this is estimated by monitoring dissolved organic carbon (DOC) levels in influent and effluent waters and comparing with a control. With this analytical method, enough carbon must be available from the test substance to be able to detect it above the carbon from the feed of the test unit. This often leads to test concentrations far above the levels expected in the environment and may show unrealistic toxic effects. As its name indicates, this method only measures dissolved organic carbon which limits its applicability to soluble materials. In the case of insoluble compounds, this technique can only indicate the release of water soluble biodegradation intermediates. Applied to DHTDMAC, this DOC technique can thus only reveal the presence of soluble intermediates but is not adequate to measure the parent compound.

- **Use of ^{14}C radiolabelled compounds:** This method demonstrates the fate of the radiolabelled carbon during the treatment. Determination of trapped radiolabelled CO_2 is an efficient technique to determine the fraction of parent ^{14}C converted into CO_2 and for performing a mass balance. It does not allow parent compound and breakdown products to be distinguished but may be used to follow the carbon associated with the biomass. The radioactivity found in the sludge or in the effluent is not necessarily that of the intact parent compound. When coupled to thin layer chromatography (TLC), this method can give information on the identity of the intermediates deriving from the parent material in activated sludge treatment.

Laboratory Simulation The combination of two or more of the above techniques can provide a precise understanding of the fate of a chemical in waste water treatment. For example, analytical methods coupled with the use of a radiotracer may permit detection of

the presence of biodegradation intermediates. A knowledge of the method used to monitor the test is essential for the understanding of test results.

Janicke and Hilge (1979) studied the fate of DHTDMAC complexed with LAS following an OECD confirmatory test and analytical monitoring by colorimetry (DBAS, see section 3). Two replicates with an influent concentration of 16.4mg DHTDMAC/l and 10mg/l LAS showed 96.5% and 95.5% removal respectively relative to the filtered effluent.

Gerike *et al* (1978) performed OECD confirmatory tests 303A on DDdDMAC at 5mg/l and DHTDMAC at 10mg/l. Excess LAS was present in all cases. Removal was measured as 94.8% and 94.5% respectively. Another OECD confirmatory test using 5mg DHTDMAC/l gave removals ranging from 58.6% to 98.2%. With DHTDMAC at 20mg/l in the presence of 20mg/l LAS, DOC removal in two tests was 108 ±9% (versus a 20mg/l LAS control) and 83 ±3% (versus a "blank" control). These DOC measures indicate that no significant levels of soluble intermediates are released.

In an OECD confirmatory test (303A) performed by ATOCHEM (1990a), DOC removal of DHTDMAC averaged 98.4% over 28 days and analytical monitoring by HPLC showed 95.6% elimination of the parent material. Since both DOC readings and specific analytical monitoring provide similar results, this provides evidence of high removal of DHTDMAC and of absence of water soluble biodegraded intermediates.

May and Neufahrt (1976) ran two continuous activated sludge tests monitored with DBAS. The first used a DHTDMAC concentration of 2.78mg/l with 8mg/l paraffin sulphonate. The removal was 91%. The second test had 1.75mg/l DHTDMAC with a mixture of LAS, alkyl sulphonate and alcohol ethoxylate. The removal was 93%.

Täuber (1988) followed the cumulative elimination of DSDMAC in continuous activated sludge systems both with and without Secondary Alkyl Sulphonate (SAS). Removal of DSDMAC appears to have reached 95% when alone and 92% when associated with the anionic surfactant. The test was monitored with DBAS.

Ruffo *et al* (1989) ran a series of continuous activated sludge tests (OECD confirmatory test 303A) on DHTDMAC alone and mixed with C_{12} LAS. DHTDMAC removal monitored by DBAS was 91.3% and 96.2% in two tests run for 28 days without adaptation and 42 days with adaptation of the biomass, respectively. At 28 days, the test with adaptation yielded 96.5% removal compared to 91.3% without adaptation. Also, in the test without adaptation, removal increased from about 80% at the start to more than 91% after 28 days while in the test with adaptation, the removal remained between 96% and 97% for

the whole test duration. This shows that adaptation occurs and may be an important factor in the environmental fate of DHTDMAC. In the tests with LAS, DHTDMAC removal monitored by DBAS was 80.7% after 28 days without adaptation and 93.5% after 42 days with adaptation. Again, after 28 days, removal in the test with adaptation was higher than in the test without (93.4% versus 80.7%) and removal increased in the test without prior adaptation while it remained constant in the test with adaptation. Monitoring of DHTDMAC in the sludge showed that accumulation occurred in the first three weeks of the tests with adaptation but decreased after that. The same was seen in the non-adapted systems. This was most probably due to increased biodegradation as biomass adaptation improved. The analytical method used was DBAS (Kupfer and Waters, 1976).

Shimp (1992) monitored the removal of alpha alkyl labelled DSDMAC at 0.01mg/l in a continuous activated sludge test (CAS). Partitioning of DSDMAC between the suspended solids and the water was studied together with the radiolabel removal of DSDMAC and its mineralization. On average, 71.2% of the ^{14}C was adsorbed onto the solids, 0.6% was in the liquid and 13.9% in the effluent during the 5 day test. The radiolabelled CO_2 was not trapped but an 11% mineralization of DSDMAC was calculated. It should be noted that sludge wastage in the CAS unit was stopped during the test period leading to an increase in solids from about 2,000mg/l at the beginning of the test period to about 5,000mg/l at the end.

Rottiers and Papez (1987), ran two CAS tests under different conditions. One influent was spiked with 5mg/l DHTDMAC, the other with 1mg/l DHTDMAC and 2mg/l LAS. They obtained 88% removal at 5mg/l and 93% removal at 1mg/l. The concentrations of DHTDMAC found on sludge were about 50mg/g and 6mg/g respectively. The tests were monitored with HPLC.

HPLC and DBAS/TLC monitoring studies on DHTDMAC were also performed in activated sludge treatment plants in Germany and Great Britain (Matthijs *et al*, 1992; Topping and Waters, 1982). The removal results for the aeration stage of the monitored plants varies between 88.0% and 94.8%.

The results of all these studies on the secondary removal of DHTDMAC in activated sludge are summarised in Table 5.

Monitoring In Municipal Activated Sludge Waste water Treatment The monitoring studies also examined the overall (combined primary + secondary) removal in full scale waste water treatment plants. The earliest (Topping and Waters, 1982) were carried out using a semi-quantitative analytical method - DBAS followed by thin layer chromatography

Table 5 Elimination of DHTDMAC in the secondary removal (aeration stage) of activated sludge treatment

Test method	Conc. (mg/l)	With	Analytical	Removal (%)	Reference
OECD conf.	16.4	10 mg/l LAS	DBAS	96.5	Janicke and Hilge, (1979)
OECD conf.	16.4	10 mg/l LAS	DBAS	95.5	Janicke and Hilge, (1979)
OECD conf.	10	LAS	DBAS	94.5	Gerike *et al*, (1978)
OECD conf.	5	10 mg/l LAS	DBAS	58.6-98.2	Gerike *et al*, (1978)
OECD conf.	28.8		DOC	98.4	Atochem (1990a)
			HPLC	95.1	Atochem (1990a)
OECD conf.	10		DBAS	91.3	Ruffo *et al*, (1989)
OECD conf.	10		DBAS	96.2	Ruffo *et al*, (1989)
OECD conf.	10	LAS	DBAS	80.7	Ruffo *et al*, (1989)
OECD conf.	10	LAS	DBAS	93.5	Ruffo *et al*, (1989)
Coupled units	20	20 mg/l LAS	DOC	108±9	Gerike *et al*, (1978)
Coupled units	20	20 mg/l LAS	DOC	83±3	Gerike *et al*, (1978)
CAS	N.A.		DBAS	~95	Täuber (1988)
CAS	N.A.	2M SAS	DBAS	~92	Täuber (1988)
CAS	2.78	8 mg/l Alk. Sulpho	BAS	91	May and Neufahrt, (1976)
CAS	1.75	9 mg/l LAS/AS/AE	DBAS	93	May and Neufahrt, (1976)
CAS	0.01		Radiolabel	86.1	Shimp, (1992)
CAS	5.0		HPLC	88	Rottiers and Papez, (1987)
CAS	1.0	2 mg/l LAS	HPLC	93	Rottiers and Papez, (1987)
Monitoring	±1		HPLC	91.3	Matthijs *et al*, (1992)
Monitoring	±1		HPLC	94.8	Matthijs *et al*, (1992)
Monitoring	1.4		HPLC	95.3	Matthijs *et al*, (1992)
Monitoring	1.38		DBAS/TLC	88.0	Topping and Waters, (1982)
Monitoring	1.57		DBAS/TLC	91.8	Topping and Waters, (1982)

(TLC). More recent studies by Matthijs *et al* (1992) and by Versteeg *et al* (1992) have used HPLC.

The total removal of DHTDMAC in two activated sludge plants, one in Germany (Duelmen) and one in Great Britain (Alderley Edge), was 94% and 92.8% respectively as monitored with DBAS/TLC. More than 98% DHTDMAC removal was reported in the monitoring of two other activated sludge plants in Germany (Topping and Waters, 1982).

A study at two plants using two sampling campaigns at Lüdinghausen and one at Waltrop showed that the overall removals analyzed by HPLC were 94.6%, 96.0% and 97.7% respectively (Matthijs *et al*, 1992).

HPLC analysis in 1987 on a plant treating waste from a detergent manufacturing plant and domestic waste in Lima, Ohio showed 98% removal by activated sludge treatment, confirming the values obtained in previous investigations (Versteeg *et al*, 1992).

Monitoring studies in the US between 1975 and 1986 demonstrated total removals of 89% to 98% DHTDMAC (Versteeg *et al*, 1992). Only grab samples were analyzed; results can only be considered approximate although they do confirm data from other sources.

The results of these monitoring studies are summarised in Table 6

Table 6 Monitoring results on the fate of DHTDMAC in full scale activated sludge plants.

Plant	Raw sewage conc. (mg/l)	Analytical	Primary (%)	Secondary removal (%)	Total removal (%)	References
Lüdinghausen 1	1	HPLC	38.7	91.3	94.6	Matthijs *et al*, (1992)
Lüdinghausen 2	1	HPLC	23.0	94.8	96.0	Matthijs *et al*, (1992)
Waltrop	1.4	HPLC	50.5	95.3	97.7	Matthijs *et al*, (1992)
Duelmen	1.57	DBAS/TLC	26.6	88.0	94	Topping & Waters, (1982)
Alderley	1.38	DBAS/TLC	40.2	91.8	92.8	Topping & Waters, (1982)
Germany	3.07	DBAS			98.2	Topping & Waters, (1982)
Germany	4.2	DBAS			98.9	Topping & Waters, (1982)
Lima	4.4	HPLC			98	Versteeg *et al*, (1992)
Bellevue	0.94	HPLC			93	Versteeg *et al*, (1992)
Lincoln	0.36	HPLC	28.0	87.5	91	Versteeg *et al*, (1992)
Kenton	1.09				98	Versteeg *et al*, (1992)
Hornell[1]	0.51				89	Versteeg *et al*, (1992)
Bilthoven[1]	0.56				92	Versteeg *et al*, (1992)

1 Monitoring work incomplete, only grab samples were analysed.
Note: Wherever possible, primary and secondary removals were separated to give the relative importance of each stage in the overall removal. In some cases, only the overall removal was monitored.

4.2.3. Relative Importance of Biodegradation and Adsorption in Removal by Activated Sludge Treatment

As was seen in section 4.2.1, removal in first settling occurs by adsorption to the primary sludge. At this stage biodegradation is negligible.

Biodegradation of DHTDMAC occurs in activated sludge treatment. Only limited data are available which distinguish between removal by biodegradation and removal by adsorption. Depending on the test system monitored the mass balances for the aeration stage vary between 9.5% adsorption/90.5% biodegradation and 71.2% adsorption/10.8% biodegradation.

The studies by Matthijs *et al* (1992) at Lüdinghausen and Waltrop monitored by HPLC are the main source of information on the relative importance of biodegradation and adsorption in the aeration stage of activated sludge treatment. Täuber (1988) also derived data monitored by DBAS in a CAS system with pure DSDMAC and a 1:2 molar DSDMAC/SAS mixture line. Using radioactive counts, Shimp (1992) calculated biodegradation as mineralization from the difference between 100% radioactivity and the counts measured in water and in sludge, assuming that this difference represents $^{14}CO_2$ formation. $^{14}CO_2$ was not measured directly. Rottiers and Papez (1987) did not use a radiolabel but monitored these tests with HPLC and calculated primary biodegradation.

They found 56% degradation at 5mg/l DHTDMAC and about 30% at 1mg/l. These studies are summarised in Table 7.

Table 7 Relative importance of adsorption and degradation in the removal of DHTDMAC in the aeration stage of activated sludge waste water treatment.

(Summary of the findings - mass balances for the aeration stage only)

Test system	Adsorption	Degradation	SRT	Reference
Lüdinghausen I	33.5%	66.5%	10 days	Matthijs *et al*, (1992)
Lüdinghausen II	33.9%	66.1%	13 days	Matthijs *et al*, (1992)
Waltrop	9.5%	90.5%	7.5 days	Matthijs *et al*, (1992)
CAS	44%	56%	5.5 days	Rottiers and Papez, (1987)
CAS DHTDMAC + LAS	70%	30%	5.5 days	Rottiers and Papez, (1987)
CAS DSTDMAC	20.5%	79.5%	N.A.[1]	Täuber (1988)
CAS DSTDMAC + SAS	18.2%	81.5%	N.A.[1]	Täuber (1988)
CAS	71.2%	10.8%	N.W.[2]	Shimp (1992)

1 Not Available.
2 No sludge wastage

In these studies, the flows of water and solids were determined as well as the concentrations of DHTDMAC in the water and in the sludges. From these data a mass balance was calculated; the amount of DHTDMAC which disappeared between the influent and effluent (sludge and water) was attributed to primary biodegradation. Although primary biodegradation may account for up to 90% of the elimination in the aeration stage of activated sludge waste water treatment, the average from Table 7 is 60%. Unfortunately, the studies performed to date do not allow an unequivocal distinction to be made between primary and ultimate biodegradation. Only Shimp (1992) measured mineralization. At present, only studies with radiolabelled material can provide such information.

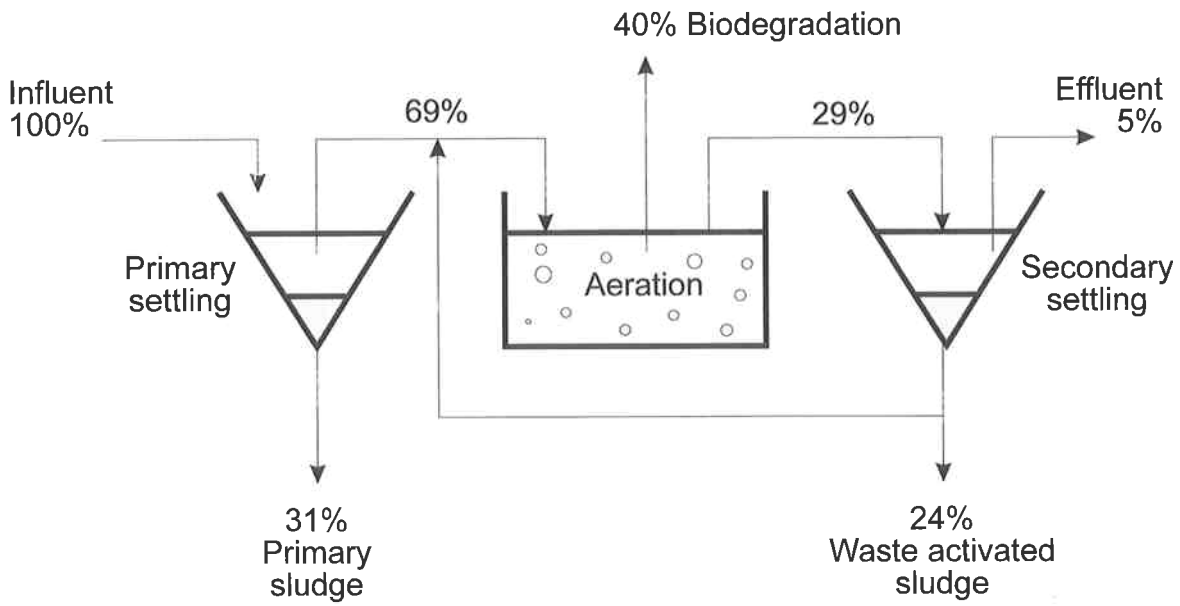
Because DHTDMAC adsorbs strongly onto activated sludge, its residence time in an activated sludge system is closer to the Sludge Retention Time (SRT) of the system than to its Hydraulic Residence Time (HRT). Unfortunately, not enough data on the SRT are available to confirm correlation of this parameter with the biodegradation of DHTDMAC.

Evidence from the test conducted by ATOCHEM (1990a) and from batch laboratory tests presented in Section 4.1.3 (Sullivan, 1983) indicate that primary biodegradation of DHTDMAC is the limiting step in the mineralization of the parent molecule. Therefore, it is likely that the biggest part of what is shown under "Degradation" in Table 7 is indeed mineralization.

The various data presented in this section are not in conflict. The results presented often depend on the type of analytical monitoring used for the tests. In other words, primary biodegradation (disappearance of parent), average carbon mineralization and ^{14}C mineralization may all proceed at difference rates. Also, the experimental procedures used were not all equally stringent, thereby introducing a source of variability. In any case, all the data indicate that biodegradation does occur in activated sludge waste water treatment. As a general rule, tight operating conditions should always be used in continuous activated sludge testing to facilitate a clear interpretation of the results. Also, with sorptive substances like DHTDMAC, the SRT should always be mentioned since it has a potentially high influence on the level of biodegradation measured.

The fate of DHTDMAC in activated sludge waste water treatment. The diagram presented in Figure 1 summarises the information available on the fate of DHTDMAC in activated sludge waste water treatment. These figures, based on averages from the reported studies indicate the order of magnitude for each of the fluxes of DHTDMAC through a treatment plant suggesting that 31% of the substance may be removed in the primary stage, essentially by adsorption onto primary sludge.

Figure 1 The fate of DHTDMAC in activated sludge waste water treatment



At least 64% (40% primary biodegradation + 24% adsorption) may be eliminated in the aeration stage, leaving less than 5% in the effluent.

Thus about 55% is eliminated by adsorption in both primary and secondary sludge, 40% is degraded and 5% released in the effluent. Of course, these figures should not be taken as absolute values. They are only indicative and may vary with factors like the loading of the plant, the activated sludge retention time (SRT), the level of suspended solids in the effluent, etc.

4.2.4. Trickling Filters

Topping and Waters (1982) reported that removal of DHTDMAC by a combined primary and trickling filter was 86.1% with an influent concentration of 0.77mg/l. Additional data from the USA were reported by Versteeg *et al* (1992) and are summarised in Table 8. There was an average removal of 74% (44% to 94%).

Table 8 Trickling filters removal data

Plant	Influent conc. (mg/l)	Effluent conc. (mg/l)	Removal %	Analytical	Reference
Lincoln, NEB	0.360	0.023	94	HPLC	Versteeg *et al*, (1992)
Council Bluffs, NEB	0.745	0.175	77	HPLC	Versteeg *et al*, (1992)
Springfield, OH	0.480	0.150	69	HPLC	Versteeg *et al*, (1992)
Gardner, MA	0.390	0.220	44	HPLC	Versteeg *et al*, (1992)
Rapid City, SD	0.335	0.084	75	HPLC	Versteeg *et al*, (1992)
Duffel, B	0.770	0.090	86	DBAS	Topping and Waters, (1982)

4.2.5. Other Systems

Monitoring data on other waste water treatment systems were collected between 1975 and 1986 and published by Versteeg *et al* (1992). They cover oxidation ditches, rotating biological contactors (RBC) and ponds. In most cases, the measurements for the ponds and the oxidation ditch were carried out on grab samples and are therefore only indicative. In the case of the oxidation ditch and of the RBC, the removal figures include primary settling. It should be noted that in the oxidation ditch system, (similar to an activated sludge system but with a very high SRT (>30 days)), DHTDMAC removal exceeds 99% (Table 9).

Table 9 DHTDMAC removal in other types of wastewater treatment (Versteeg *et al*, 1992)

Location	Treatment type	Influent (mg/l)	Effluent (mg/l)	Analytical	Removal %
Mechanicsburg, OH	Pond	0.980	0.055	HPLC	94
Rose Hill, K	Pond	0.111	0.055	HPLC	94
Derby, K	Ox.ditch	0.371	<0.004	HPLC	>99
Hastings, NEB	RBC	0.573	0.125	HPLC	78

4.3. DHTDMAC IN SLUDGE TREATMENT AND DISPOSAL

Sludge is usually treated before its end use or disposal. There are six main types of sludge treatment:

- thickening,
- dewatering,
- drying,
- incineration or melting,
- composting or aerobic digestion,
- anaerobic digestion.

The treated sludge, ash or compost which results from these treatments is then disposed of in one the following ways:

- soil amendment or agricultural use,
- landfill,
- discharge at sea.

The first three types of sludge treatment are not expected to produce any significant degradation of DHTDMAC. With incineration under good conditions (high temperature, complete combustion), DHTDMAC will be destroyed. Composting or aerobic digestion is usually carried out before soil amendment. Because conditions are aerobic, this treatment may create conditions for the degradation of DHTDMAC that are similar to those encountered in soils, as discussed below.

Disposal in a landfill will lead to the presence of DHTDMAC under strictly anaerobic conditions (see section 4.3.1).

Within a few years, the dumping of sewage sludge at sea will be completely banned in Europe and this route of discharge of DHTDMAC will thus disappear.

4.3.1. DHTDMAC in Anaerobic Digestion

A 72-day anaerobic degradation study was performed using ^{14}C methyl and alkyl labelled DHTDMAC. The concentrations tested were 20 (with the methyl label), 20 (with the alkyl label), 200, and 1,500 (with non radioactive material)mg/l. The solids concentration in the digesters was 30,000mg/l. No adverse effects were observed on the performance of the digesters. The level of radioactivity in the gas after 10 weeks was 0.1% for the alkyl label and 0.04% for the methyl label and could be due to the degradation of impurities. About 90 to 95% of the DHTDMAC were associated with the solids (Fieler, 1975a).

Several tests carried out on the effect of DHTDMAC on anaerobic digestion showed no adverse effects. No adverse effect was observed on gas production or the performance of the digesters up to 1,500mg/l (Van Ginkel and Van Rij, 1990).

Topping and Waters (1982) also found no effect of DHTDMAC on anaerobic digesters under various conditions and reported that there is no evidence that DHTDMAC undergoes anaerobic degradation.

Thus there is no evidence that DHTDMAC degrades under anaerobic conditions. It does not affect the operation of anaerobic digesters.

4.3.2. Biodegradation of DHTDMAC in Soils

The studies performed on the biodegradation of DHTDMAC in soils indicate a significant potential for mineralization.

Fieler (1975b) studied the biodegradation rate of ^{14}C methyl tagged DSDMAC using a batch incubated flask method. In this technique, soil containing some radiolabelled material is incubated at room temperature in a sealed flask in which a NaOH CO_2 trap is suspended. The content of the trap is counted by liquid scintillation counting (LSC) every week. A loam and a sandy loam were tested; the loam contained some digested sewage sludge as a conditioner. The dosage rate of 50mg/kg dry soil was calculated to be equivalent to typical field application rates. The test duration was 55 weeks. There was no significant difference between the findings with the two soils. $^{14}CO_2$ production was approximately 48% at the end of the test. A parallel test in the presence of 38mg LAS/kg yielded 38% $^{14}CO_2$ from DSDMAC at the end of the test. There was no evidence of a lag phase. The initial rate of degradation was slightly higher in the presence of LAS.

A similar test by the same author was performed on alpha alkyl tagged DSDMAC. Four different soils were used: two loams (one with and one without sewage sludge), a sandy

loam and a silt loam. Three doses were investigated: 0.5, 5.0 and 50mg/kg. Parallel tests were performed with 0.38, 3.8 and 38mg LAS/kg added to DSDMAC on the sandy loam. The tests lasted 62 weeks. At 0.5mg/kg, two soils (sandy loam and loam with sludge) gave about 27% CO_2 production from DSDMAC and two others (loam without sludge and silt loam) approximately 18%. At 5mg/kg, the loam without sludge gave 31% $^{14}CO_2$, the silt loam gave about 42% and the other two about 50%. At 50mg/kg, the loam without sludge gave 31%, the silt loam 50%, the sandy loam 60% and the second loam about 63% $^{14}CO_2$. The tests with LAS on the sandy loam gave exactly the same results as those with DSDMAC (Fieler, 1975b).

Another study with radiolabelled DSDMAC was performed by Weston (1987). The test duration was 116 days. Triplicate tests were made using 0.1 and 1mg/kg soil. The $^{14}CO_2$ production was 27%, 33% and 18% at 0.1mg/kg and 37%, 38% and 34% at 1mg/kg.

Weston (1989) conducted a similar study to investigate the degradation of DHTDMAC in sludge amended soil (sandy loam from Pennsylvania, USA). This study lasted 120 days and used alpha alkyl labelled DSDMAC. Test concentrations were 0.1 and 1mg/kg, run in duplicate. The final CO_2 productions were about 52% and 36% at 0.1mg/kg and 38% and 41% at 1mg/kg.

The various biodegradation studies of DSDMAC in soils performed by Procter and Gamble (1992) were made using several types of dispersions of the test substance. Figure 2 shows the final CO_2 yields of these studies with an indication of the type of dispersion used: solvent dose (solution of DSDMAC with a solvent), emulsion of DSDMAC with lecithin or aqueous dispersion (Larson, 1992). Figure 3 plots the different levels of CO_2 produced at the end of the test versus test duration. Two main remarks can be made around this graph. The first is that overall, the longer the test, the higher the CO_2 production. This suggests that the true biodegradation plateau is not reached even after long test duration. The second is that the way the test substance is dosed to the soil appears to have a major influence on the test results. The solvent dose appears to be the least favourable to biodegradation and the aqueous dispersion the most favourable. All this points to the fact that given enough time, DHTDMAC biodegrades in soils and that physical chemistry (bioavailability) is likely to play a major role in the kinetics of biodegradation of DHTDMAC.

The conclusion of a monitoring study on sludge amended fields in England (Matthijs, 1990a) was that concentrations of DHTDMAC in agricultural soils treated with sludge from biological waste water treatment were overall lower than what was expected from the rate of sludge application (50mg/kg average). The 43% of samples taken from fields that had

Figure 2 Biodegradation of DSDMAC in Sludge-Amended Soil

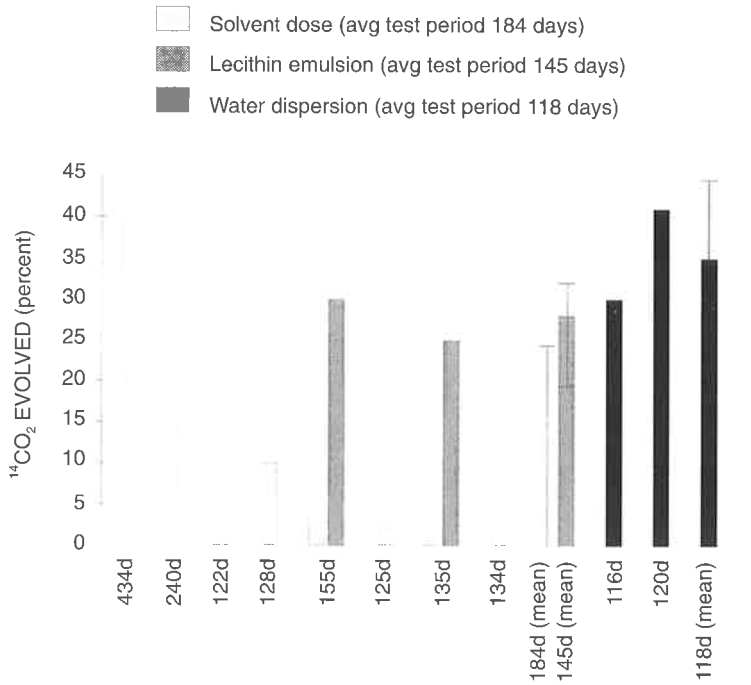
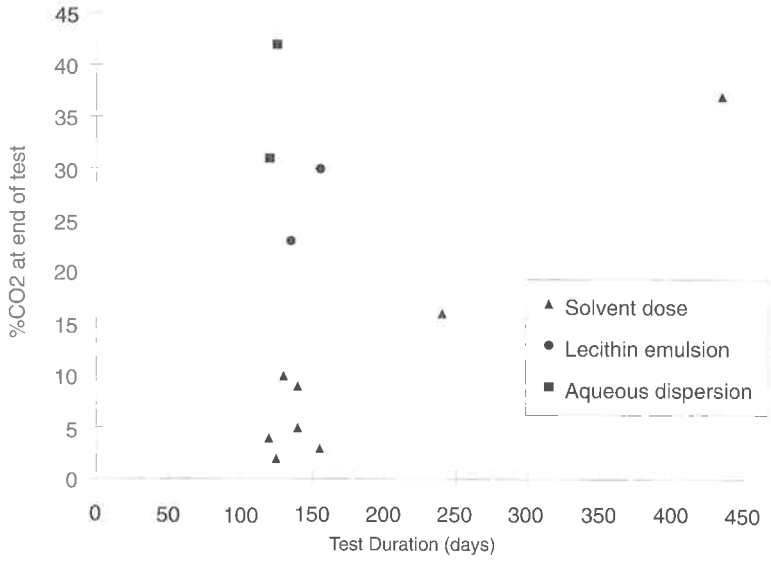


Figure 3 CO_2 Production in Various Biodegradation Tests for DSDMAC in Sludge Amended Soils



received their last application of sludge the year preceding that of sampling had a DHTDMAC level lower than 20% of the expected load. The samples containing the highest concentrations of DHTDMAC were from fields that had received among the lowest amounts of sludge (one or two amendments). This again is an indication that adaptation

of the biomass may be needed before significant biodegradation can occur. This adaptation may require a certain amount of DHTDMAC to occur. The average level of removal from the fields that had received their last application of sludge in the year of sampling was 74%. For the other fields, average removal was 64%. On the whole, the data from this study show the potential for DHTDMAC to biodegrade although they are only directional and are insufficient to draw firm conclusions about the rate of disappearance of DHTDMAC in sludge amended soils.

The results of biodegradation studies of DSDMAC in soils are summarised in Table 10. These results show that DHTDMAC has the potential to biodegrade slowly in soils and that adaptation of the biomass is possible.

Table 10 Summary of biodegradability tests for DHTDMAC in soils

Conc.	Duration (days)	Radiolabel	Test compound	CO_2 prod. (%)	Reference
0.1	120	Alpha alkyl	DSDMAC	52	Weston, (1989)
0.1	120	Alpha alkyl	DSDMAC	36	Weston, (1989)
0.1	116		DSDMAC	27	Weston, (1989)
0.1	116		DSDMAC	33	Weston, (1989)
0.1	116		DSDMAC	18	Weston, (1989)
0.5	434	Alpha alkyl	DSDMAC	27	Fieler, (1975b)
0.5	434	Alpha alkyl	DSDMAC	27	Fieler, (1975b)
0.5	434	Alpha alkyl	DSDMAC	18	Fieler, (1975b)
0.5	434	Alpha alkyl	DSDMAC	18	Fieler, (1975b)
0.5	434	Alpha alkyl	DSDMAC + LAS	27	Fieler, (1975b)
1	120	Alpha alkyl	DSDMAC	38	Weston, (1989)
1	120	Alpha alkyl	DSDMAC	41	Weston, (1989)
1	116		DSDMAC	37	Weston, (1989)
1	116		DSDMAC	38	Weston, (1989)
1	116		DSDMAC	34	Weston, (1989)
5	434	Alpha alkyl	DSDMAC	50	Fieler, (1975b)
5	434	Alpha alkyl	DSDMAC	50	Fieler, (1975b)
5	434	Alpha alkyl	DSDMAC	31	Fieler, (1975b)
5	434	Alpha alkyl	DSDMAC	42	Fieler, (1975b)
5	434	Alpha alkyl	DSDMAC + LAS	50	Fieler, (1975b)
50	385	Me	DSDMAC	48	Fieler, (1975b)
50	385	Me	DSDMAC	48	Fieler, (1975b)
50	385	Me	DSDMAC + LAS	38	Fieler, (1975b)
50	385	Me	DSDMAC + LAS	38	Fieler, (1975b)
50	434	Alpha alkyl	DSDMAC	60	Fieler, (1975b)
50	434	Alpha alkyl	DSDMAC	63	Fieler, (1975b)
50	434	Alpha alkyl	DSDMAC	50	Fieler, (1975b)
50	434	Alpha alkyl	DSDMAC	31	Fieler, (1975b)
50	434	Alpha alkyl	DSDMAC + LAS	60	Fieler, (1975b)

4.3.3. Biodegradation of DHTDMAC in Sediments

Few data are available on the biodegradation of DHTDMAC in sediments. Federle and Pastwa (1988) studied the fate of various surfactants in a pond directly receiving the effluent from a public laundry. The average concentration of DHTDMAC in the top 1m of

sediment, determined by HPLC, was 10 times higher than in the overlying water but decreasing with depth. Beyond a depth of 1m DHTDMAC was no longer detectable.

Mineralization assays using alpha alkyl labelled ^{14}C DSDMAC were performed with the sediment taken from various depths. No mineralization occurred in the sediments from a pond which had never been exposed to DHTDMAC. However, slow but significant mineralization occurred in sediment from a pond receiving DSDMAC. The rate of mineralization decreased in samples from increasing depths and with decreasing DHTDMAC concentration.

This study had limitations making it difficult to assess with confidence the biodegradation of DHTDMAC in sediments. The mineralization studies were not performed *in situ* but on samples which had been extracted and disturbed. Some exposure to oxygen occurred and no counts of the anaerobic bacterial flora were performed. The richness of endogenous anaerobic and aero-anaerobic florae (especially of the symbiotic associations of anaerobic bacterial groups which are necessary for the complete mineralization of organic compounds in sediments) may have been disturbed.

Nevertheless the study provides evidence that biodegradation of DHTDMAC may occur in sediments and it may be enhanced by adaptation of the biomass.

4.4. PHOTODEGRADATION OF DHTDMAC

Neufahrt and Pleschke (1984) have developed some evidence for photodegradation of DHTDMAC and other quaternary ammonium compounds. Substances were adsorbed on silica gel and irradiated. The photoproducts were then either oxidized and measured as CO_2 or separated by thin layer chromatography and tested for biodegradation with the OECD screening test using DOC and DBAS measurements. Two experimental systems were used: one producing Pyrex filtered UV light and a second producing quartz filtered UV light. After 72 hours of Pyrex filtered UV light exposure, 43% of the DHTDMAC had been degraded (DBAS response). Photomineralisation appeared to be wavelength dependent. The products obtained from DHTDMAC after 16 hours irradiation under quartz filtered UV light produced 63% DOC disappearance in the OECD screening test after 10 days but no change in the DBAS response. The authors considered that only part of the decomposition products obtained from the quartz filtered UV exposure of DHTDMAC can be used by bacteria as a source of carbon and energy. The products obtained from the Pyrex filtered UV exposure were largely and rapidly biodegraded, reaching 81% mineralization after 28 days. The DBAS response disappeared after 10 days.

In general, the longer the test substance is exposed to UV light, the higher the level of degradation. While it is conceivable that UV degradation of DHTDMAC may occur in the environment it is probably not a significant degradation mechanism.

4.5. THE BIODEGRADATION PATHWAY OF QUATERNARY AMMONIUM COMPOUNDS

Little has been published on fundamental aspects of biodegradation of cationic surfactants in general and quaternary ammonium compounds in particular.

Baleux and Caumette (1977) investigated the comparative biodegradation of a number of cationic surfactants. A series of papers by Cruz (1979, 1981) go further in attempting to rationalize the findings. They investigated the influence of the structure of the molecules, the test concentration, the adaptation of the biomass and some external factors on biodegradability. The rationalisation was carried even further by van Ginkel (1991, 1992), who published the mechanisms by which quaternary ammonium compounds are broken down by bacteria.

4.5.1. Experimental Evidence, Trends

Several publications have considered the biodegradation of quaternary ammonium compounds. The main findings are summarised below.

(Baleux and Caumette, 1977)

- The counter ion may be important: the lag period before biodegradation of C_{16} Tallow Methyl Ammonium Chloride is longer than with C_{16} Tallow Methyl Ammonium Bromide;
- Adaptation improves biodegradation, sometimes dramatically;
- Monoalkyl quaternary ammonium compounds degrade more easily than dialkyl compounds:

(Cruz, 1979; Cruz and Garcia, 1979; Cruz, 1981)

- The counter ion has no influence;
- The longer the monoalkyl chain, the slower the adaptation;
- For dialkyl, the longer the chain, the slower the degradation;
- The higher the number of chains, the slower the degradation;
- The more branching of the alkyl chain, the slower the biodegradation;

- The higher the test concentration, the longer the lag period and the slower the degradation;
- Same trend for decreasing inoculum concentration;
- Adaptation occurs markedly;
- Adaptation is reversible;
- Degradation becomes slower if easily metabolized nutrients are available;
- Temperature improves degradation markedly between 5 and 15°C; little difference between 25 and 35°C;
- Degradation improves slightly as aeration increases;
- Sunlight has no effect on the lag phase but slows biodegradation:

(Van Ginkel, 1991)

- An increased test concentration lengthens the lag phase and decreases biodegradation rate;
- Increased alkyl chain length decreases biodegradation kinetics;
- Branched chains biodegrade more slowly than linear chains;
- Unsaturated chains biodegrade faster than saturated chains;
- Dialkyl compounds biodegrade more slowly than monoalkyl compounds.

The most important features demonstrated by the papers are the need for adaptation before biodegradation can proceed at a practical rate and the slowing of biodegradation with increasing numbers of alkyl chains. The importance of adaptation is universally recognized and in line with all the evidence provided earlier in this section. Many points cannot be verified on DHTDMAC only but this molecule seems to gather the characteristics that make biodegradation and biomass adaptation slow. Some points are in line with evidence existing for other types of surfactants (e.g. the adverse effect of alkyl chain branching on biodegradation). One point of disagreement between two publications is the influence of the counter ion on biodegradation. However, the weight of evidence points to little effect from the counter ion. Even Baleux and Caumette (1977) only mention its influence on the lag phase before the biodegradation of monoalkyl quaternary ammonium compounds.

4.5.2. Biochemical Mechanisms of Biodegradation of Quaternary Alkyl Ammonium Compounds

Four publications consider the fundamental mechanism of biodegradation of quaternary ammonium compounds (Dean-Raymond and Alexander, 1977; van Ginkel 1991, 1992; van Ginkel *et al*, 1992)

Tetramethyl ammonium chloride. The degradation of tetramethyl ammonium chloride provides a basis for understanding the breakdown of quaternary alkyl ammonium compounds. Various microorganisms can use tetramethyl ammonium chloride aerobically as a sole source of carbon and energy (van Ginkel, 1992).

The first step in degradation is oxidation; the formation of formaldehyde and trimethyl amine catalyzed by a mono-oxygenase. NADH is an essential oxido-reducer.

$$N^{+}(CH_3)_4 + O_2 + NADH + H^{+} \rightarrow N(CH_3)_3 + CH_2O + NAD^{+} + H_3O^{+}$$

The catabolism of trimethylamine may proceed by two different pathways. The first involves oxidation to trimethylamine N oxide, the oxide being then cleaved to yield dimethylamine and formaldehyde.

The second needs a specific trimethylamine dehydrogenase which leads directly to the formation of formaldehyde and dimethylamine. Dehydrogenases are the most common oxidizing enzymes which catalyze the oxidation of a substrate by removing one hydrogen.

$$N(CH_3)_4 + NAD^{+} + H_2O \rightarrow HN(CH_3)_2 + CH_2O + NADH + H^{+}$$

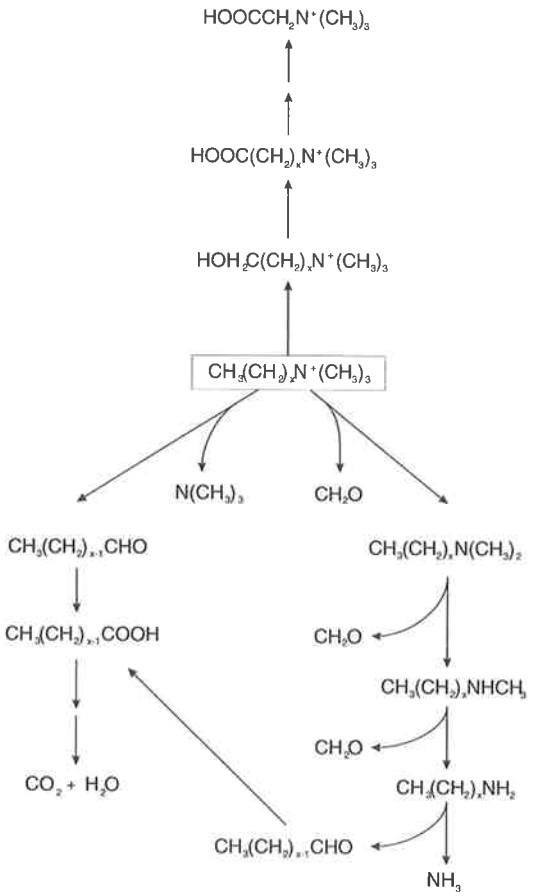
A further methyl is then cleaved by a secondary amine mono oxygenase and the last by a primary amine dehydrogenase. The formaldehyde and formate thus obtained are mineralized.

Ethyl trimethyl ammonium chloride. The initial attack by a mono oxygenase gives either acetaldehyde or formaldehyde and a tertiary amine (van Ginkel, 1992). The reaction then proceeds to complete mineralization. It is unclear whether the cleavage of the ethyl unit occurs first, second or third. It cannot occur last because ethylamine is not a growth substrate for the bacteria isolated in the study.

Alkyl trimethyl ammonium salts. Catabolism may occur at two points in the molecules:

- Omega oxidation of the chain ends
- Cleavage of the C1-N bond, separating the alkyl chain from the nitrogen.

Dean-Raymond and Alexander (1977) support the existence of the first while the publications of van Ginkel (1992) and van Ginkel *et al* (1992) prove the existence of the second. The following scheme shows the various theoretical biodegradation pathways of quaternary ammonium compounds.



Evidence from these publications points to the fact that no intermediates are formed during the biodegradation of alkyl quaternary ammonium compounds that are resistant to catabolism.

Dialkyl dimethyl ammonium compounds. Unfortunately, no information is available on the biodegradation pathway of dialkyl dimethyl ammonium compounds (DDAC). However, the evidence from the studies performed on monoalkyl trimethyl ammonium compounds provides interesting insights for the DDAC. Clearly, all the mechanisms necessary for the complete biological break down of DDAC exist. However, the experimental evidence currently available does not allow conclusions to be reached on what is the sequence of events in the mineralization of DDAC or if there are competing mechanisms. This is clearly an area where more fundamental experimentation is needed to gain an ultimate understanding.

4.6. OVERALL CONCLUSIONS ABOUT THE FATE OF DHTDMAC IN THE ENVIRONMENT

Much data are available on the environmental fate of DHTDMAC. Removal averages around 31% in primary sewage treatment, 95% in activated sludge treatment and 74% in trickling

filters. Limited data available for other types of waste water treatment indicate that DHTDMAC is also likely to be extensively removed in rotating biological contactors, ponds and oxidation ditches.

Biodegradability screening tests show that DHTDMAC is not readily biodegradable as defined by the EC. Adaptation of the biomass to DHTDMAC is slow but largely increases the biodegradation rate of DHTDMAC. DHTDMAC has been shown to be completely biodegradable.

Fate studies on both real and lab-scale activated sludge treatment systems suggest that 40% primary biodegradation may occur during biological waste water treatment. Other studies show that primary biodegradation is the rate limiting step for the mineralization of DHTDMAC but the nature of this step is still unknown. DHTDMAC does not degrade under strictly anaerobic conditions. It does not affect the performance of anaerobic digesters (methane production or general operation).

DHTDMAC biodegrades slowly in soils. Evidence indicates that soil microorganisms adapt to its presence.

Radiolabelled material show that DHTDMAC eventually mineralizes completely and does not leave any residue resistant to biodegradation. The biodegradation of DHTDMAC is usually very slow in river water die-away tests but may be considerably enhanced with adapted sediment biomass.

Few data are available on the fate of DHTDMAC in sediments. They point to a slow mineralization.

SECTION 5. AQUATIC TOXICITY OF DHTDMAC

The results of studies on freshwater organism are listed in Table 11 (acute toxicity) and Table 12 (chronic and subchronic toxicity) and for marine and estuarine species in Table 13.

5.1. VARIABILITY OF THE RESULTS IN AQUATIC TOXICITY TESTING

The results of the aquatic toxicity tests with DHTDMAC show an unusually high level of variability; large differences have been found both between species and in different tests with the same species. For example, the EC_{50} values for green algae ranged from 0.026mg/l (*Selenastrum capricornutum*) to 1.8mg/l (*Scenedesmus pannonicus*) and from 0.065mg/l to 3.6mg/l for *Daphnia magna*. For fish, *Lepomis macrochirus* the LC_{50} ranged from 0.56mg/l to 14.0mg/l.

A wide range in species sensitivity to a given compound is not unusual. However, experimental variation in results from the same type of test on one species does not normally exceed a factor of 10. For DHTDMAC this experimental variability is extremely high. For example the NOECs from tests with *Selenastrum capricornutum* vary by a factor of 42, the acute EC_{50} for *Daphnia magna* by a factor of 55 and the acute LC_{50} for *Lepomis macrochirus* by a factor of 25. Table 14 demonstrates that variability ranges from a factor of 2 to a factor of 214.

These variations are mostly due to differences in the bioavailability of DHTDMAC in various test systems. In toxicity tests, the bioavailable fraction of DHTDMAC can be considered as the fraction of the material present in a test system that is free to exert its toxicity on the organisms. The main causes of these variations in bioavailability are discussed in section 5.2.

5.2. FACTORS INFLUENCING THE TOXIC EFFECTS OF DHTDMAC

5.2.1. Adsorption

As described earlier (section 2.2.3) DHTDMAC readily adsorbs onto surfaces; this can lead to a rapid fall in the concentration of dissolved or finely dispersed DHTDMAC in static test solutions. Pre-treatment of the test vessels (e.g. pre-conditioning at the appropriate concentration with DHTDMAC) may be necessary to prevent unacceptable losses to vessel walls by allowing saturation of the adsorbent surfaces before the start of the test. A flow-through procedure is the best to achieve stable test concentrations although this is only possible for large enough organisms (e.g. fish, *Daphnia magna*). Algal toxicity tests

Table 11 Results of acute aquatic toxicity tests of DHTDMAC

Invertebrates

Species	Test duration (hours)	Water used	Suspended solids (mg/l)	EC_{50} or LC_{50} (mg/l)	Impurities	Reference
Daphia magna	48	laboratory		0.16-1.06		Kappeler (1982)
	48			2.6-3.1		Kappeler (1982)
	24			0.9		Fina (1989)
	48			0.24		Kao Corp. (1990)
	48			0.35		Berol Nobel (1990b)
	48			0.1		Unilever (1990, 1991)
	48			0.48		Atochem (1990b)
	96			0.48		Procter & Gamble (1974-1986)
	48			0.065	4.6% MHTTMAC	Procter & Gamble (1974-1986)
	48			0.28	5.5% MHTTMAC[1]	Procter & Gamble (1974-1986)
	48			0.19	8% MHTTMAC	Procter & Gamble (1974-1986)
	48	well		1.06		Procter & Gamble (1974-1986)
	48	river	9.2	2.1	5.5% MHTTMAC[1]	Procter & Gamble (1974-1986)
	48	river	9.2	3.6	8% MHTTMAC	Procter & Gamble (1974-1986)
Chironomus riparius	72[2]	lab.+ sed		11.3	1.7% MHTTMAC	Pittinger *et al*, (1989)
	96[3]	lake		7.1	1.7% MHTTMAC	Roghair *et al*, (1991)
Lymnea stagnalis	96	lake		13.9	1.7% MHTTMAC	Roghair *et al*, (1991)

Fish

Species	Test duration (hours)	Water used	Suspended solids (mg/l)	EC_{50} or LC_{50} (mg/l)	Impurities	Reference
Salmo gairdneri	96	laboratory		2.6		Akzo (1987)
	96	laboratory		1.7		Kao Corp. (1990)
Gasterosteus aculeatus	96	lake		3.5	1.7% MHTTMAC	Roghair *et al*, (1991)
Oryzias latipes	96	lake		5.2	1.7% MHTTMAC	Roghair *et al*, (1991)
Pimephales promelas	96	laboratory		0.29-0.558		Versteeg (1989)
Lepomis macrochirus	96h	laboratory		0.62-2.17		Procter & Gamble (1974-1986); Kappeler (1982)
	96	river		10.1-14.0		Procter & Gamble (1974-1986); Kappeler (1982)
	96	laboratory		0.56-3.2		Procter & Gamble (1974-1986)
	96	laboratory		0.64	8% MHTTMAC[4]	Procter & Gamble (1974-1986)
	96	river	380	14	8% MHTTMAC	Procter & Gamble (1974-1986)
	96	river	9.2	13	8% MHTTMAC[1]	Procter & Gamble (1974-1986)
	96	river		7.7		Procter & Gamble (1974-1986)

1 Solubility problem.
2 Newly hatched larvae.
3 Second instat larvae
4 Isopropanol

Table 12 DHTDMAC - Results of chronic/subchronic toxicity tests

Species	Test method	Water quality	Suspended solids (mg/l)	NOEC (mg/l)	EC_{50} (mg/l)	LOEC (mg/l)	MiAC[1] (mg/l)	Comments/ Impurities	Reference
Scenedesmus pannonicus[2]	growth inhib. 96h	lake		0.58	1.8				Roghair *et al* (1991)
Microcystis aeruginosa[2]	growth inhib. 96h	laboratory			0.05				Procter & Gamble (1974-1986)
	growth inhib. 5d	laboratory		0.13			0.32	8% MHTTAC[4]	
		laboratory		0.075			0.12	4.6% MHTTAC	
		river water	68	0.078			0.21	8% MHTTAC	
Selenastrum capricornutum[2]	growth inhib. 96h	laboratory		0.12	0.21			4% MHTTMAC	Akzo (1991b)
		laboratory		0.006	0.026	0.012		sonication	Akzo (1991a)
		laboratory			0.06				Lewis (1990)
		effluent		20.3					) Versteeg &
		effluent		10.7					) Woltering (1990)
	growth inhib. 5d	laboratory		0.075			0.13	4.6% MHTTMAC	Procter & Gamble
		laboratory		0.078			0.23	8% MHTTMAC[4]	(1974-1986)
		river water	139	0.25			2.6	8% MHTTMAC[4]	
		river water	139	0.12			>4	8% MHTTMAC[4]	
		river water	68	0.062			0.71	8% MHTTMAC[4]	
Navicula seminulum[2]	growth inhib. 5d	laboratory		0.05			0.146	4.6% MHTTMAC	Procter & Gamble (1974-1986)
Chlorella vulgaris[2]	growth inhib. 96h	laboratory			0.4				Unilever (1990-1991)
		laboratory			0.27				
Daphnia Magna[2]	reprod. 21d	laboratory		0.180	0.599	0.320			Akzo (1991c)
		river		0.380		0.76			Lewis & Wee (1983)
Ceriodaphnia dubia[2]	reprod. 7d	river			0.78			adults	Taylor (1984)
		river			0.2[5]				
		effluent		4.53				reproduction	) Versteeg &
		effluent		10.7					) Woltering (1990)
Chironomus riparius[3]	egg hatching	laboratory	sediment	>21.5				1.7% MHTTMAC	Pittinger *et al* (1989)
	lethal 28d	lake		1.03				1.7% MHTTMAC	Roghair (1991)
Lymnea stagnalis[3]	lethal 28d	lake		0.25				4% MHTTMAC	Roghair (1991)
Gasterosteus aculeatus[3]	lethal 28d	lake		0.58				1.7% MHTTMAC	Roghair (1991)
Pimephales promelas[3]	ELS 33d	river water		0.23		0.45		8% MHTTMAC[6]	Procter & Gamble (1974-1986)
	ELS 34d	well water		0.053		0.09	>4	8% MHTTMAC[4]	Lewis & Wee (1983)

1 MiAC = minimum algistatic concentration
2 chronic
3 subchronic
4 Isopropanol
5 EC_{20}
6 Triethylene glycol

Table 13 DHTDMAC - Results of Toxicity Tests with Estuarine and Marine Species

Species	Test method	Water quality	Salinity (%)	EC_{50}/LC_{50} (mg/l)	Comments/ Impurities	Reference
Crassostrea virginica, lv[1]	acute, 48h	5µm filtered sea water	16-26	2.0		Kappeler (1982) Lewis and Wee (1983)
Penaeus duorarum[1]	acute, 96h	5µm filtered sea water	23-25	36.0	IPA[3]	) Procter & Gamble (1974-1986)) Kappeler (1982)) Lewis and Wee (1983)
			22	3.1	4.6% MHTTMAC[3]	Procter & Gamble (1974-1986)
		Sea water	25-26	1.3	5.5% MHTTMAC[3]	Procter & Gamble (1974-1986)
			30	>1000		
Mysidopsis bahia[1]	acute, 96h	5µm filtered sea water	24	0.22-0.42	IPA, 8% MHTTMAC[3]	Procter & Gamble (1974-1986) Kappeler (1982) Lewis and Wee (1983)
Callinectes sapidus[1]	acute, 96h	5µm filtered sea water	16-26	>50	8% MHTTMAC	Kappeler (1982) Lewis and Wee (1983)
Cyprinidon variegatus[1]	acute, 96h	5µm filtered sea water	16-26	24.0		Kappeler (1982) Lewis and Wee (1983)
			23-25	4.8	5.5% MHTTMAC[3]	Procter & Gamble (1974-1986)
Mysidopsis bahia[1]	Life cycle, 28d	Sea water	24	0.075 NOEC 0.166 LOEC	8% MHTTMAC	Procter & Gamble (1974-1986)
Dunaliella tertiolecta[1]	5d growth	filtered artificial sea water	20	0.5-1.0[4] 1.0-10.0[5]		Kappeler (1982) Lewis and Wee (1983)

1 Estuarine
2 Marine
3 Solubility problems
4 Algistatic
5 Algicidal

typically involve a 72 or 96 hour static exposure to various concentrations. At the start of the test the algal cell concentration is relatively low and the availability of the test substance high. However, as the test proceeds the surface area of algal cells can increase by several orders of magnitude. This, combined with adsorption of DHTDMAC onto the test vessel, will result in decreasing bioavailability and toxicity, and may lead to a recovery in growth rate in the later stages of the test. It may also be possible to see a difference in the test result according to whether the algae are added to the test solution (some DHTDMAC already lost to the test vessel walls) or the test substance is added to the algal suspension (competition for adsorption between the test vessel walls and the algae). Unfortunately, the information usually available on reported tests does not provide such details.

The presence of other forms of suspended solids such as activated sludge, silica or clay particles can lead to absorption and adsorption of DHTDMAC and thereby reduce the

Table 14 DHTDMAC - Range of values resulting from different aquatic toxicity tests

Organisms	NOEC (mg/l)		EC_{50} or LC_{50} (mg/l)		MIAC (mg/l)		factor
	from	to	from	to	from	to	
All Species (chronic/subchronic)	0.006	1.03	0.026	4.0			171 153
Total Algae (chronic/subchronic)	0.006	0.58	0.026	1.8	0.12	>4.0	97 69 >33
Microcystis aeruginosa	0.075	0.13	0.075	0.13	0.12	0.32	~2 ~2 ~3
Selenastrum capricornutum	0.006	0.25	0.026	0.21	0.12	>4.0	42 8 >33
Total Invertebrates (chronic)	0.18	1.03	0.2	4.0			6 20
Daphnids	0.18	0.38	0.2	0.78			2 4
Total Fish (subchronic)	0.053	0.58					10
Pimephales promelas	0.053	0.23					4
Total Invertebrates (acute)			0.065	13.9			214
Daphnids			0.065	3.6			55
Total Fish (acute)			0.29	14			48
Lepomis macrochirus			0.56	14			25

toxic effects (Waters *et al*, 1982).

5.2.2. Preparation and Dosing of the Samples

Differences in the results of toxicity tests with DHTDMAC, using the same test method may be caused in part by the difficulties of preparing test solutions. Since concentrations of DHTDMAC that show toxic effects exceed its solubility in water it is common practice to prepare a stock dispersion to be diluted to the appropriate test concentrations. Differences in the preparation of these dispersions may lead to significant differences in the availability of DHTDMAC. For example, dispersions prepared with laboratory water by 8-10 minutes ultra-sonication were 20 times less toxic to *Selenastrum capricornutum* than

dispersions prepared by 1 hour ultra-sonication. The respective NOECs were 0.12mg/l and 0.006mg/l (Akzo, 1991a).

The use of solvents to aid test solution preparation, or the presence of isopropylalcohol in commercial DHTDMAC, may also lead to differences in the availability of DHTDMAC. It is unclear whether the solvent content has significant influence on the toxicity of DHTDMAC (see tables 11 and 13).

5.2.3. Concentration of Mono Alkyl By products (MHTTMAC)

Pure MHTTMAC is more toxic than DHTDMAC. For example NOEC and EC_{50} values for DHTDMAC in *Daphnia* reproduction tests were respectively 0.18mg/l and 0.69mg/l and for MHTTMAC respectively 0.03mg/l and 0.05mg/l; the latter compound was thus 6 to 14 times more toxic in standard single species laboratory tests. Acute toxicity data in laboratory water for *Pimephales promelas* give some support to the fact that MHTTMAC present in commercial DHTDMAC may significantly contribute to its toxicity. Nevertheless some *Daphnia* reproduction and algal tests with DHTDMAC containing 0 to 4% MHTTMAC showed no trend of increasing toxicity with increasing MHTTMAC concentration (Table 15). This discrepancy probably results from a combination of several factors:

- Commercial DHTDMAC samples do not always contain the same levels of MHTTMAC (from about 4 to more than 8%), making comparisons difficult.
- The colloidal properties of DHTDMAC may allow it to 'hide' at least some of the MHTTMAC in its liquid crystals or colloidal vesicles. This 'hiding' may be subject to phase changes and may not be proportionally related to concentration. This is likely also to be influenced by the method of preparation of the test dispersions.
- In static chronic tests, MHTTMAC may be biodegraded preferentially and show less effect than in acute tests.

The data currently available do not allow firm conclusions to be drawn but again point to the extreme caution needed for the interpretation of toxicity test results on DHTDMAC. These factors also show the artificiality of simple laboratory tests in comparison to environmental matrices.

Table 15 Results of Toxicity tests of DHTDMAC, DSDMAC, MHTTMAC and their Mixtures

Substance	Compound or concentration	Algae test[1]	*Selenastrum capricornutum*[1]				*Daphnia* acute[2]	*Daphnia* Reprod. test[2]		*Pimephales promelas* acute	*Pimephales promelas* subchronic
		EC_{50} (mg/l) lab. water	NOEC (mg/l)		EC_{50} or MiAC (mg/l)		EC_{50} (mg/l) lab. water	NOEC (mg/l) lab. water	EC_{50} (mg/l) lab. water	LC_{50} (mg/l) lab. water	LOEC (mg/l) river water
			lab. water	river water	lab. water	river water					
C_{18}DSDMAC			0.16	0.6	0.46	1.17				4.08[3]	>12.7[3]
DHTDMAC+	0% MHTTMAC	0.014					0.45	0.18	0.68		
								0.18	0.69		
	1% MHTTMAC	0.021						0.32	0.90		
	2% MHTTMAC	0.017						0.32	0.75		
	4% MHTTMAC	0.026	0.12		0.21			0.18	0.60		
DHTDMAC	Commercial		0.078	0.062-0.25	0.23	0.71-2.6	0.9	0.18	0.60	0.45	0.45
MHTTMAC	100%	0.009	0.04		0.05		0.19	0.03	0.05	0.06	

1 Akzo, (1990a, b)
2 Akzo, (1991b)
3 Versteeg, (1989)
All other values taken from Tables 11 and 12

5.2.4. Complexation

DHTDMAC readily forms complexes with anionic substances such as anionic surfactants, eg LAS, and humic acids (see section 2.2.4). The lower toxicity of these complexes has been clearly demonstrated in several studies (Table 16 and 17). Typically, complexed DHTDMAC is 3 to 100 times less toxic to aquatic organisms in natural systems than uncomplexed DHTDMAC. This is discussed further in the following section.

Table 16 Results of Acute Aquatic Toxicity Laboratory Tests of DHTDMAC complexed by LAS and Humic Acid (laboratory water)

Species	Time	Molar ratio DHTDMAC/LAS	Humic acid[1] (mg/l)	EC_{50} or LC_{50} (mg/l)	Reference
Daphnia magna	48h	1:1 1:1 1:1		0.72 0.79 1.6	Procter & Gamble (1974-1986)
Lepomis macrochirus	96h	1:2 2:1 1:1 1:1 1:1 1:1		17.6 7.1 113.5 16.0 171 7.9	Procter & Gamble (1974-1986)
Pimephales promelas	96h		4.1 6.9 11.5	6.46 10.3 22.9	Lewis and Wee (1983)

1 Humic acid concentrations expressed as total organic carbon

5.2.5. Water Quality

Since the form of DHTDMAC presented to the test organism influences toxicity, factors such as the concentrations of suspended solids, organic carbon, dissolved anionic surfactants and humic substances in the test medium can have a significant influence on toxicity results. The clearest example of modification of toxic effects by differences in water quality is with synthetic laboratory media and natural surface water (Tables 11 to 13). In laboratory water containing no suspended solids, the EC_{50} of DHTDMAC to *Daphnia magna* was 0.25mg/l but in river water with 9.2mg/l suspended solids, the EC_{50} was 2.75mg/l (Procter and Gamble 1974-1986). This 11-fold difference was most probably caused by adsorption losses and complexation with dissolved or colloidal anionic surfactants and humic substances.

Table 17 Comparison between acute aquatic toxicity laboratory results of DHTDMAC in the presence and absence of LAS or humic acids

Species	DHTDMAC only EC_{50} or LC_{50}		DHTDMAC + anionic compound EC_{50} or LC_{50}		
	Range (mg/l)	Geom. mean (mg/l)	Single results (mg/l)	Geom. mean (mg/l)	Molar ratio or concentration of anionic compounds
Daphnia magna		0.36		0.97	1:1 LAS
Lepomis macrochirus	0.56-3.2		17.6 7.1	39.5	1:1 LAS 1:2 LAS 2:1 LAS
Pimephales promelas	0.29-0.558		6.46 10.3 22.9		4.1mg/l Humic acid 6.9mg/l Humic acid 11.9mg/l Humic acid

A similar lowering of toxic effects in river water was demonstrated in tests with *Lepomis macrochirus* (13 times less toxic in river water). However, a high concentration of suspended solids, 380mg/l, did not cause a further reduction in toxicity from that in river water containing 9.4mg/l suspended solids. (LC_{50}s were 14mg/l and 13mg/l respectively) (Procter and Gamble 1974-1986). This indicates that the type of suspended solids or dissolved organic substances have an important influence on bioavailability and toxic effects.

The suspended solids concentration (0 to 139mg/l) of test water had only a minor influence on the toxicity of DHTDMAC to *Selenastrum capricornutum* (Table 12). It may be that adsorption of DHTDMAC onto algae effectively reduces the influence of the suspended solids.

Although testing in river water improves the predictive value of toxicity test results, it does not model the realistic exposure of organisms to DHTDMAC in the environment. When tested in a mixture of sewage treatment plant effluent and river water, DHTDMAC was not toxic to *Ceriodaphnia dubia* (7 day reproduction test) at concentrations up to 4.5mg/l (Versteeg and Woltering, 1990). These values should be compared to EC_{50} values of less than 1mg/l for the same type of test in river water (see table 12). This suggests that under the most realistic simulated exposure conditions, DHTDMAC must be even less bioavailable than in river water dosed with the surfactant.

5.3. ESTUARINE AND MARINE SPECIES

Comparison of the toxicity data of estuarine and marine species with those of fresh water suggests that both groups have a similar range of sensitivity to DHTDMAC; the results of acute and chronic toxicity tests with marine and estuarine species are given in Table 13.

5.4. BIOCONCENTRATION

Lewis and Wee (1983) reported the whole-body bioconcentration factor (BCF) of DHTDMAC to be 32 for *Lepomis macrochirus* exposed in well water; the BCF in river-water was 13. The BCFs in muscle and viscera were <5 and 256 respectively (well-water study). The high visceral burden is consistent with ingestion and a low level of absorption from the gut and with absorption and excretion via the gall bladder, as is observed with other surfactants.

Comparable results were obtained by Neufarth *et al* (1978) who exposed carp to ^{14}C labelled DSDMAC. They demonstrated that absorbed DSDMAC was almost completely eliminated in 7 to 14 days when fish were transferred to clear water. Similar behaviour can be expected for DHTDMAC suggesting that this chemical will not bioaccumulate.

5.5. SEDIMENT

The acute toxicity of DHTDMAC and DSDMAC to larvae of the midge *Chironomus riparius*, in the absence and presence of sediment, is relatively low. The reported EC_{50} values are 7.1mg/l (Roghair *et al*, 1991) and 11.3mg/l (Pittinger *et al*, 1989) respectively. In partial life-cycle tests with spiked sediment, Pittinger *et al* (1989) found the LOEC in the range 0.88mg/l to 2.7mg/l. The NOEC was approximately 1,000 times greater than the NOEC in water only.

Lewis and Wee (1983) reported no effects on larval survival or adult emergence of the midge *Paratanytarsus parthenogenica* exposed to DHTDMAC concentrations up to 67mg/l. Lee (1986) also reported the low toxicity of sediment containing 0.8% DHTDMAC (on dry solids), or 20% on total organic matter. These results indicate that adsorption onto sediment greatly reduces the availability of DHTDMAC to chironomids. These values for sediment do not conflict with the general level of toxicity found in other complex mixtures of water, suspended solids and other contaminants (Versteeg and Woltering, 1990).

5.6. CONCLUSIONS

Aquatic toxicity of DHTDMAC as shown in laboratory tests is influenced by many factors. These factors can be grouped in three categories: presence of MHTTMAC, sample

preparation and test conditions. They explain the variability of the toxicity data available for DHTDMAC.

The toxic effects in the aquatic environment are reduced by several factors linked to the inherent physical-chemical properties of DHTDMAC such as:

- low solubility;
- complexation with anionic surfactants;
- adsorption onto organic solids and humic substances.

These factors have a major influence on the bioavailability of DHTDMAC in the environment. Since standard laboratory tests do not assess this influence, they can be taken as indicating the inherent toxicity of a substance but not the risk of harm to organisms under more natural conditions. Aquatic toxicity data using realistic environmental test conditions which more closely mimic realistic environmental situations should be used for risk assessment purposes.

SECTION 6. TERRESTRIAL TOXICITY OF DHTDMAC

6.1. PHYTOTOXICITY

Plant toxicity studies have shown that as much as 40,000mg/kg DHTDMAC (on dry solids) contained in a Levington compost (ex Fison Ltd) has no significant effect on the growth and productivity of four commercially important crop plants i.e. tomato (*Lycopersicum esculentum*), lettuce (*Lactuca sativa*), barley (*Hordeum vulgare*) and radish (*Raphanus sativa*). Parameters measured included the fresh and dry weights of shoots, roots, tap roots, leaves, fruit and grain as appropriate. Thus a margin of 1,000-10,000 exists between the predicted environmental levels of DHTDMAC in soil and the highest levels tested, which were found to have no toxic effects to plants (Topping and Waters, 1982).

In addition, it has also been established under conditions simulating crop irrigation that as much as 50mg/l of DHTDMAC in water has no significant effect on the germination and early growth stages of the seeds of the above plants. This concentration is approximately 1,000 times greater than that found in UK and German rivers (Topping and Waters, 1982).

Stanley and Tapp (1982) examined the effect of Distearyl dimethyl ammonium chloride (presumably DHTDMAC) on oats (*Avena sativa*) and turnip (*Brassica rapa*) using a draft OECD Guideline 'Growth Test with Higher Plants' (OECD, 1981). No phytotoxicity was observed at concentrations in excess of 1,000mg/kg.

Windeatt (1987) studied the effect of DHTDMAC on sorghum (*Sorghum bicolor*), sunflower (*Helianthus annuus*) and mung bean (*Phaseolus aureus*). The method used was OECD Test Guidelines 208, (OECD, 1984). The highest test material concentrations which caused no growth reduction at the 95% significance level were ≥1,000mg/kg for sorghum and sunflower and ≥10,000mg/kg for mung bean. The 21-day EC_{50} values for growth of seedlings from seed sown 28 days before (with emergence occurring around seven days after sowing), as mg/kg active ingredient in dry soil were:

sorghum	2,530
sunflower	2,930
mung bean	>10,000

The 7-day EC_{50} for seed emergence (the concentration at which seedling emergence was 50% of that recorded in the control by the seventh day after sowing) was greater than the highest concentration tested (10,000mg/kg) for all species. No trends towards an EC_{50} were

observed, emergence being in the range 72-97% in the controls and the test soils, regardless of concentration of DHTDMAC.

In an unattributed article (Anon, 1991) the EC_5 of DHTDMAC for the growth of mustard was found to be 1,400mg/kg in soil and the EC_{50} 3,540mg/kg.

6.2. EARTHWORM STUDY

Coulson *et al* (1989) exposed clitellate adult earthworms (*Eisenia foetida*) to 1,000mg/kg DHTDMAC for 14 days. In this period there were no deaths, behavioral effects or significant losses of bodyweight.

6.3. SOIL RESPIRATION STUDIES

Täuber *et al* (1986) showed that the oxygen uptake and carbon dioxide production in soils were unaffected over a period of 28 days when DHTDMAC (adsorbed onto sludge) was added at concentrations greatly in excess of typical application rates (365mg/kg). In a similar but longer-term study Procter and Gamble (1992) showed that over a 14 week period soil containing 400mg/kg DHTDMAC with or without 304mg/kg LAS produced 117 and 96% respectively of the carbon dioxide produced by control soil. In a different, much more active soil, the equivalent percentages were 105 and 119 respectively.

6.4. UPTAKE OF DSDMAC INTO PLANTS

The uptake of DSDMAC was studied by Loetzsch *et al* (1984) using a model compound. They grew tomatoes, cucumbers (*Cucurbita pepo*), beans and radishes from seeds or seedlings in soil containing DHTDMAC derived from sewage sludge at an original concentration of 2mg/g DHTDMAC dry weight (uniformly labelled stearyl-U-^{14}C). After mixing the sludge with the soil the DHTDMAC concentration was about 4mg/kg. In the experiment, which was of up to 38 days duration, the uptake of ^{14}C-labelled DSDMAC by the plants was determined. Uptake was found to be a maximum of 0.03% in shoots and less than 0.03% in radish roots. This shows there is little of biomagnification by this route.

6.5. CONCLUSIONS

Studies of the toxicity of DHTDMAC to a range of economically important plants exposed as seeds or seedlings either by soaking in concentrated solutions of the cationic or by being grown in sludge-amended soil produced no significant adverse effects even at levels grossly in excess of those expected during the normal practice of sludge-amendment of soil.

Similarly, there was no evidence of bioaccumulation, of toxicity to earthworms or of impairment of the general metabolism of soil as a result of contamination with DHTDMAC. The results are summarised in Table 18.

Table 18 Summary of Terrestrial Toxicity Data

Species	Concentration (mg/kg)	Notes	Reference
Barley Lettuce Radish Tomato	40,000 40,000 40,000 40,000	No effect on growth or productivity	Topping and Waters, (1982)
Oats Turnip	1,000	No effect on growth	Stanley and Tapp, (1982)
Mung bean	10,000	No effect on growth over 28d	Windeatt, (1987)
Sorghum Sunflower	2,530 2,930	28d EC_{50} 28d EC_{50}	Windeatt, (1987)
Mung bean Sorghum Sunflower	10,000 10,000 10,000	No effect on seedling emergence (7d)	Windeatt, (1987)
Mustard	1,400 3,540	EC_5 EC_{50}	Anon, (1991)
Eisenia (earthworm)	1,000	No effect over 14d	Coulson *et al*, (1989)
Soil	400	No effect on soil respiration	Proctor and Gamble, (1992)
Soil	365	No effect over 14d	Täuber *et al*, (1986)

SECTION 7. ENVIRONMENTAL CONCENTRATION OF DHTDMAC

The determination of the concentrations of pollutants in the environment is required for performing sound hazard assessment of such substances. The availability of such data also makes it possible to check concentrations predicted by simple models.

Numerous environmental data related to DHTDMAC have been published. The earlier studies used analytical methods which were not so specific to DHTDMAC, unlike the methods now used (see section 3.1).

In the following sections only those measurements of DHTDMAC concentration are discussed which originate from studies where sample sites and sampling methods are sufficiently characterised and reliable DHTDMAC-specific analytical methods have been used. These methods usually measure total DHTDMAC in the sample (free and complexed bound).

It should be noted that most of the measured concentrations reported were determined prior to the major decrease in the consumption of DHTDMAC in Europe which started in 1990 (see section 2.3).

7.1. OCCURRENCE OF DHTDMAC IN SEWAGE TREATMENT PLANTS (STP)

As described earlier (sections 2 and 4) most DHTDMAC used is discharged into sewers and complexed with anionic compounds. The main part is then removed in sewage treatment plants. The concentrations reaching the aquatic environment in sewage effluent are further reduced by dilution, adsorption onto sediment and biodegradation. DHTDMAC will also reach the soil environment as a result of spreading sewage sludge on farmland where it can continue to biodegrade.

7.1.1. Influent/Effluent Concentrations

Since the influent and effluent concentrations of DHTDMAC in STPs can vary with the season, the day of the week (washing days) and weather conditions it is important to follow these variations in a monitoring exercise to obtain a realistic average concentration.

The measurements from eight monitoring studies indicated a typical average influent concentration ranging from 0.5 to 1.6mg/l. The influent concentrations found in Europe (1.0mg/l) are higher than those reported from the US (0.5mg/l). The lower figure in the US can be related to the higher per capita use of water which generally results in lower influent concentrations (Table 19).

Table 19 DHTDMAC Concentrations in the Influent of Sewage Treatment Plants

Year	Location	Observations	DHTDMAC (mg/l)		Reference
			Range	Mean	
1975-85	USA, (x12)[1]	139		0.5	Rapaport, (1987)
1975-85	USA, (x5)[1]		0.3-0.7	0.5	Versteeg *et al*, (1992)
1975-85	USA, (x5)[2]		0.4-2.6	1.0	Versteeg *et al*, (1992)
1978	UK, Alderley Edge[2]		0.7-1.9	1.4	Topping and Waters, (1982)
1979	D, Duelmen[2]		0.8-2.4	1.6	Topping and Waters, (1982)
1986	D, Lüdinghausen[2]			1.1	Matthijs *et al*, (1992)
1987	D, Lüdinghausen[2]		0.6-1.4	1.0	Matthijs *et al*, (1992)
1987	D, Waltrop[2]			1.4	Matthijs *et al*, (1992)
1991	NL, Bilthoven[3]		0.4-4.3		van Leeuwen *et al*, (1992)

1 Trickling filter plant
2 Activated sludge plant
3 Conditions not specified

The measured effluent concentrations of DHTDMAC from activated sludge plants summarised on Table 20 are very consistent around 0.05mg/l. The lower efficiency of trickling filters (see section 4.2.4) results in a higher average effluent concentration, around 0.14mg/l.

In Western Europe, the activated sludge process is the most prevalent method of waste-water treatment.

7.1.2. Waste and Anaerobic Digested Sludge

Monitoring studies conducted by Topping and Waters (1982), Rapaport (1987) and Matthijs (1992) detected mean DHTDMAC concentrations around 5g/kg in dry solids in waste sludge of STP's in Europe, and around 1g/kg in the USA. (See Table 21). The same authors found slightly higher concentrations of DHTDMAC (5-9g/kg in dry solids) in anaerobic digested sludge. (See Table 22)

Table 20 DHTDTMAC Concentrations in the Effluent of Sewage Treatment Plants

Year	Location	Observations	DHTDMAC (mg/l)		Reference
			Range	Mean	
1975-85	USA, (x7)[1]	59	0.02-0.22	0.14	Rapaport, (1987) Versteeg *et al*, (1992)
1975-85	USA, (x6)[2]	84	0.02-0.06	0.05	Rapaport, (1987)
1978	UK, Alderley Edge[2]		0.003-0.09	0.04	Topping and Waters, (1982)
1979	D, Duelmen[2]		0.04-0.12	0.09	Topping and Waters, (1982)
1986	D, Lüdinghausen[2]			0.06	Matthijs *et al*, (1992)
1987	D, Lüdinghausen[2]		0.03-0.05	0.04	Matthijs *et al*, (1992)
1987	D, Waltrop[2]			0.03	Matthijs *et al*, (1992)
1991	NL, Bilthoven[3]		0.01-0.06	0.04	van Leeuwen *et al*, (1992)

1 Trickling filter plant
2 Activated sludge plant
3 Conditions not specified

Table 21 DHTDMAC Concentrations in Aerobic Sludge

Year	Location	Observations	DHTDMAC (g/kg dry solids)		Reference
			Range	Mean	
1975-80	USA, (x4)	59		1	Rapaport, (1987)
1978	UK, Alderley Edge		2-4	3	Topping and Waters, (1982)
1979	D, Duelmen		6-10	8	Topping and Waters, (1982)
1986	D, Lüdinghausen			4	Matthijs *et al*, (1992)
1987	D, Lüdinghausen			4	Matthijs *et al*, (1992)
1987	D, Waltrop			4	Matthijs *et al*, (1992)

Table 22 DHTDMAC Concentrations in Anaerobic Sludge

Year	Location	Observations	DHTDMAC (g/kg dry solids)		Reference
			Range	Mean	
1975-80	USA, (x2)	19	2-4		Rapaport, (1987)
1986	D, Lüdinghausen			5	Matthijs *et al*, (1992)
1987	D, Lüdinghausen			4	Matthijs *et al*, (1992)
1987	D, Koblenz			9	Hellmann, (1989)

7.2 CONCENTRATION OF DHTDMAC IN SURFACE WATERS AND SEDIMENTS

Surface waters. Data from measurements of concentrations in the environment are difficult to interpret and to compare since they depend on many parameters such as sampling procedure, location (type of surface water, distance from a STP outlet) and time (season, day of the week).

As mentioned before, only the most recent measurements for which the sampling procedures are known and the methods of analysis are DHTDMAC-specific, have been taken into account and are summarised in Table 23.

The measured concentrations reported in surface waters are total concentrations, including DHTDMAC adsorbed onto suspended solids. The typical amounts of suspended solids in European rivers range from 5 to 50mg dry solids/litre. (Hellmann, 1990).

The total concentrations of DHTDMAC found depends on the type of surface water investigated (Klotz, 1990c; Matthijs, 1990b). In Europe, measured concentrations of DHTDMAC in large rivers are typically between 2 and 15µg/l depending on location. In small rivers the concentration range covers 20-30µg/l, and in surface waters with slow flow velocities and low dilution factors (canals and polders) the measured concentrations are higher. The highest concentrations (up to 116µg/l) were measured in a canal (Vallei kanaal) below the outfall of a sewage treatment plant and in a polder (Valburg). In these waters the oxygen concentration was only 67% and 42% of the saturation values respectively. The corresponding conductivities were 1,159µS/cm and 956µS/cm. Such DHTDMAC concentrations are therefore atypical since they were measured in highly polluted waters (Matthijs, 1990b; Versteeg *et al*, 1992).

Table 23 DHTDMAC Concentrations in River Water Including Suspended Solids

Year	Location	Observations	DHTDMAC (µg/l)		Reference
			Range	Mean	
1976-86	USA 3 below outfall 4 above outfall	43 31		56 34	Rapaport, (1987)
1978-81	USA, (x4)	38	<2-40		Lewis and Wee, (1983)
1981	D, (Rhein x 30 plus tributaries)		4-27	15	Kappeler, (1982)
1989/90	D, Main, Frankfurt Rhein, Main mouth	13 7	2-9 2-6	4 4	Klotz, (1990c)
1990	D, Rhein, NL-border			10	Hellmann, (1990)
1990	NL, large rivers rivers tributaries canals polders		15-25 22-52 11-48 15-116 17-114	20 30 27 43 56	Matthijs (1990b) Versteeg *et al*, (1992)
1990/91	NL Rhine, Meuse, Schelde		2-34		van Leeuwen *et al*, (1992)

Kappeler (1982) and Hellmann (1990) indicated that more than 90% of the DHTDMAC found in surface water is adsorbed onto particulates and suspended solids, the amount adsorbed being dependent on the amount and nature of the particles present.

Usually the "adsorbed" fraction of a substance is considered as not being available to exert its toxicity. As described in section 2 non-adsorbed DHTDMAC in surface waters may be present either in the form of colloidal particles or complexed with anionic constituents of the surface water. Therefore most of the non-adsorbed DHTDMAC may also have a bioavailability to aquatic species different from the parent compound tested in laboratory conditions.

Suspended solids and sediments. Both suspended solids and sediments vary in composition and particle size according to season and weather conditions. Generally suspended solids are more organic in character with a smaller particle size than sediments. Due to the fact that a smaller particle size leads to a higher degree of adsorption per unit weight and a slower settling rate, a large variation of concentrations in the sediment and

suspended solids can be expected. Thus measured concentrations of DHTDMAC in sediments will vary with the location and the sampling procedure (i.e. the depth of sediment sampling). This is supported by the concentrations measured in sediments (Tables 24 and 25) which vary from almost zero to 200mg/kg. Klotz (1990c) found levels of DHTDMAC in suspended solids comparable to the levels reported for sediments.

Table 24 DHTDMAC Concentrations in River Sediments

Year	River	Observations	DHTDMAC (mg/kg)		Reference
			Range	Mean	
1976-82	USA 3 below outfall 1 above outfall	47 13	2-5	115	Rapaport, (1987)
1978-81	USA Rapid Creek		3-67		Lewis and Wee, (1983)
1987	B Dender, Schelde, Zwalm	3	11-67		Waters *et al*, (1992)
1987	D Rhein, Iffezheim	1		78	Klotz, (1990c)

Table 25 DHTDMAC Concentrations in River Suspended Solids

Year	River	Observations	DHTDMAC (mg/kg)		Reference
			Range	Mean	
1989-90	D, Main	13	11-201	85	Klotz, (1990c)
1990	D, Elbe Weser Mittelrhein		 80-100 50-150	20	Hellmann, (1990)

7.3. CONCENTRATIONS IN SLUDGE-AMENDED SOIL (FARMLAND)

Measurements were performed in soils in the USA in 1979, at a time when semi- quantitative analytical methods were employed (probably DBAS/TLC). The average of 86mg/kg dry weight reported at the surface is an average of analyses in three grab-samples taken at depths of between 0 and 8cm. At a depth of 90cm, the reported average concentration was less than 1mg/kg, indicating that DHTDMAC is unlikely to penetrate deeply in soils (Rapaport, 1987).

These values seem high compared to the more recent values reported in Europe (averages of 10 to 15mg/kg dry weight). Five fields were monitored at regular time intervals to follow

the disappearance of DHTDMAC after sludge application using DHTDMAC specific analytical methods as described in section 3.1 (Matthijs, 1990a).

An accumulation of 15mg/kg soil per year was calculated assuming a soil bulk density of 1,200kg/m^3 and 6mg DHTDMAC/g sludge. This corresponded to an average expected level in soil of 50mg/kg assuming no disappearance. The range of concentrations measured went from less than 2mg/kg to a maximum of 37mg/kg. In the 42 fields that received their last sludge application prior to 1987 (the year of sampling), 95% of the samples contained less than 20mg DHTDMAC/kg and 62% had less than 10mg DHTDMAC/kg. The average concentration was 9.5mg/kg. In the 9 fields that received their last application of sludge in 1987, concentrations ranged from about 2mg/kg to 33mg/kg with an average of about 3.3mg/kg. These data are in agreement with those reported by Klotz (1987b, 1988) for various locations in Germany (Table 26).

Table 26 DHTDMAC Concentrations in Sludge Amended Solids

Year	Location	Observations	DHTDMAC (mg/kg)		Reference
			Range	Mean	
1979	USA Rapid City (ND)	3 3	32-164	86 <1	Rapaport, (1987)
1987	D, Lüdinghausen	6	<1-24	12	Klotz, (1987b)
1987	UK, Thames Water Authority	42 8	<2-37 2-33	9.5 13.3	Matthijs, (1990a) Matthijs, (1990a)
1987	D Around Frankfurt	3	2-22	10	Klotz, (1988)

Overall, these data indicate that DHTDMAC does not accumulate in soils.

7.4. CONCENTRATIONS IN GROUND WATER AND DRINKING WATER

Due to the highly adsorptive character of DHTDMAC, a very low penetration is expected into ground water.

This is supported by the data published by Lewis *et al* (1989). They report levels in ground water lower than detection limit (4µg/l). At one location samples were taken directly beneath the tile field of a septic tank and at a distance of 70 metres down-gradient. At another

location samples were taken immediately below the sand bed of a trickling filter effluent discharge and 4,500 metres down-gradient.

Versteegh *et al* (1992) published measured levels of DHTDMAC in raw water used for the production of drinking water. Two types of water were investigated: surface waters and surface waters after bank filtration. While the surface waters showed concentrations ranging from less than 1.1µg/l to 21.8µg/l (average 8.4µg/l), the bank filtrates showed a range from less than 1.1µg/l to 14.4µg/l (average 3.3µg/l).

Versteegh *et al* (1992) also reported concentrations of DHTDMAC in drinking water produced from both the surface waters and the bank filtrates. The levels of DHTDMAC after treatment range from less than 1.1µg/l (detection limit) to 6.0µg/l with averages of 2.8µg/l in the water from surface waters and 1.9µg/l from the bank filtrates. The analytical method used by Versteegh *et al* was state of the art HPLC but the sample preparation was not optimal and the reported values may deviate from the true values by ± 15% (Matthijs *et al*, 1992).

A concentration of 0.6µg/l DHTDMAC has been reported for an American drinking water which was prepared from a raw water containing 3.2µg/l (Rapaport, 1987). These figures are averages of eight measurements by DBAS/TLC. The source of the raw water was probably surface water.

SECTION 8. ENVIRONMENTAL HAZARD ASSESSMENT

8.1. INTRODUCTION

Environmental hazard assessment is a two-step process in which exposure assessment and effects assessment are integrated.

Exposure Assessment. The objective of exposure assessment is to identify the environmental compartments of concern and to predict environmental concentrations (PEC) of a chemical in these compartments. Various models have been developed to predict environmental concentrations (OECD, 1989; ECETOC, 1993).

Effects Assessment. The objective of effects assessment is to estimate the concentration of a chemical in the environment which will have no adverse effects on ecosystems (NEC). Since this concentration cannot be determined exactly the estimate is known as the predicted NEC (PNEC).

The PNEC is generally derived from No-Observed-Effect Concentrations (NOEC) or LC_{50} and EC_{50} values determined in single-species laboratory tests. The PNEC for ecosystems is derived from these laboratory data by applying arbitrary "extrapolation factors" or "safety factors" which (in published methods) range between 5 and 1,000 depending on the number of species tested and on whether the tests were acute or chronic. Safety factors are intended to allow for the limited variety of responses covered by laboratory tests and their limitations in representing ecosystems.

The various extrapolation techniques which are currently available to estimate the NEC have recently been critically evaluated and described by OECD (1991a) and ECETOC (1993).

Hazard Assessment. Hazard assessment in the sense agreed at the EEC Workshop (Ispra, 1990) is generally expressed as a comparison of the predicted environmental concentration with the predicted no effect concentration in comparable systems.

For simple substances in simple situations this may be done by comparing laboratory aquatic toxicity results with predictions (PEC) or measurements (MEC) of aquatic concentrations in the environement.

Such simple straight-forward treatment is not possible for DHTDMAC because of :

- strong adsorption to solid material in the envrionment (see Section 2.2.3);
- irreversible adsorption (see Section 2.2.3);

- complexation (see Section 2.2.4);
- physical state (see Section 2.2.1 - 2.2.2);
- the manner in which the substance enters the environment which cannot be reproduced in laboratory tests (see Section 2.3; 2.4). This has a profound effect on how toxic effects may be expressed (see Section 5.2).

Hence for PEC/PNEC comparisons with such substances it is necessary to adopt pragmatic but valid approaches in which the effects of these different factors are integrated. This is done below in the following discussion of the derivation of PNEC and PEC values.

8.2. PREDICTION OF ENVIRONMENTAL CONCENTRATIONS - EXPOSURE ASSESSMENT

Until 1990, fabric softeners accounted for over 90% of the tonnage released to the environment. In this ECETOC report, the tonnage figures from Table 27 are used. They are based on the use of DHTDMAC in fabric softeners during the period 7/1989-6/1990. Since then, market changes have resulted in significant reductions in tonnages (Section 2.3).

In order to predict average concentrations in rivers just below sewage outfalls, classic modelling concepts for detergent chemicals can be used (Holman, 1981). Predictions are derived from estimates of the tonnage of chemical usage, per capita waste water flow rate, incidence and type of sewage treatment (i.e. primary treatment only, primary + secondary and/or tertiary treatment) and dilution by receiving waters.

Dilution by receiving waters is the main source of uncertainty in predicting environmental concentrations just below sewage outfalls. In the conclusions of the EEC Workshop on Environmental Hazard and Risk Assessment (Ispra, 1990), an average dilution factor of 30 was recommended for the worst-case initial environmental exposure assessment of new chemicals. However, more stringent conditions are assessed below.

The predicted waste water treatment plant influent and effluent concentrations of DHTDMAC and river concentrations below the mixing zone have been calculated for European countries (Table 27).

Average concentrations in river water below the mixing zone were predicted under various dilution conditions, i.e. dilution factors 1:30, 1:10 and 1:3. Figures on per capita waste water flow rate were taken from OECD (1991b).

Table 27 Prediction of Environmental DHTDMAC Concentrations (mg/l) in Various European Countries

European country:	Germany	Netherlands	France	U.K.	Italy
Tonnage of DHTDMAC (Jul.89-Jun.90)	12,000	2,000	9,400	8,600	4,300
Population x10^6 1990[1]	61	15	56	57	58
Influent STPs (1/capita/day)[2]	285	252[4]	265	328	458
Degree of connection to STPs (%)[3]	90	92	52	84	60
Incidence of STPs (%of total capacity)[3]					
- Primary	2.3	2	6.6[5]	7.1	30[7]
- Secondary and/or tertiary	97.7	91	86.1[5]	92.9	70[7]
- Trickling filters		7	7.3[5]		
% Removal of DHTDMAC during sewage treatment steps[6]					
- Primary	31				
- Primary + secondary and/or tertiary	95				
- Trickling filters	74				
Sewage treatment concentrations (mg/l)					
- Influent conc. (C_i; raw sewage)	2.156	1.468	1.825	1.640	0.818
- Primary treatment effluent	1.487	1.013	1.259	1.132	0.564
- Secondary and/or tert. treatment effluent	0.108	0.073	0.091	0.082	0.041
- Trickling filter treated effluent		0.382	0.475		
River water conc. (mg/l) at dilution 1:30 at the mixing zone					
- below primary treatment outfall	0.049	0.034	0.042	0.038	0.019
- below sec. and/or tert. treatment outfall	0.004	0.002	0.003	0.003	0.001
River water conc. (mg/l) at dilution 1:10					
- below primary treatment outfall	0.149	0.101	0.126	0.003	0.056
- below sec and:or tert. treatment outfall	0.011	0.007	0.009	0.008	0.004
River water conc. (mg/l) at dilution 1:3					
- below primary treatment outfall	0.496	0.338	0.420	0.377	0.188
- below sec; and/or tert. treatment outfall	0.036	0.024	0.030	0.027	0.014

1 Taken from 'Le Monde Bilan Economique et Social 1991, Dossiers et Documents du Monde'. Jan 1992 - 17e année. (Figure of Germany is for the year 1988.)

2 Calculated from 'OECD Envrionmental Data 1991' on total water withdrawal by major uses in the late 1980s by using the following formula:-

$$\left[\frac{\text{total water withdrawal/capita/year}}{\text{365 days/year}}\right] \times \left[\frac{\text{\% of population served by STP}}{100}\right] \times \ldots$$

$$\ldots \frac{(\text{\% Public water supply}) + (\text{\% Industrial water supply [no cooling]})}{100}$$

All calculations of sewage treatment concentrations and river water concentrations are based on a sewage flow of 250l/inhabitant/day for the various countries.

3 Taken from 'OECD Environmental Data 1991'

4 Taken from the Netherlands 'Environmental Statistics' (CBS, 1990, part b)

5 Taken from 1986 French data on the available wastewater treatment plants

6 Average removal based on monitoring data in several STP's in Germany, UK and US.

7 Estimated figures

The waste water treatment plant influent concentration (raw sewage, C_i) was calculated as follows:

$$C_i = \frac{T \times 10^9}{D \times F \times P}$$

T = estimated tonnage of DHTDMAC released to the environment from July 1989 to June 1990.

10^9 = factor for conversion to mg/l

D = number of days in a year (365)

F = per capita waste water flow rate (l/capita/day)

P = population of country

From the DHTDMAC concentration in influent waste water, the waste water treatment plant effluent concentration (C_e) can be calculated as follows:

$$C_e = C_i \times (1 - R)$$

C_i = concentration in influent waste water (mg/l)

R = fraction of DHTDMAC removed from the water by sewage treatment (% removed/100)

The average sewage treatment removal efficiency for DHTDMAC was based on monitoring data in several activated sludge treatment plants in Germany, UK and the US. The removal efficiency after primary treatment (mechanical) ranged from 19% to 51% with an average of 31%. The combination of mechanical and biological treatment (primary + secondary and/or tertiary treatment) gave an average removal efficiency of 95%, whereas trickling filters had an average removal efficiency of 74% (section 3.1.3). These removal figures based on monitoring data are in agreement with removal calculated by a general fate model for detergent chemicals (Cowan *et al*, 1992), which predicts 35% and 95% removal after primary and primary secondary treatment respectively.

The calculated concentrations in sewage treatment plant influent (raw sewage) are 0.82-2.16mg/l in the various European countries (Table 27). Similar concentration ranges (0.3-4.3mg/l) were measured in sewage treatment plant influents in Germany, The Netherlands, UK and the USA (Table 19). Calculated concentrations in the effluent after primary treatment (0.56-1.49mg/l) or secondary treatment (0.041-0.1mg/l) are also in agreement with concentrations measured in effluents from sewage treatment plants, i.e. 0.26-1.70mg/l

(Topping and Waters, 1982) versus 0.010-0.120mg/l in secondary effluent (Table 20) (Topping and Waters, 1982, Van Leeuwen *et al*, 1992).

The average predicted concentration in rivers below the mixing zone ranges from 0.004 to 0.007mg/l, assuming a 1:30 dilution. These values correspond to the measured environmental concentrations in large rivers in Germany, UK and the Netherlands, (Table 23). The highest concentrations can be expected after discharge into receiving waters with low flow rates, like small rivers, canals or tributaries. Assuming a worst-case dilution of 1:3, the predicted average surface water concentration will reach up to 0.038-0.066mg/l (Table 27). Recent measurements of DHTDMAC concentrations in receiving waters with low flow rates in the Netherlands (Table 23) lie within that range.

8.3. AQUATIC EFFECTS ASSESSMENT

A considerable amount of data is available for DHTDMAC.

Various methods have been proposed for the extrapolation from single-species laboratory toxicity tests to safe concentrations for aquatic ecosystems. They are extensively discussed in the report of the OECD Workshop on the Extrapolation of Laboratory Aquatic Toxicity Data to the Real Environment (OECD, 1991a). For the reasons described earlier (Section 5.2) toxicity data derived from laboratory studies using artificial aquatic media indicate the inherent toxicity of a substance such as DHTDMAC and not the degree of toxicity likely to occur in any more natural systems.

Care must therefore be exercised in applying any extrapolation technique, statistical or by the use of factors, to derive PNECs from such data.

Statistical extrapolation techniques may eventually provide an effective way of estimating PNECs from relatively small data bases but for DHTDMAC the assumptions required are not met. A discussion of these assumptions is set out below. The main points for discussion on the use of the OECD extrapolation methods in this case are:

- It is assumed that test species are randomly selected from an ecosystem. In fact, the selection of species for use in single-species laboratory toxicity studies is based on their relative ease of maintenance in the laboratory and their sensitivity towards toxicants. Relatively tolerant species tend not to be used.

- It is assumed that experimental variation (measuring errors, varying test conditions, etc.) is negligible compared to the variation in sensitivity between species. However, the results presented in section 5.1 show that the experimental variation with

DHTDMAC can be considerable. In addition, the use of NOEC values in the statistical extrapolation methods introduces an additional source of variation as different effects (or 'endpoints', e.g. growth, reproduction or behaviour) are measured for each species and sometimes within a species. NOEC-values are empirically defined as the highest concentration which has no effect in a toxicity test. Their values therefore depend on the design of the experiment (e.g., the ratio selected between the test concentrations).

- Interactions between species in ecosystems are not taken into account.

- It is questionable whether the assumption of a log-logistic or log-normal distribution of NOEC data is true because there are rarely enough data to prove or disprove the nul-hypothesis. (A typical "rule of thumb" when determining whether or not data are parametrically distributed is that at least 30 data points are required). For chemicals with a specific mode of action (e.g. pesticides) it has been shown that the available data do not fit the log-logistic distribution (Leeuwangh, 1992).

- Intra-species genetic variation is not considered.

- The possibility that a species may become tolerant to a toxic material in the environment is not considered.

The most important problem to address in deriving a PNEC for DHTDMAC is the difference between bioavailability in laboratory studies and the real environment. Given this issue, and those discussed above, it was decided to resort to the simple comparison of PEC and PNEC where the latter was obtained from the most realistic long-term study so far available (Versteeg and Woltering, 1990). This study was characterised by the most realistic means of addition of DHTDMAC to a test system, i.e. via a sewage treatment plant. In these experiments, CAS units were fed with sewage dosed with artificially high levels of DHTDMAC from detergent manufacturing plant wastes. The effluent from these CAS units was then used both undiluted and diluted with river water to perfrom chronic toxicity tests. The lowest NOEC determined for *Selenastrum capricornutum* (96h., growth inhibition) and *Ceriodaphnia dubia* (7d., mortality and reproduction) where 20.3mg/l and 4.53mg/l respectively. The range of suspended solid levels in these tests was from ~2mg/l to ~95mg/l, covering typical ranges of suspended solids from clean to heavily polluted rivers.

Thus, the chronic "practical" NOEC value for the most sensitive species (*Ceriodaphnia*) tested in realistic conditions is 4.53mg/l. This result can be taken as base value recognising however, that the solubility limit of DHTDMAC is 1μg/l and that the mechanisms of bioavailability of DHTDMAC under such conditions are not understood.

This chronic NOEL of 4.53mg/l for *Ceriodaphnia* is 20 to 25 fold higher than EC_{20} and NOEC values obtained in other tests with *Ceriodaphnia* and *Daphnia magna* (Table 12) but where DHTDMAC was directly added to the dilution water. These studies, demonstrate that the chronic toxic effects of DHTDMAC are significantly reduced after activated sludge treatment and release in an effluent, even when compared with the results from standard laboratory toxicity studies in river water.

8.4. ENVIRONMENTAL HAZARD ASSESSMENT

8.4.1. Surface Water

From Section 8.3 the lowest chronic "practical" NOEC value was 4.53mg/l. Using the approach outlined by ECETOC (1993) a PNEC from chronic studies is derived by dividing the NOEC by 5, to give, in this case, a PNEC of 0.906mg/l. The ratios between the PNEC or NOEC and the PEC are given below. The PEC values (0.002-0.11mg/l) are derived from the data summarised in Table 23.

PEC (mg/l)	NOEC (mg/l)	PNEC (mg/l)	Ratio (Safety factor)	
			NOEC/PEC	PNEC/PEC
0.002 - 0.11	4.53	0.906	41.2 - 2,250	8.2 - 450

In order to demonstrate worst-case conditions the following presents the same type of calculation but using figures for undiluted and poorly diluted effluent following secondary sewage treatment in Germany (Table 27).

PEC (mg/l)	NOEC (mg/l)	PNEC (mg/l)	Ratio (Safety factor)	
			NOEC/PEC	PNEC/PEC
Undiluted 0.108	4.53	0.906	41.9	8.4
3-fold dilution 0.036	4.53	0.906	125	25.2
10-fold dilution 0.011	4.53	0.906	412	82.4

Given the conservative nature of both PEC and PNEC, any factor greater than 1.0 should be predictive of no significant harm in the freshwater environment.

Furthermore given that marine species do not demonstrate any greater sensitivity to DHTDMAC than freshwater species (Tables 11 to 13), and that river waters will be diluted many-fold as they enter estuaries and the sea, no significant harm is predicted from the presence of DHTDMAC in the marine environment.

A well documented case of risk assessment of DHTDMAC has been discussed for the Netherlands.

In the Netherlands the maximum permissible level of a chemical for the 'general environmental quality' is reached if the concentration of the chemical in the environment reaches the 95% species protection level (Premises for Risk Management, 1989). The Directorate-General for Environmental Protection in the Netherlands concludes in its ecotoxicological risk evaluation for DHTDMAC that the maximum permissible risk level for DHTDMAC (range 0.02-0.1mg/l, average of 0.05mg/l based on 5 different extrapolation models) is exceeded in 20% of the surface waters in the Netherlands (van Leeuwen *et al*, 1992). However, at the time the risk assessment was made the following was not considered:

- the real solubility of DHTDMAC in water is extremely low (see section 2) and single species toxicity studies were conducted at concentrations which exceeded the solubility in water by at least a factor 1,000. As discussed in section 5, the bioavailability of DHTDMAC in the environment is much lower than in laboratory toxicity tests.

- the dilution model used to calculate the environmental concentrations of DHTDMAC did not account for instream removal processes (e.g., biodegradation, adsorption, settling of suspended sediments) nor for background concentrations of DHTDMAC due to discharge from upstream locations.

Based on a new environmental fate model, which accounts for instream removal and background concentrations of DHTDMAC, Versteeg *et al* (1992) calculated the 90th percentile river DHTDMAC concentration at 1,000m below waste water outfalls as 0.021mg/l. This means that only at 10% of the sites in The Netherlands would this level be exceeded and that less than 3% of the sites (i.e., only 13 locations) would have DHTDMAC concentrations above 0.05mg/l (Versteeg *et al*, 1992). The latter concentration represents the maximum permissible level or 95% species protection level as calculated by statistical extrapolation from NOEC's derived from toxicity tests with various aquatic species (van Leeuwen *et al*, 1992).

It should be noted that these 13 locations are representative of systems with low dilution (dilution factor < 5) and low water velocity (10-200m/day) such as polders, canals and tributaries which mainly receive discharges of poorly treated effluent from primary or trickling filter waste water treatment plants. These surface waters do not meet basic water quality criteria due to the presence of high BOD and nitrogen concentrations (see section 7.2).

Based on a comprehensive environmental exposure model, which accounts for instream removal and upstream background DHTDMAC concentrations, it was predicted that less than 3% of the sites in The Netherlands would have DHTDMAC levels greater than 0.05mg/l at 1,000m below sewage treatment outfall (Versteeg *et al*, 1992). This predicted value corresponds to the highest measured concentrations of DHTDMAC in low dilution polders and tributaries in The Netherlands (range 0.011-0.1mg/l; (Matthijs, 1990b; Versteeg *et al*, 1992). If this worst-case environmental concentration is compared with the chronic no-effect concentration determined for the most sensitive species under environmentally realistic exposure conditions (section 8.3), the DHTDMAC safety factor for aquatic species is 90 (PNEC/PEC = 4.53/0.050).

Even with a conservative approach, using an average stream dilution factor, not correcting for instream removal and background concentrations, and comparing the predicted environmental concentration with the 95% species protection level extrapolated from single-species laboratory toxicity studies, the no effect concentration for DHTDMAC is only approached at low dilution factor streams (dilution factor ≤3) or at sites which receive poorly treated effluents. At these sites water quality is compromised mainly by other factors such as high BOD and ammonia concentrations. It can be expected that the situation at these locations will improve significantly in the forthcoming years, since the EC has adopted a Directive (91/271/EEC) on the implementation of adequate waste water treatment in all EC member states.

Laboratory studies have shown that DHTDMAC is inherently and completely biodegradable under aerobic conditions. No stable intermediates are formed in the environment (section 4.6). Measurements of DHTDMAC in superficial sediments (Osburn, 1982 and Federle and Pastwa, 1988: discussed in Section 4.3.3) suggest that DHTDMAC is also biodegraded in this environmental compartment. However, a realistic study of the biodegradation of DHTDMAC in sediments, under conditions that will preserve the sedimentary structure and microbial diversity, has never been conducted.

8.4.2. Soil

Studies on terrestrial organisms (i.e., higher plants and earthworms) have indicated that there exists a large safety margin (three to four orders of magnitude) between the predicted environmental levels of DHTDMAC in soil and the highest levels tested, which were not toxic to these species (see section 6). In addition, no risk of bioaccumulation via the food chain can be expected. Similarly, benthic organisms (i.e. larvae of the midge *Paratanytarsus parthenogenica*) exposed to sediments containing 2 to 67ppm DHTDMAC showed no effects on the survival and adult emergence (Lewis and Wee, 1983). Even levels of DHTDMAC up to 20% on organic sediments did not cause adverse effects on the larval stages, the pupa or emergence of adults in life-cycle toxicity tests with the midge *Chironomus riparius* (Lee, 1986). These data show that substantial safety margins (100-1,000) exist for benthic organisms. This is in agreement with the results of toxicity tests with mid-water species (algae and *Daphnia*) under realistic environmental conditions described earlier (section 5.2). The data confirm that adsorption of DHTDMAC to activated sludge or sediments greatly reduces the bioavailability and thereby the toxic effects to aquatic species.

8.4.3. Conclusions

Based on the above considerations, it can be concluded that the environmental concentrations of DHTDMAC discussed do not pose a hazard to aquatic and terrestrial ecosystems.

ABBREVIATIONS USED

AC	Algicidal Concentration
BAS	Batch Activated Sludge test
BOD	Biochemical Oxygen Demand
C	Chronic
CAS	Continuous Activated Sludge unit
CEC	Cationic Exchange Capacity
DCDMAC	DiCoco DiMethyl Ammonium Chloride
DDdDMAB	DiDodecyl DiMethyl Ammonium Bromide
DDDMAC	DiDecyl DiMethyl Ammonium Chloride
DdTMAC	Dodecyl TriMethyl Ammonium Chloride
DHDMAC	DiHexadecyl DiMethyl Ammonium Chloride
DHTDMAA	DiHydrogenated Tallow DiMethyl Ammonium Acetate
DHTDMAC	DiHydrogenated Tallow DiMethyl Ammonium Chloride
DOC	Dissolved Organic Carbon
DODMAB	DiOctadecyl DiMethyl Ammonium Bromide
DODMAC	DiOctadecylDiMethyl Ammonium Chloride
DSBAS	Di Sulfine Blue Active Substances
DSDMAC	DiStearyl DiMethyl Ammonium Chloride
DTdDMAC	DiTetradecyl Dimethyl Ammonium Chloride
DTDMAC	DiTallow DiMethyl Ammonium Cloride
GLP	Good Laboratory Practice
HPLC	High Pressure Liquid Chromatography
HTMAB	Hexadecyl TriMethyl Ammonium Bromide
IPA	IsoPropyl Alcohol
LAS(ABS)	Linear Alkylbenzene Sulfonate
LOEC	Lowest 'Observed' Effect Concentration
LSC	Liquid Scintillation Counter
MEC	Measured Environmental Concentration
MHTTMAC	monoHydrogenated Tallow TriMethyl Ammonium Chloride
MiAC	Minimum Algistatic Concentration
MLSS	Mixed Liquor Suspended Solids
MTTMAC	monoTallow TriMethyl Ammonium Chloride
NEC/NOEC	No Effect Concentration
NOEC	No 'Observed' Effect Concentration
OTMAC	Octadecyl TriMethyl Ammonium Chloride
PEC	Predicted Environmental Concentration
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
RAFS	Rinse Added Fabric Softener
SAS	Secondary Alkane Sulfonate
SC	Sub Chronic
SCAS	Semicontinous Activated Slugde Unit
SDS	Sodium Dodecyl Sulfonate
SS	Suspended Solids
STP	Sewage Treatment Plant
TEG	Tri Ethylene Glycol
TLC	Thin Layer Chromatography
TOC	Total Organic Carbon

BIBLIOGRAPHY

Akzo (1987). Acute toxicity of Arquad 2HT-75 to rainbow trout. Report NA 86 9835/3. Akzo Chemicals International. BV. Amersfoort. The Netherlands.

Akzo (1990a). Algal growth inhibition test with distearyl dimethyl ammonium chloride (DSDMAC). Report CRL F90096. Chemicals International. BV. Amersfoort. The Netherlands.

Akzo (1990b). Algal growth inhibition test with octadecyltrimethylammonium chloride (MSTMAC). Report CRL F90097. Chemicals International. BV. Amersfoort. The Netherlands.

Akzo (1991a). Algal growth inhibition test with DHTDMAC. Report CRL F91004. Chemicals International. BV. Amersfoort. The Netherlands.

Akzo (1991b). Alga growth inhibition test with DHTDMAC. Report R90/364. Chemicals International. BV. Amersfoort. The Netherlands.

Akzo (1991c). *Daphnia* reproduction study with DHTDMAC. Report R90/287. Chemicals International. BV. Amersfoort. The Netherlands.

Anon (1991). Pflanzenschaedigende Wirkung ausgewaehlter Tenside. Umwelt, 9/91, 395.

Atochem (1990a). Test report: Biodegradabilité "ultime" et "primaire" du NORAMIUM M2SH en maquettes de laboratoire de station d'épuration à boues activées,. Atochem. Centre d'application de Levallois. France.

Atochem (1990b). Acute toxicity of Noramium M2SH to Daphnia magna. Report 1/10/90. Atochem centre d'application de Levallois. France.

Baleux, B., and Caumette, P. (1977). Biodégradation de quelques agents de surface cationiques, Water Research, 11, 833 - 841.

Berol Nobel (1990a). Internal data. Personal communication. Dr. J. Rosenblom. Berol Nobel, Nacka, Sweden.

Berol Nobel (1990b). Acute toxicity of DHTDMAC to *Daphnia magna*. Report 116/52. Berol Nobel 4B, Nacka Sweden.

Brinkman, U.A.Th., De Ruiter, C., Hefkens, J.C.H.F., Frei, R.W., Evers, M., Matthijs, E., Meijer, J.A. (1987). Liquid chromatographic determination of cationic surfactants in environmental samples using a continous post-column ion-pair extraction detector with sandwich phase separator. Intern. J. Environ. Anal. Chem. 31, 325-339.

Brown, G. W. (1975) Studies on the ultimate biodegradation of DTDMAC using 14C tagged distearyl and ditallow dimethyl ammonium chloride in an aqueous system, Procter and Gamble internal report, December 24. Procter and Gamble European Technical Centre, Professional and Regulatory Services, Brussels.

Buecking, H.W., Lötzsch, K. and Täuber G. (1978). Sorption and desorptions phenomena of DSDMAC. VIII Jornadas del Comité Español de la Detergencia, 33-46.

Capovilla, L., Labbé, P. and Reverdy, G. (1991). Formation of Cationic/Anionic Mixed Surfactant Bilayers on Laponite Clay Suspensions, Langmuir, 7, 2000-2003.

CBS (1990). Dutch Central Office for Statistics. Water Quality Management. Part B. Sewage Treatment, 1988.

Clancy, S. F., and Tanner, D. A. (1991). Determination of surfactant biodegradability, Sherex, internal data. Sherex Chemical Co. Inc. Dublin OH 43017-0646.

Coulson, J.M., Yearsdon, H.A., Edwards, P.J., Hill, R.W. (1989). Determination of toxicity to the earthworm *Eisenia foetida*. ICI Contract Report BL/B/3559 for Unilever Research - BIOD/82/06. 13pp.

Cowan, C.E., Larson, R.J., Feijtel, T.C.J. and Rapaport, R.A. (1992). An improved model for predicting the fate of consumer product chemicals in waste water treatment plants. Wat. Res. (Submitted).

Cruz, R. J. (1979). Contaminación de los cursos de aguas naturales por los detergentes sintéticos. XVI. Influencia de la de la concentración de tensioactivo y de la aclimatación previa de los microorganismos en la biodegradación de agentes tensioactivos catiónicos en agua de rió, Grasas y Aceites, 30, 293-299.

Cruz, R. J. (1981). Contaminación de los cursos de aguas naturales por los detergentes sintéticos. XVII. Influencia de la temperatura y otras variables experimentales en la biodegradación de agentes tensioactivos catiónicos en agua de rió, Grasas y Aceites, 32, 147-153,

Cruz, R. J. and Garcia, D. M. C. (1979). Contaminación de los cursos de aguas naturales por los detergentes sintéticos. XV. Relación entre estructúra y biodegradación de tensioactivos cationicós en agua de rió, Grasas y Aceites, 30, 67-74.

Dean-Raymond, D. and Alexander, M. (1977). Bacterial metabolism of quaternary ammonium compounds, Applied and Envrionmental Microbiology, 33, 1037-1041.

Dubois, M., and Zemb Th. (1991). Phase behaviour and Scattering of Double-Chain Surfactants in Diluted Aqueous Solutions; Langmuir, 7, 1352-1360.

EC (1991). Council Directive of the European Community 91/271/EEC. Official Journal of EEC Nr. L 135/40 (1991).

ECETOC (1993). Environmental Hazard Assessment of Substances. Technical Report 51, ECETOC , Brussels, Belgium.

Evans E. and Needham D. (1987). Physical properties of surfactant bilayer membranes: thermal transitions, elasticity, rigidity, cohesion and colloidal interactions. J. Phys. Chem., 91, 4219-4228.

Federle, T. W., Pastwa, G. M. (1988). Biodegradation of surfactants in saturated subsurface sediments: a field study, Ground Water, 26, 761-770.

Fieler, G. M. (1975a). The fate of DHTDMAC in sewage treatment processes. Internal report. Procter and Gamble Technical Centre, Professional and Regulatory Services, Brussels.

Fieler, G. M. (1975b). DTDMAC soil degradation studies, Procter and Gamble internal report. Procter and Gamble Technical Centre, Professional and Regulatory Services, Brussels.

Fina (1989). Acute toxicity of dihydrogenated tallow dimethyl ammonium chloride to *Daphnia magna* (DHTDMAC). Report B.7113. Sanofi Research, Montpellier, France.

Fontell, K., Ceglie, A., Lindman, B. and Ninham, B. (1986). Some Observations on Phase Diagrams and Structure in Binary and Ternary Systems of Didodecyldimethylammonium Bromide; A. Chem. Scand., A40, 247-256.

Games L. M., King, J.E. and Larson, R.J. (1982). Fate and Distribution of a Quaternary Ammonium Surfactant, Octadecyltrimethylammonium Chloride (OTAC), in Waste Water Treatment; Environ. Sci. Techn., 16, 483-88.

Gerike, P., Fischer W. K., Jasiak, W. (1978). Surfactant quaternary ammonium salts in aerobic sewage digestion, Water Res., 12, 1117-1122.

Hellmann, H. (1989). Fortschritte bei der Bestimmung von Kation- und Aniontensiden in Sedimenten, Schwebstoffen und Schlaemmen z. Wasser-Abwasser-Forsch. 22, 131-137.

Hellmann, H. (1990). Konzentration von Tensiden in Oberflaechengewässern, Tenside Surf. Det. 27, 318-323.

Hoechst (1991). Octadecaminium; Dimethyl-Octadecyl-Ammonium Chloride AIDA. Grunddatensatz Publ. VCI, Frankfurt, Germany.

Hohm, G. (1990). Duennschichtchromatographische Methoden bei der qualitativen und quantitativen Untersuchung von tensidhaltgien Praeparaten. Seifen-Ole-Fette-Wachse 116, 273-280.

Holman, W. F. (1978). The biodegradability of 14C labelled DSDMAC and Varisoft 445 in activated sludge, Procter and Gamble Technical Report. Procter and Gamble, European Technical Centre, Professional and Regulatory Services, Brussels.

Holman, W.F. (1981). Estimating the environmental concentrations of consumer product components. In: Aquatic Toxicology and Hazard Assessment: Fourth Conference. ASTM STP 737. Eds. Branson, D.R. and Dickson, K.L., American Society for Testing and Materials, p.159.

Hopping, W.D. (1975). DHTDMAC treatability by activated sludge. Procter and Gamble, Internal report. European Technical Centre Professional and Regulatory Services, Brussels

Ispra (1990). Environmental Hazard and Risk Assessment. EEC Workshop Ispra October 1990. Document XI/730/89.

Janicke and Hilge (1979). Biologisches Abbauverhalten von Anion/kationtensidekomplexe unter Aeroben und Anaeroben Bedingungen der Abwasser Tenside Detergents 16, 117-122.

Kao Corp. (1990). Acute toxicity of DHTDMAC to *Daphnia* and Rainbow trout. Report AT309/004 and AT 309/005 Kao Corporation SA, Puig dels Tudons, 10: 08210 Barbera de Vallès (Barcelona).

Kappeler, T. U. (1982). Die Aquatische Toxicität von DSDMAC und ihre Ökologische Bedeutung. Tenside Deterg., 19, 169-176.

Klotz, H. (1984). Bestimmung und spektrospische Charakterisierung von kationischen Tensiden. Proceedings of the World Surfactants Congress, München, CESIO und TEGEWA, 3, 305-316.

Klotz, H. (1987a). Bestimmung kationischer Tenside in der Umwelt Tenside Surfactants Detergents 24, 370-373.

Klotz, H. (1987b). Bestimmung von DSDMAC in mit/ohne Klaerschlamm beaufschlagten - Ackerboeden, Hoechst AG, internal report. No AL 186-87(B), No AL 014-87(B)

Klotz, H. (1988). Bestimmung von DSDMAC in mit/ohne Klaerschlamm beaufschlagten -Ackerboeden, Hoechst AG, internal report. No AL 88-89(B), No AL 200-88(B), No AL 242-88(B)

Klotz, H. (1990a). Analytische Neuentwicklungen zur Bestimmung von kationischen Tensiden in der Umwelt. Muenchner Beitraege zur Abwasser-, Fischerei- und Flussbiologie 44, 205-217.

Klotz, H. (1990b). Bestimmung von Mono-, Tri- und Distearylquat mit HPLC und DC, Hoechst AG, Internal Report. No L 80-90(B)

Klotz, H. (1990c). Bestimmung der DSDMAC Konzentrationen und Frachten im main und Rhein 1989-1990; Hoechst AG, internal report. No AL 1-90(B), No AL 120-90(B)

Kunieda, H. and Shinoda K. (1978). Solution behaviour of dialkyldimenthylammonium chloride in water. Basic properties of antistatic fabric softener; J. Phys. Chem. 82, 1710-1714.

Kupfer, W. (1982). Spurenanalytik von kationischen Tensiden unter den speziellen Bedingungen im Wasser und Abwasser. Tenside Detergents 19, 158-161.

Kupfer, W. and Waters, J. (1976). The determination of cationic surfactants in the presence of anionic surfactant in biodegradation test liquors. Analytica Chimi. Acta 85, 241-251.

Larson, R. J. (1983). Comparison of biodegradation rates in laboratory screening studies with rates in natural waters, Residue Reviews, 85, 159-171.

Larson, R. J. (1992). Personal communication, Procter and Gamble, European Technical Centre, Professional and Regulatory Services, Brussels.

Larson, R. J. and Vashon, R. D. (1983). Adsorption and Biodegradation of Cationic Surfactants in Laboratory and Environmental Systems, Dev. Ind. Microbiol., 24, 425.

Laughlin, R.G., Munyon, R.L., Fu, Y.C. and Fehl, A.J. (1990). Physical Science of the Dioctadecyldimethylammonium Chloride-Water system. 1. Equilibrium Phase behaviour; J. Phys. Chem, 94, 2546.

Laughlin, R.G., Munyon, R.L., Fu, Y.C. and Emge, T.J. (1991). Physical Science of the Dioctadecyldimethylammonium Chloride-Water system. 2. Kinetic and Mechanistic Aspects; J. Phys. Chem, 95, 3852.

Laughlin, R.G., Munyon, R.L., Burns, J.L., Coffindaffer, T.W., Talmon, Y. (1992). Physical Science of the Dioctadecyldimethylammonium Chloride-Water system. 3. Colliodal Aspects; J. Phys. Chem. 96, 374-83

Le Monde (1992). Bilan économique et social 1991. In: Dossiers et Documents du Monde, Paris, Jan '92 - 17^{e} annee, p.70

Lee, C. M. (1986). Toxicity of dihardened-tallow dimethyl ammonium chloride adsorbed on sediments to the aquatic midge *Chironomus riparius*. Tenside Detergents, 23, 196.

Leeuwangh, P. (1992). Operational Environmental Criteria: Toxicity to Aquatic Organisms. Introduction during Seminar "Environmental Criteria for Assessing Agricultural Pesticides", 20-21 February 1992, Wageningen, The Netherlands.

Levsen, K. and Schneider, E. (1987). Verfolgung des biologischen Abbaus von Tensiden mit Hilfe der Felddesorptionsmassenspektrometrie. Fresenius Z. Anal. Chem. 326, 43-48.

Lewis, M. and Wee, V.T. (1983). Aquatic safety assessment for cationic surfactants. Environ. Toxicol. and Chem. 2, 105-118.

Lewis, M.A. (1990). Chronic toxicities of surfactants and detergent builders to algae : A review and risk assessment. Ecotoxicology and Envrionmental Safety, 20, 123.

Lewis, M., Rapaport, R., Shimp, R. (1989). Environmental data base summary for softener actives, P&G internal report.

Lindman, B. (1992). Personal Communication. Dept. of physical chemistry, University of Lund, Sweden.

Lindman, B. and Wennerström H. (1980). Micelles. Amphiphile Aggregation in Aqueous Solution; Topics in Current Chemistry, 87, 1-84, Springer-Verlag, Berlin.

Loetzsch, K., Neufahrt, A., Gantz, D. (1984). Radiometric studies of the ecological behaviour of the cationic sufactant distearyldimethylammonium chloride. Commun. Jorn. Com. Esp. Deterg. 15, 445-61.

Matthijs, E. Rottiers, A., De Henau, H., Jendreyko, H., Korber, H.-G. (1989). The effect of the emergence of a new heavy duty liquid detergent category on the removal of surfactants and on the toxicity of the effluent of a municipal sewage treatment plant, Z. Wasser-Abwasser-Forsch., 22, 151-157.

Matthijs, E. (1990a). The levels and fate of ditallow dimethyl ammonium chloride in sludge amended soils, Procter and Gamble internal report. Procter and Gamble European Technical Centre, Professional and Regulatory Services, Brussels.

Matthijs, E. (1990b). DHTDMAC concentrations in the river waters ofthe Netherlands; Procter and Gamble internal report. Procter and Gamble European Technical Centre, Professional and Regulatory Services, Brussels.

Matthijs, E. and Hennes, E. C. (1991). Determination of surfactants in environmental samples. Tenside Surf. Det. 28, 22-27.

Matthijs, E., Gerike, P., Klotz, H., Kooijman, J. G. A., Korber, H. G, Waters, J. (1992). Removal and mass balance of the cationic fabric softener ditallow dimethyl ammonium chloride in activated sludge sewage treatment plant. Submitted to Water Research.

May, A. and Neufahrt, A. (1976). Zum Öxikologischen Verhalten von Kationtensiden, 3: Über das Verhalten von Distearyldimethylammoniumchlorid in Belebtschlammanlagen, Tenside Surf. Det., 13, 65-69.

Metcalf and Eddy Inc. (1979). Waste water Engineering: Treatment/Disposal/Reuse 2nd edition. McGraw Hill.

Michelsen, E. R. (1978). Quantitative determination of quaternary ammonium bases in water and waste water by TLC. Tenside Detergents 15, 169-175.

Neufahrt, A., Eckert, H.G., Kellner H.M. and Lötzsch K (1978). Untersuchungen über die Aufnahme und Verteilung von Radioaktiv Markiertem Distearyldimethylammoniumchlorid (DSDMAC) in Karpfen; Spanischer CED-kongress/Madrid.

Neufahrt, A. and Pleschke, D. (1984). Studies of Abiotic and Biological Degradation Process on Cationic Surfactants. XIII Jornadas del Comité Español de la Detergencia, Barcelona, Spain.

Neufahrt, A. and Täuber, G. (1984). Ueber Pruefungen der Ökologischen verträglichkeit von DSDMAC alleine und in einem Tensidsystem im "Biosimulator", Welt-Tensid Kongress, Munich.

OECD (1981). Growth Test with Higher Plants. OECD Chemical Testing Programme, Ecotoxicology Group. Third Draft (January 1981).

OECD (1984). Guidelines for Testing of Chemicals. 208: Terrestrial Plants Growth Test.

OECD (1989). Compendium of environmental exposure assessment methods for chemicals. OECD Environmental Monographs No. 27. OECD, Paris, 350 pp.

OECD (1991a). Draft report of the OECD workshop on the extrapolation of laboratory aquatic toxicity data to the real environment. OECD Environmental Directorate.

OECD (1991b). Environmental Data, OECD Paris 1991, 44.

Osburn, Q.W. (1982). Analytical method for a cationic fabric softener in waters and wastes. Journal of American Oil Chemists Society, 59, 453-457

Pittinger, C.A., Woltering, D.M. and Masters, J.A. (1989). Bioavailability of sediment-sorbed and aqueous surfactants to *chironomus riparius* (midge). Environ. Toxicol. Chem., 8, 1023.

Premises for Risk Management (1989). Risk limits in the context of environmental policy. Annex to the Dutch National Environmental Policy Plan (To Choose or to Loose) 1990-1994. Second Chamber of the States General, session 1988-1989, 21137, 29.

Procter & Gamble-data (1974-1986) as supplied to the ECETOC Task Force on DHTDMAC 1992. Data available on request from Procter & Gamble European Technical Centre, Professional and Regulatory Services, Brussels.

Procter & Gamble (1992). Unpublished. Procter and Gamble European Technical Centre, Professional and Regulatory Services. Brussels.

Rapaport, R. (1987). Historical DTDMAC report (Data base, 1975-1986). Procter and Gamble Human and Environmental Safety Division. Cincinnati OH 45217 USA.

Roghair, C.J., Buijze, A. and Schoon, H.N.P. (1991). Maximum permissible level of the cationic surfactant DTDMAC for aquatic ecosystems. Report of the Dutch National Institute of Public Health and Environmental Protection. Report nr. 719102007.

Rottiers, A. and Papez, M. (1987). Continuous activated sludge (CAS) test on new cationic softener actives, Procter & Gamble internal report. Procter and Gamble European Technical Centre Professional and Regulatory Services, Brussels.

Ruffo, C., Bernardini, M., Arpino, A. (1989). Valutazione del comportamento ambientale del distearildimetil ammonio cloruro (DSDMAC) principale componente degli ammorbidendi ad uso domestico, La Rivista delle Sostanze Grasse, 66, 269-272.

Schneider, E. and Levsen, K. (1987). Verfolgung des biologischen Abbaus von Tensiden mit Hilfe der Felddesorptionsmassenspektrometrie, Fresenius Z. Anal. Chem., 326, 43-48

Schoeberl, P., Bock, K. J., Huber, L. (1988). Ökologisch relevante Daten von Tensiden in Wasch- und Reinigungsmitteln., Tenside Surf. Det., 25, 86-98

Schoester, M. and Kloster, G. (1991). Optimization of a chromatographic post-column ion pair extraction system for the determination of cationic surfactants. Vom Wasser, 77, 13-20.

Shimp, R. J. (1992). Treatability and biodegradation of several surfactants by activated sludge waste water treatment, manuscript submitted to Water Research.

Simms, J.R., Keough, T., Ward, S.R., Moore, B.L., Banduraga, M.M. (1988). Quantitative determination of trace levels of cationic surfactans in environmental matrices using FAB-MS. Anal. Chem. 60, 2613-2620.

Stanley, R. D. and Tapp, J. F. (1982). An assessment of ecotoxicological test methods: Part VIII. The effect of nine chemicals on the growth of *Avena sativa* and *Brassica rapa*. ICI Internal Report BL/A/2164, February 1982, 26pp.

Sullivan, D. E. (1983). Biodegradation of a cationic surfactant in activated sludge, Water Res., 17, 1145-1151.

Täuber, G., Klotz, H., Neufahrt, A. (1986) Unpublished data from Hoechst AG; Environmental aspects of cationic fabric softeners. 6pp. Hoechst AG, Frankfurt, Germany.

Täuber, G. (1988). Zum Umweltverhalten von Kationtensiden, Tenside Surf. Det., 25. 2

Taylor, M.J. (1984). Comparative sensitivity of *Ceriodaphnia sp.* and *Daphnia magna* to selected surfactants. Procter & Gamble Research and Development Report TDR-84002.

Topping, B.W. and Waters, J. (1982). Monitoring of cationic surfactants in sewage treatment plants. Tenside Detergents, 19, 164-170.

Unilever (1990-1991). Ecotoxicity data for surfactants. Data as supplied to the AIS/CESIO Task Force. Port Sunlight Laboratory.

Van Leeuwen, K., Roghair, C., de Nijs, T., and de Greef, J. (1990). Wasverzachters II. Resultaten van aanvullent onderzoek. H_2O, 23, 295-299.

Van Leeuwen, K., Roghair, C., de Nijs, T., and de Greef, J. (1992). Ecotoxicological risk evaluation of the cationic fabric softener DTDMAC. III. Risk Assessment. Chemosphere, 24, 629-639.

Van Ginkel, C. G. and Stroo (1990). Biodegradability of ARQUAD 2HT acetate in a SCAS test, Akzo Technical Report, Akzo Chemicals International BV, Amersfoort, The Netherlands.

Van Ginkel, C. G. and van Rij, K. C. (1990). Toxicity of Arquad 2HT to anaerobic microorganisms, Akzo Internal Report, Akzo Chemicals International BV, Amersfoort, The Netherlands.

Van Ginkel, C. G. (1991). Relations between the structure of quaternary alkyl ammonium salts and their biodegradability, Chemosphere, 23, 281-289.

Van Ginkel, C. G. and Stroo (1991). Biodegradability of ARQUAD 2.18, Akzo Technical Report. Akzo Chemicals International BV, Amersfoort, The Netherlands.

Van Ginkel, C. G.(1992). Biodegradation of cationic surfactants, in "Biodegradability", edited by D. R. Karsa and M. R. Porter, Elsevier Science Publishers.

Van Ginkel, C. G., van Dijk, J. B., Kroon, A. G. M. (1992). The metabolism hexadecyl trimethyl ammonium chloride in Pseudomonas B1, Applied Environmental Microbiology, in press.

Versteeg, D. J. (1989). Toxicity of ditallowdimethylammonium chloride to aquatic organisms. Procter & Gamble Internal Notebook ZE 1340.

Versteeg, D. J. and Woltering, D. M. (1990). A laboratory-scale model for evaluating effluent toxicity in activated sludge waste water treatment plants. Water Res., 24, 717.

Versteeg, D. J., Feijtel, T. C. J., Cowan, C. E., Ward, T. E. and Rapaport, R. A. (1992). An environmental risk assessment for DTDMAC in The Netherlands. Chemosphere, 24, 641-662.

Versteegh, J. F. M., Bergers, P. J. M. and De Groot, A. C. (1992). Wasverzachters; ook een bedreiging voor de drinkwater productie ?. H_2O, 25, 564-569.

Waters, J., Gerike, P., Klotz, H., Kooijman, J. G. A., Matthijs, E. (1992). The determination of DHTDMAC in environmental matrices using trace enrichment techniques and HPLC with conductometric detection. Waters Res., submitted.

Wee, V. T., (1984). Determination of cationic surfactants in waste-and river waters. Water Research 18, 223-225

Wee, V. T. and Kennedy, J. M., (1982). Determination of trace levels of quaternary ammonium compounds in river water by liquid chromatography with conductometric detection. Anal. Chem. 54, 1631-1633.

Weston R. F. (1987). Inc., $^{14}CO_2$ production test on S1093.01R, S1094.01R, S1095.01R, S1096.01R and S1097.01R in sludge amended soil, Project No 87-054 for Procter & Gamble, 1987. Procter & Gamble Human and Environmental Safety Division. Cincinnati, OH 452 17 USA.

Weston R. F. (1989). Inc., $^{14}CO_2$ production test on S1189.01R, S1190.01R, S1191.01R, S1192.01R and S1193.01R in sludge amended soil, Project No 88-015 for Procter & Gamble Company. Human and Safety Division. Cincinnati, OH 452 17 USA.

Windeatt, A. J. (1987). Effect on the growth of *Sorghum bicolor*, *Helianthus annuus* and *Phaseolus aureus*. ICI Contract Report BL/B/3181 for Unilever Research - BIOD/82/06. 19pp.

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CORRECTION

Please add at the end of page 30 : .... This ratio of sediments/muds will also be an important parameter in determining the fate of incoming chemicals. In these compartments....