Human and Environmental Risk Assessment
on ingredients of Household Cleaning Products

Esterquats
Human Health Risk Assessment Report

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Executive Summary

Esterquats are a widely used class of cationic surfactants. They were introduced in the early 1980s when concerns were raised about the environmental profile of DHTDMAC (Di-Hardened Tallow Di-Methyl Ammonium Chloride) a fabric conditioner. Esterquats are similar to DHTDMAC except that ester links were introduced into the head-group of the molecules, making them more subject to degradation by hydrolysis and greatly facilitating biodegradation. Most, if not all, fabric conditioners marketed in Europe are now comprised of the three Esterquat groups, TEAQ, DEEDMAC, and HEQ ((Z)-2-hydroxy-3-[(1-oxo-9-octadecenyl)oxy]propyltrimethylammonium chloride). They combine a good environmental profile, especially in terms of ready and ultimate biodegradability, with the structural features required for an effective fabric conditioner.

The total volume of Esterquat surfactants used in Europe is estimated to be 130,000 tonnes/year on an active matter basis [HERA, 2004].

Environmental assessment

The environmental section has been published in July 2008.
See the document on the HERA website.
http://www.heraproject.com/

Human health assessment

Consumers are exposed to esterquats through their presence in fabric conditioners mainly via the dermal route, but to some minor extent also via the oral route. Skin exposure occurs mainly in hand-washed laundry and through esterquats being present on the fabric of laundry treated with fabric conditioner. Consumers are orally exposed to esterquats through residues in drinking water or eating foods that have taken up esterquats through their presence in surface waters. The maximum total aggregate exposure of consumers to esterquats has been estimated to be 36.9 µg/kg bw/day.

A substantial amount of toxicological studies demonstrate that esterquats are of low toxicity. Esterquats were found to be mildly to moderately irritating to rabbit skin and eyes. The degree of irritation was concentration dependant as dilutions in water resulted in proportionally lower level of irritation. Local dermal effects due to skin contact with esterquat containing handwasing solutions or esterquat residues on skin are not of concern because esterquats are neither considered skin sensitizer nor expected to be irritating under in-use conditions. Accidental eye contact with undiluted esterquat containing fabric conditioner formulation may cause mild irritation which is, however fully reversible shortly after exposure. As other components in the fabric conditioner formulation may contribute to these effects, immediate rinsing with plenty of water is recommended and will mitigate any potential eye irritation effects.

With regard to repeated dose toxicity, existing subacute and subchronic toxicity studies with esterquats coherently demonstrate a low level of systemic toxicity of all types of esterquats. No major clinical effects were observed in any of the studies, even at dose levels up to 1,000 mg/kg bw/day. There is further no information suggesting that esterquats are genotoxic, mutagenic or toxic to the foetus. Although no carcinogenicity study has been conducted with esterquats yet, the absence of genotoxicity and the overall low toxicity of esterquats do not raise any carcinogenicity concern. Likewise, although no multigeneration studies are available, the absence of any effects on gonads in well-conducted

subacute and subchronic toxicity studies, does not raise an immediate concern for a possible effect of esterquats on fertility.

For assessing risks associated with human exposure to esterquats in context of their use in fabric conditioner, a conservative NOAEL of 300 mg/kg bw/day was established on the basis of 90-day oral toxicity study with a TEA-based esterquat. The comparison of the aggregate exposure of 36.9 µg/kg bw/day and the NOAEL results in an MOE of 8,100. Taking into account the conservatism in the exposure calculation and the assigned NOAEL for esterquats, this margin of exposure is considered to be large enough to account for the inherent uncertainty of the database and variability of the database.

In summary, the human health risk assessment has demonstrated that the use of esterquats in fabric conditioners is safe and does not cause concern with regard to consumer use.

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2. SUBSTANCE CHARACTERISATION

The “Substance Characterisation” has been published in July 2008.
See the document on the HERA website.
http://www.heraproject.com/

3. ENVIRONMENTAL ASSESSMENT

The environmental section has been published in July 2008.
See the document on the HERA website.
http://www.heraproject.com/

4. HUMAN HEALTH ASSESSMENT

4.1 Consumer exposure

4.1.1 Product types

In line with the objectives of the HERA initiative, this human health assessment will focus on the use of esterquats in household cleaning products. In this sector, esterquats are primarily used in fabric conditioners. Table 13 lists the different fabric conditioner product types as well as the range of and typical esterquat concentrations used in the respective applications.

Table 13: Fabric conditioner types and esterquat (EQ) concentrations (AISE, 2002)

Applications of Fabric Conditioner	Range of EQ level in finished product	Typical content of EQ in finished product
Liquid Regular	0.0 – 5.3 %	3.5 – 5.1 %
Liquid Concentrate	6.8 – 23%	11.2 – 23%
Sheets	25%	25%
"Two in One"	7.4 – 20.3%	7.4%

Fabric conditioners are generally added to the laundry to soften the fabric. The liquid forms like the Liquid Regular or Liquid Concentrate or also the "Two in One" are added to the washing machine. In addition to softening the fabric, the "Two in One" acts also as an ironing aid. Fabric conditioner sheets are pieces of cloth containing an esterquat concentrate which are added to the tumble drier.

4.1.2 Consumer contact scenarios

For the uses of esterquats the following consumer exposure scenarios were identified and assessed:

1. Direct skin contact with diluted formulations during hand-washing laundry, transfering softened wet laundry to a tumble drier or contact with esterquat-containing sheets during loading of tumble driers. The latter two contacts are, however, short and limited to exposure to fingertips with the wet laundry or a near-solid and the contact time is very short. The systemic uptake of esterquats under these circumstances is considered negligible;
2. Direct skin contact from wearing softened clothes;
3. Indirect oral exposure via drinking water or eating food items such as vegetables that have taken up esterquats through its presence in surface waters;
4. Accidental or intentional over-exposure.

4.1.3 Consumer exposure estimates

There is a consolidated overview concerning habits and practices of the use of household cleaning products in Western Europe which has been tabulated in Appendix F of the HERA guideline

document (HERA, 2005). This table reflects consumers' use of household cleaning products including fabric conditioners in g/task, tasks/week, duration of task and other uses of products and is largely the basis for the exposure estimates in the following paragraphs. In some instances, lacking information was complemented by additional information provided by the member companies of AISE. The calculations of the estimated consumer exposures are based on the highest relevant concentrations that consumers can be exposed to.

4.1.3.1 Direct skin contact from hand-washing laundry

The use of fabric conditioners in hand-washing laundry has been identified as a common consumer habit. In this task, the esterquat containing fabric conditioner formulations comes in direct contact with the skin of hands and forearms.

The contact time with esterquats in the course of hand-washing laundry is very short (10 minutes; AISE, 2002) and the percutaneous absorption is very low (*i.e.*, at maximum, only 2% of an HEQ-based esterquat was absorbed in an *in vivo* rat dermal penetration study over a 48 hrs exposure period; Unilever, 1992chh; other types of esterquats have been shown to penetrate the skin at lower levels; see Chapter 5.2.1.1). Therefore, it can be assumed that the amount of esterquat systemically available, if any, is very low.

According to the following algorithm from HERA guidance document, the dermal systemic consumer exposure (Exp_{sys}) to esterquats under hand-washed laundry conditions can be estimated:

$$\mathbf{Exp_{sys} = F_1 \times C' \times F_2 \times F_3 \times F_4 \times t \times S_{der} \times n / BW} \qquad \mathbf{(1)}$$

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

F_1	Percentage weight fraction of substance in product	***23 %*** (Table 13; liquid concentrate; worst case)
C	Product concentration	***10 mg/cm^3*** (1%; AISE, 2002)
F_2	Percentage of EQ transfer from solution to skin	**100%** (worst case)
F_3	Percentage of EQ remaining on skin	**100%** (worst case)
F4	Percentage of EQ absorbed through skin	**2%** (Unilever, 1992chh)
S_{der}	Surface area of exposed skin	***1980 cm^2*** (TGD, 2003)
n	Product use frequency (tasks per day)	***1.4*** (HERA, 2005)
BW	Body weight	***60 kg*** (TGD, 2003)
Tder	Thickness of product layer in contact with skin	**0.01cm** (TGD, 2003)
C'	Product load in mg/cm^2; **C' = C x Tder**	10 mg/cm^3 x 0.01 cm = **0.1 mg/cm^2**

$$\mathbf{Exp_{sys\ (hand\ laundering)}} = [0.23 \times (0.1\ mg/cm^2) \times (1980\ cm^2) \times 1.4/day \times 2\%] / 60\ kg$$
$$\mathbf{= 0.021\ mg/kg\ bw/day}$$

The exposure algorithm (1) described above allows estimating the systemic uptake of esterquats following use of fabric conditioner in hand-washing laundry suitable for assessing the health risks associated with potential systemic toxicity of esterquats.

However, concerns have been raised as to the skin sensitisation potential of esterquats. While this issue is addressed in more detail in section 5.3.1.5 and a weight of the evidence analysis did not suggest an increased risk of esterquats causing skin sensitisation in humans, for risk assessment purposes it may in addition be useful to characterise the actual skin exposure under in-use conditions quantitatively on a dose per unit ($\mu g/cm^2$) area skin basis. This dose metric has been recommended for use in dermal sensitisation risk assessments of fragrances used in consumer products (Kimber I. *et al.*, 2008). This concept is also considered to be suitable for the skin sensitisation risk assessment of other ingredients present in consumer products.

The esterquat dose per unit area skin can be calculated according to the following algorithm:

$$\mathbf{Exp_{skin\ (hand\ laundering)} = F_1 \times C \times T_{Der}} \qquad \mathbf{(2)}$$

F_1	Percentage weight fraction of substance in product	***23 %*** (Table 13; liquid concentrate; worst case)
C	Product concentration	***10 mg/cm³*** (1%; AISE, 2002)
Tder	Thickness of product layer in contact with skin	**0.01cm** (TGD, 2003)

$$\mathbf{Exp_{skin\ (hand\ laundering)}} = 0.23 \times 10\ mg/cm^3 \times 0.01cm = \mathbf{0.023\ mg/cm^2 = 23\ \mu g/cm^2}$$

4.1.3.2 Direct skin contact from wearing clothes

The consumer can further be directly exposed to esterquats via the skin by wearing clothes that have been laundered and softened with fabric conditioners. Esterquats function by cross-linking the fibres of clothes and thereby softening the fabric. Consumers may be exposed to esterquats through their presence on the fabric and their release to the human skin.

The dermal systemic consumer exposure (Exp_{sys}) to esterquats resulting from the transfer of esterquats from the fabric to the skin can be estimated according to the following algorithm:

$$\mathbf{Exp_{sys} = F_1 \times C' \times S_{der} \times N \times F_2 \times F_3 \times F_4 / BW,}\ \text{where}\ \mathbf{C' = M \times F' \times FD/W} \qquad \mathbf{(2)}$$

The terms used in this algorithm are defined as follows:

F_1	Percentage weight fraction of substance in product	***23 %*** (Table 1, compact detergent, gel)
C'	Product load in mg/cm^2	

S_{der}	Surface area of exposed skin	***17600 cm²*** (TDG, 2003)
N	Exposure frequency	1 event per day
F_2	Percent weight fraction transferred to skin	***1 %*** (Vermeire *et al.*, 1993)
F_3	Percent weight fraction remaining on skin	***100 %*** (worst case)
F_4	Percent weight fraction absorbed via skin	**2 %** (Unilever, 1992chh)
M	Amount of undiluted product used	***90 g*** (Liquid concentrate; AISE, 2002)
F'	Percentage of weight fraction of substance deposited on fabric	***100 %*** (worst case)
FD	Fabric density	***10 mg/cm²*** (Procter and Gamble, 1996)
W	Total weight of fabric	***1 kg*** (worst case)
BW	Body weight	***60 kg*** (TGD, 2003)

C' = M x F' x FD/W = (90,000mg x 10mg/cm^2)/1,000,000mg = **0.9 mg/cm^2**

$Exp_{sys\ (fabric\ wearing)}$ = [0.23 x 0.9 mg/cm^2 x 17600 cm^2 x 1 x 0.01 x 1 x 0.02] / 60 kg
= **0.012 mg/kg bw/day**

The esterquat dose per unit area skin can be calculated according to the following algorithm:

$Exp_{skin\ (fabric\ wearing)}$ = F_1 x C' x F_2, where **C' = M x F' x FD/W** **(2)**

The terms used in this algorithm are defined as follows:

F_1	Percentage weight fraction of substance in product	***23 %*** (Table 1, liquid concentrate, worst case)
C'	Product load in mg/cm^2	
F_2	Percent weight fraction transferred to skin	***1 %*** (Vermeire T.G. *et al.*, 1993)
M	Amount of undiluted product used	***90 g*** (Liquid concentrate; AISE, 2002)
F'	Percentage of weight fraction of substance deposited on fabric	***100 %*** (worst case)
FD	Fabric density	***10 mg/cm²*** (Procter & Gamble, 1996)
W	Total weight of fabric	***1 kg*** (worst case)

C' = M x F' x FD/W = (90,000mg x 10mg/cm^2)/1,000,000mg = **0.9 mg/cm^2**

$Exp_{skin\ (fabric\ wearing)}$ = 0.23 x 0.9 mg/cm^2 x 0.01 = **0.0021 mg/cm^2 = 2.1 µg/cm^2**

4.1.3.3 Systemic oral exposure in humans

Oral exposure to esterquats can originate from uptake via drinking water or eating food items such as vegetables that have taken up esterquats through its presence in surface waters. Based on the EUSES calculations (see Environmental Assessment, Chapter 4), the estimated "reasonable worst case" assumption for indirect uptake of esterquats through residues in drinking water and via food is 3.88 x 10^{-3} mg/kg/day.

4.1.3.4 Total exposure

In the unlikely event of maximum worst case exposure from all sources, the total exposure to esterquats from its use in fabric conditioner would be 36.9 µg/kg bw/day. The individual sources of exposures leading to the overall exposure are summarised in Table 14.

Table 14: Worst case exposure estimates for different consumer contact scenarios

Task	Worst case exposure estimate (EXP_{sys}) [µg/kg bw/day]
Direct skin contact from hand washing laundry	21
Direct skin contact from wearing laundered clothes	12
Oral exposure to Esterquats	3.9
Total exposure	**36.9 µg/kg bw/day**

4.2 Hazard assessment

4.2.1 Summary of available toxicological data

4.2.1.1 Absorption, Distribution, Metabolism and Excretion Studies

Dermal studies

The absorption, distribution, metabolism and excretion of HEQ- or MDEA-based esterquats as well as the DEEDMAC's precursor 2,3 dihydroxy propyl trimethylammonium chloride (DHPT) following dermal exposure have been studied in *in vitro* and *in vivo* investigations (Unilever, 1992chh; Unilever, 1993dhh; Unilever, 1997ehh).

In an *in vitro* study, epidermal slices of porcine skin were exposed to an aqueous solution of a ^{14}C-labelled HEQ-based esterquat. The supernatant was monitored for ^{14}C-hydrolysis products of the esterquat. The investigators demonstrated that the esterases present in porcine skin rapidly hydrolysed approximately 40% of HEQ over 24 hours, which, under the testing conditions, equalled a rate of about 86 ng/hr/cm^2 (Unilever, 1993dhh). The esterquat was only slightly hydrolysed under the control conditions (*i.e.*, buffer solutions alone and boiled skin). The study is summarised in Table 15:

Table 15: In vitro HEQ hydrolysis and dermal penetration study

Substance	Substrate	Radiolabel	Route	Absorption
HEQ 67846-68-8 [Unilever 1993dhh]	Porcine skin *in vitro*	^{14}C in C3 position of propyl group in HEQ	Epidermal slices incubated with 0.75 mg/ml HEQ	Skin hydrolyses of about 40% of HEQ at a rate of 86 ng/hr/cm^2

The dermal absorption and excretion of the ^{14}C-labelled MDEA-based esterquat DEEDMAC and a ^{14}C-labelled HEQ-based esterquat in ethanol were investigated in two independent studies in rats.

In the DEEDMAC study, female rats were treated topically with approximately 0.17mg/cm^2 of a ^{14}C-labelled DEEDMAC test solution (a total of 0.1mL on 9.6cm^2 of skin). The treatment sites were fully occluded and the urine, faeces and expired CO_2 were collected and assayed for ^{14}C activity over 48 hours after treatment. The results obtained indicate that following 48 hours of occluded topical application, about 0.2% of the ^{14}C-dose, was dermally absorbed. Of the absorbed ^{14}C-dose, approximately 30% was excreted in the urine and 10% in the faeces, the rest remained in the carcass. Expired $^{14}CO2$ and terminal ^{14}C-blood levels were all below detection limit. After 48 hours, approximately 58% of the ^{14}C was rinsed from the skin, leaving about 38% associated with the skin. The overall ^{14}C-recovery was about 99%. Under the study conditions, the investigators concluded that only very low levels of DEEDMAC were percutaneously absorbed (Unilever, 1997ehh).

In another investigation in the rat, a ^{14}C-labelled (in C3 position of the propyl group) HEQ-based esterquat or its ^{14}C-labelled precursor, 2,3 dihydroxy propyl trimethylammonium chloride (DHPT), was topically applied under occlusive conditions to separate groups of rats. While the total amount of HEQ penetrating the skin was 0.7%, a total of 2% of the topically applied DHPT penetrated the rat skin under the study conditions. The HEQ-based esterquat was applied at 140 µg/cm^2 onto the skin, resulting in a penetration rate of approximately 0.98 µg HEQ/cm^2 over 48 hours. The respective penetration rate of DHPT was determined to be 3.52 µg DHPT/cm^2 over the same exposure period following a topical application of 176 µg/cm^2 (Unilever, 1992chh). In an additional investigation of similar design, but with the ^{14}C-label in the fatty acid tail position, the total absorption of a HEQ-based esterquat was estimated to be 2% (Unilever, 1992dhh). The data are summarised in Table 16.

Table 16: ADME of esterquats following dermal application

Substance	Species	Radiolabel	Route	Absorption	Excretion
DEEDMAC 67846-68-8 [Unilever, 1997ehh]	DR-CD Rat	^{14}C in methyl group of dimethyl moiety	Epidermal (occlusive)	0.2%	Of original dose 0.06% excreted in urine; 0.02% in faeces and 0.12% in carcass
HEQ 67846-68-8 [Unilever, 1992chh]	OLAC Wistar Rat; 5	^{14}C in C3 position of propyl group in HEQ	Epidermal (occlusive)	0.7%	Of original dose 0.1% expired as $^{14}CO2$, 0.1% excreted in urine; 0.02% in faeces and 0.43% in carcass
DHPT 34004-36-9 [Unilever, 1992chh]	OLAC Wistar Rat; 5	^{14}C in C3 position of propyl group in HEQ	Epidermal (occlusive)	2%	Of original dose 0.02% expired as $^{14}CO2$, 1.3% excreted in urine; 0.05% in faeces and 0.63% in carcass
HEQ 67846-68-8 [Unilever, 1992dhh]	OLAC Wistar; 3/3	^{14}C on fatty acid tail of esterquat	Epidermal (occlusive)	2%	Of original dose 0.27% expired as $^{14}CO2$, 0.02% excreted in urine; 0.01% in faeces and 1.67% in carcass

Taken all data together, it can be concluded that even under conservative occlusive conditions, HEQ or DEEDMAC is only poorly absorbed through intact rat skin [Unilever, 1997ehh]. With a total dermal absorption of 2%, 0.7% and 0.2%, HEQ- and MDEA-based esterquats behave relatively similar. The percutaneous absorption of the HEQ-based esterquat precursor DHPT was slightly higher than that of HEQ. However a dermal penetration of 2% of the applied dose under occlusive exposure conditions is still considered to be low taking into account that, in realistic scenarios, usually non-occlusive exposure conditions are applied.

Oral studies

The absorption, distribution, metabolism and excretion of an HEQ-based esterquat, DEEDMAC, MTEA-I and MTEA-methosulfate (*i.e.*, N-Tris-(2-hydroxyethyl)-methylammonium iodide or methosulfate), the metabolite of a TEA-based esterquat, was evaluated in a total of 3 oral studies in rats (Henkel, 1991bhh; Unilever, 1992dhh; Unilever, 1997dhh).

The HEQ-based esterquat was extensively absorbed after oral gavage. The total absorption was about 73% of the totally applied dose. About 33% (males) to 46% (females) of the radio-labelled carbon was detected in expiratory air within 48 hours; urine and faeces contained approximately 5%. The remainder was resident in the carcass, with radio-TLC indicating that, in faeces, most radio-labelled carbon was present as the expected hydrolysis products of the HEQ-based esterquat (Unilever, 1992dhh).

In a similar oral gavage study conducted with DEEDMAC, female rates excreted approximately 57% of the dose in urine, 25% in faeces and 0.4% as $^{14}CO_2$. At 48 hours after dosing, a total of

approximately 4.5% of the dose remained in the body, with 0.9% in the liver and 0.5% in the kidneys. In male rats, approximately 36% of the dose was excreted in urine, 51% in faeces and 0.4% as $^{14}CO_2$. 48 hours after exposure, the investigators determined that about 4% of the total dose remained in the body, with 1.1% in the liver and 0.2% in the kidneys. These results indicated that intestinal absorption was higher in females (63% of dose) than in males (41% of dose). The investigators identified the major urinary metabolites of DEEDMAC to be the de-esterified form of DEEDMAC (*i.e.*, ^{14}C-dimethyl diethanolammonium chloride; DDEA) as well as possibly some further oxidation products of DDEA (*i.e.*, carboxylic acid of DDEA). A small degree of decarboxylation occurred to produce $^{14}CO_2$. Non-absorbed ^{14}C material was metabolised, probably by gut esterases, to liberate the monoester of DEEDMAC and eventually DDEA (Unilever, 1997dhh).

In a further absorption and excretion study in rats, ^{14}C-labelled MTEA-I, the iodide form of the metabolite of a TEA-based esterquat, was given orally in a single dose at 100 mg/kg bodyweight. About 50% of the radioactive material was excreted with the faeces and 40% in urine within the first 3 days of exposure. The elimination via the urine was mostly within the first 24 hrs after exposure. Generally, the radioactivity determined in the organs was very low. With 0.3% of the totally administered dose, the highest amount was found in the gastro-intestinal tract. About 0.2% was determined in the liver. In a second experiment, 100 mg/kg of a 50/50 mixture of radiolabelled MTEA-I with unlabelled MTEA-methosulfate was given orally in a single dose to rats. Similar results were observed: about 61% of the radiolabel was excreted with faeces in three days, about 28% percent was found in urine with the same time-excretion pattern as for MTEA-I alone (Henkel, 1991bhh). While the cationic moiety in MTEA-I and MTEA-methosulfate is the same, the anionic counter-ion is different. This difference is, however, not expected to change absorption characteristics of the MTEA-moiety. The following Table 17 summarises the available ADME studies following oral administration.

Table 17: ADME following oral administration

Substance	Species M/F	Radiolabel	Dose	Absorption	Excretion
DEEDMAC 67846-68-8 [Unilever, 1997dhh]	DR-CD rat; 8/8	^{14}C in methyl group of DMDA moiety	17 mg/kg bw	63% of total administered dose	36 -57% urine (M/F) 25-51% faeces (M/F) 0.4% exhaled CO_2 4 – 4.5% remainder
HEQ 67846-68-8 [Unilever, 1992dhh]	OLAC Wistar rat; 3/3	^{14}C on fatty acid tail	0.95 mg	73% of total administered dose	33-46% exhaled CO_2 5% faeces /urine balance incorporated
MTEA-I CAS-No: N/A [Henkel, 1991bhh]	Sprague-Dawley rat; 8/8	^{14}C-labelled methyl group in MTEA-I	100 mg/kg bw	About 40% of total administered dose	48-52% in faeces; 37-45% in urine

These studies show apparent differences in the kinetics between the HEQ-based esterquat and DEEDMAC (main excretion in air versus in urine respectively). A possible explanation for this observation could be related to the different position of the ^{14}C-label in the test substances. In

DEEDMAC, the label was on a methyl-group of the dimethyldiethanolammonium moiety. The ^{14}C-labelled HEQ contained the ^{14}C in the octadecyl group of the fatty acid ester. The studies with different position of the ^{14}C-label show that the fatty acid moiety is either exhaled as CO_2 or incorporated most likely into body fat which is consistent with its metabolism via the physiological fatty acid pathways, while the Ammonium moiety is mainly excreted in the urine and the faeces.

Intravenous application

In addition to the oral ADME study discussed before, a single dose of ^{14}C-labelled MTEA-I was further administered intravenously to male Sprague Dawley rats. About 96% of the radioactivity was excreted in urine within the first 96 hours out of which 91% were eliminated within the first 24 hours following administration. Only 1.3% of the administered radioactivity was determined in faeces and only 0.6% in expired air. The radioactivity recovered in the organs at study completion was only very low: The highest level was found in liver (0.1%) followed by gastro-intestinal tract (0.07%) and in blood (0.05%). Table 18 summarises the key elements of the study:

Table 18: ADME following intravenous administration

Substance	**Species/strain; M/F**	**Radiolabel**	**Dose**	**Excretion**
MTEA-I CAS-No: N/A [Henkel, 1991bhh]	Sprague-Dawley; 1/2	^{14}C methyl on MTEA-I	0.9/0.6 mg/kg bw	91% urine 1.3% faeces

Conclusion

The available ADME studies on HEQ- and MDEA-based esterquats (*i.e.*, DEEDMAC) suggest that esterquats are only poorly absorbed through skin. In good quality *in vivo* dermal ADME studies, only 0.2% of the topically applied DEEDMAC and 0.7% - 2% of the topically applied HEQ-based esterquat dose was systemically available after 48 hrs exposure under occlusive conditions. Considering their chemical similarity and their comparable low water solubility (< 0.001 mg/L), TEA-based esterquats are expected to have an absorption profile similar to that of HEQ or DEEDMAC. Hence, for the purpose of calculating systemic exposure to esterquats from skin exposure, a worst case absorption of 2% as determined for the HEQ-based esterquat will be assumed.

Once dermally absorbed, approximately 30% of the systemically available dose was excreted in urine, 10% in the faeces and the remainder could be detected in the carcass. Following oral exposure studies with radiolabelled esterquats, the information suggests differences in the kinetics between HEQ and DEEDMAC. Considering total radioactivity, the largest portion of systemically available HEQ was exhaled in air whereas radiolabelled DEEDMAC and its degradation products were predominantly excreted in urine and faeces. These differences are likely to be related to the position of the label and not to an inherently difference in metabolism. The excretion of ^{14}C-labelled CO_2 in the case of the HEQ-based esterquat indicates rapid metabolism of free fatty acids following de-esterification, whereas the excretion of radioactivity in urine in the case of DEEDMAC demonstrates the rapid excretion of the dimethyldiethanolammonium moiety in urine and faeces. The latter is further supported by available ADME studies with MTEA-I, the iodide form of the metabolite of a TEA-based esterquat. The available data indicate that both the monomethyl triethanolammonium (from TEA) and the dimethyldiethanolammonium (from DEEDMAC) moieties are quickly excreted in urine.

No data are available on the elimination of the trimethyldihyroxypropylammonium from HEQ, but there is no reason to expect great differences in behaviour between these moieties. Importantly, the available data do not indicate the potential of esterquats or their metabolites to bioaccumulate.

4.2.1.2 Acute toxicity (oral/dermal)

4.2.1.2.1 Acute oral toxicity

The acute oral toxicity of TEA-, HEQ-, or MDEA-esterquats was evaluated in rats in a total of twelve acute oral toxicity studies:

- 9 studies on TEA-based esterquats (Stepan 1983ahh; Stepan, 1988ahh; Kao, 1989ahh; Kao, 1989bhh; Stepan, 1991ahh; Ceca, 1991bhh; Degussa, 1992ahh; Kao, 1997ahh; Henkel, 1994bhh)
- 1 study on HEQ-based esterquats (Unilever, 1990chh)
- 1 study on MDEA-based esterquats (Procter & Gamble, 1993ahh)

Test substance characterisation data was not available for all test materials. However, for those substances for which such characterisation data was available, the information indicated that test materials typically contained more than 77% of the active compound, the difference being solvents like isopropanol or dipropylene glycol.

The test substances were dosed by gavage in neat or diluted form at concentrations of 2,000 or 5,000 mg/kg body weight active compound. Most of the studies were conducted according to OECD protocol 401 for acute oral toxicity and compliant with Good Laboratory Practices and hence considered to be reliable without or reliable with restrictions according to the Klimisch criteria. Those studies rated with a Klimisch score of 2 ‘reliable with restrictions’ were typically lacking an appropriate characterisation of the test substance.

Following dosing, in all studies the rats were observed daily for mortality and clinical symptoms. Individual body weights were recorded at time intervals and at the end of the 14-day observation period; the animals were sacrificed and macroscopically examined.

There were no deaths following a single oral application of the esterquats investigated. At doses of 5,000 mg/kg, in some individual studies the animals displayed mild clinical symptoms like piloerection, increased salivation, diarrhea, or in two studies reduced body weight gain in one or two animals. In a single study, clinical symptoms such as sedation, rales and ruffled fur were observed at the 2,000 mg/kg dose level. In none of the studies did the macroscopic examination reveal any unusual findings. The following Table 19 summarises the available acute oral toxicity studies for the different types of esterquats as well as their validity evaluations.

Table 19: Acute oral toxicity studies

Substance	Species M/F	Dose[1)] (mg/kg bw)	LD_{50} (mg/kg bw)	Validity	Comments
TEA-based EQ CAS-No: N/A [Stepan, 1983ahh]	Rat, Albino; 5/5	5,000	> 5,000	3	Test substance not identified, characterised and purity level not provided
TEA-based EQ	Rat, Crl: CD	5,000	> 5,000	2	Test substance

91995-81-2 [Stepan, 1988ahh]	(SD) BR; 5/5				characterisation not available
TEA-based EQ 91995-81-2 85% active, 15% IPA [KAO, 1989ahh]	Rat, Crl:CD (SD) BR VAF+; 5/5	4,250 (corrected) 5,000 (nominal)	> 4,250	2	Individual animal data incomplete
TEA-based EQ 91995-81-2 85% active, 15% IPA [KAO, 1989bhh]	Rat, Crl:CD (SD) BR VAF+; 5/5	4,250 (corrected) 5,000 (nominal)	> 4,250	2	Individual animal data incomplete
TEA-based EQ CAS-No: N/A [CECA, 1991bhh]	Rat, Sprague-Dawley; 6/6	2,000	> 2,000	1	
TEA-based EQ 91995-81-2 [Stepan, 1991ahh]	Rat, ICO: OFA-SD (IOPS Caw); 5/5	2,000 and 5,000	> 5,000	2	Individual animal data incomplete
TEA-based EQ 91995-81-2 90% active; 10% isopropanol (IPA) [Degussa, 1992ahh]	Rat, Crl: (WI) BR – Wistar; 5/5	2,000	> 2,000	2	Impurities not identified
TEA-based EQ 91995-81-2 77% active [Henkel, 1994bhh]	Rat, Hsd/Win: WU; 5/5	1,540 (corrected) 2,000 (nominal)	> 1,540	2	Full characterisation of test substance not done, impurities not identified
TEA-based EQ 94095-35-9 80% active; 20% di-propylene glycol [KAO, 1997ahh]	Rat, Crl: CD (SD) BR; 5/5	2,000	> 2,000	2	Individual animal data incomplete
HEQ-based EQ CAS-No: N/A 82% active [Unilever, 1990chh]	Rat, Crl:CD (SD) BR VAF+; 5/5	4,100 (corrected) 5,000 (nominal)	> 4,100	2	Impurities not identified
MDEA-based EQ CAS-No: N/A [Procter & Gamble, 1993ahh]	Rat, HanIbm: Wist (SPF); 5/5	2,000	> 2,000	2	Test substance characterisation report available, but chemical nature of substance poorly revealed

[1)] Dose was corrected for active content if this information was available; in cases where the active level was not provided, the nominal test substance concentration was provided.

Conclusion

The acute oral toxicity of TEA-, HEQ-, and MDEA-based esterquats has been evaluated on the basis of a range of good quality and GLP-compliant studies according to OECD 401 protocol for acute oral toxicity. On the basis of these studies, it can be concluded that esterquats are of low acute oral toxicity in the rat with an LD_{50} values greater than 2,000 mg/kg body weight. No mortality was observed in those studies where doses of up to 5,000 mg/kg body weight were applied. The results are comparable for all three groups of esterquats, supporting the validity of the grouping approach.

4.2.1.2.2 Acute dermal toxicity

Two valid acute dermal toxicity studies are available to assess the acute dermal toxicity of esterquats in rats. Both studies, one conducted with a TEA-based and the other with an HEQ-based esterquat, were GLP-compliant and in accordance with OECD protocol 402 for acute dermal toxicity.

In both studies, the test substance was applied at a dose of 2,000 mg/kg by spreading it evenly on the shaved skin of the rat. The treated area was covered with a gauze which was held in place with an impermeable dressing. At the end of a 24-hour exposure period, the dressings were removed and the treated area cleaned to remove any remaining test substance.

Following the dosage, the rats were observed daily for mortality and clinical symptoms following treatment. Individual body weights were recorded at defined time intervals and at the end of the 14-day observation period, the animals were sacrificed and macroscopically examined.

In neither of the two studies any mortality occurred as a result to test substance exposure, nor any clinical or other signs of toxicity were observed. In both studies, the LD_{50} for acute dermal toxicity was greater than 2,000 mg/kg bodyweight for nominal product concentration. The following Table 20 summarises the results of the available acute dermal toxicity studies.

Table 20: Acute dermal toxicity studies

Substance	Species M/F	Dose[1)] (mg/kg bw)	LD_{50} (mg/kg bw)	Validity	Comments
TEA-based EQ 91995-81-2 Unsaturated C16-C18 [CECA, 1991ahh]	Rat; 5/5	2,000	> 2,000	4	Study details not available for review
TEA-based EQ 157905-74-3 100% active [Degussa, 2004ahh]	Rat, Crl:CD; 5/5	2,000	> 2,000	1	Characterisation of test substance provided retrospectively by study sponsor
HEQ-based EQ CAS-No: N/A 82% active [Unilever, 1990bhh]	Rat, Crl:CD (SD) BR VAF+ ; 5/5	1,640 (corrected) 2,000 (nominal)	> 1,640	2	Individual animal data incomplete and impurities unknown

[1)] Dose was corrected for active content if this information was available; in cases where the active level was not provided, the nominal test substance concentration was provided.

Conclusion

Esterquats are considered to be of low acute dermal toxicity to rats. This was demonstrated in two OECD guideline and GLP-compliant acute dermal toxicity studies with TEA- and HEQ-based esterquats which have been judged to provide reliable information on the acute dermal toxicity of esterquats in rats. The results are plausible and in line with the information received from acute oral toxicity studies considering that only a maximum of 2% of dermally applied esterquat is systemically available (see Chapter 5.3.1.1).

Although MDEA-based esterquats were not specifically evaluated in dedicated acute dermal toxicity studies, this type of esterquat is also assessed to be of low acute dermal toxicity. This assessment takes into account the similarity of the physico-chemical, toxicokinetic and toxicological characteristics of the three esterquat families. There is no evidence suggesting that MDEA-based esterquats might have an acute dermal acute toxicity different to that of TEA- or HEQ-based esterquats.

4.2.1.3 Skin irritation

4.2.1.3.1 Animal data

The potential of esterquats to cause skin irritation in experimental animals has been evaluated on the basis of a total of 19 rabbit studies. Skin irritation studies were conducted with concentrations ranging from 2% aqueous solutions to undiluted application of the test substances. The patch applications were for four hours either under semi-occluded or fully occluded conditions. Most studies were compliant with GLP or other comparable quality assurance conditions and conducted according to the OECD 404 protocol. While the available studies differed with regard to certain testing conditions, the majority of the studies were rated as 'reliable without restriction (Klimisch score 1) or 'reliable with restriction (Klimisch score 2). For those studies which were rated with a Klimisch score 2, the characterisation of the test substance were either incomplete or missing. For those few studies rated with Klimisch scores 3 or 4, the documentation provided by the study sponsors were incomplete and did not allow reproduction of the study conduct and its result. The following table 21 provides a summary of the available studies investigating the skin irritation potential of esterquats in experimental animals.

Table 21: Skin irritation in experimental animals

Substance	Species M/F	Amount applied; Concentration	Exposure Condition	Mean Irritation Scores	Validity	Comments
TEA-based EQ CAS-No: N/A [Stepan, 1983ehh]	Rabbit, NZW 3/3	0.2 ml/cm^2;	Occlusive; 24 hrs	Intact sites Erythema: 2.5/2.5/3.25/1.25/2/2 Oedema: 1/2/1/0/0.75/1 Abraded sites: Erythema: 2.5/2.25/3.5/1.5/2/2.25 Oedema: 0.75/0.75/1.25/0.75/0.5	4	No identification and characterisation of test substance available to reviewer
TEA-based EQ CAS-No: N/A 30% active [Stepan, 1988chh]	Rabbit, NZW 3	0.083 ml/cm^2;	Semi-occlusive 24 hrs	Intact sites Erythema: 2.50/2.50/2.50 Oedema: 2.00/2.00/2.00 Abraded sites: Erythema: 2.50/2.50/2.50 Oedema: 2.50/2.00/2.00	2	Incomplete test substance characterisation
TEA-based EQ 91995-81-2 20% active; 80% water [Stepan, 1990bhh]	Rabbit, NZW; 3/0	0.083 ml/cm^2;	Semi-occlusive; 4 hrs	Erythema: 0.70/0.00/0.00 Oedema: 0.00/0.00/0.00	2	Incomplete test substance characterisation
TEA-based EQ 91995-81-2 85% active; 15% IPA [CECA, 1991chh]	Rabbit, NZW; 3/0		Semi-occlusive; 4 hrs	Erythema: 1.70/2.00/2.00 Oedema: 0.00/1.30/1.30	4	Study details not available for review
TEA-based EQ 91995-81-2 90% active, 10% IPA [Henkel, 1991chh]	Rabbit, Kleinrussen Chbb:H; 3/0	0.083 ml/cm^2	Semi-occlusive; 4 hrs	Erythema: 1.67/2/2 Oedema: 2/2/1	1	Test substance identification not contained in report but provided by sponsor retrospectively

Substance	Species M/F	Amount applied; Concentration	Exposure Condition	Mean Irritation Scores	Validity	Comments
TEA-based EQ 91995-81-2 [Stepan, 1991bhh]	N.Z. NZW; 3/0	0.083 ml/cm^2	Semi-occlusive; 4 hrs	Erythema: 2.70/2.00/0.30 Oedema: 2.00/2.00/0.30	2	Incomplete test substance characterisation
TEA-based EQ 91995-81-2 90% active; 10% IPA [Degussa, 1992bhh]	Rabbit, NZW; 6/0	0.083 ml/cm^2	Semi-occlusive; 4 hrs	Erythema: 1/1/1/1/1/1 Oedema: 0.3/0/0.3/1.67/0.67/0.3	1	Test substance identification not contained in report but provided by sponsor retrospectively
HEQ-based EQ CAS-No: N/A [Unilever, 1992ehh]	Rabbit; 3/0	0.5 g/patch, moistened with water	Not specified,	Erythema: 0.33/0.00/0.00 Oedema: 0.00/0.00/0.00	4	Incomplete summary of test report; incomplete test substance characterisation
TEA-based EQ 91995-81-2 30% active, 4.5% IPA [KAO 1993ahh]	Rabbit, NZW; 3/0	0.083 ml/cm^2	Semi-occlusive; 4 hrs	Erythema: 0.66/0.33/0.66 Oedema: 0/0/0	2	Incomplete test substance characterisation
MDEA-based EQ 67846-68-8 [Procter & Gamble, 1993bhh]	Rabbit, Chbb: NZW (SPF); 1/2	0.083 ml/cm^2	Semi-occlusive; 4 hrs	Erythema: 0.33/0.00/0.33 Oedema: 0.00/0.00/0.00	1	
TEA-based EQ 91995-81-2 28% active, 2.5% IPA [KAO, 1994chh]	Rabbit, NZW; 3/0	0.083 ml/cm^2	Semi-occlusive; 4 hrs	Erythema: 0/1/0.66 Oedema: 0/0/0	2	Incomplete test substance characterisation
TEA-based EQ, 91995-81-2 85% active; 15% IPA [KAO, 1995ahh]	Rabbit, NZW; 3/0	0.083 g/cm^2	Semi-occlusive; 4 hrs	Erythema: 2/2/2 Oedema: 0.33/0.33/0.33	2	Incomplete test substance characterisation
TEA-based EQ 91995-81-2 90% active; 10% IPA [KAO, 1996bhh]	Rabbit, NZW, CRL:KBL (NZW) BR; 1/2	0.083 ml/cm^2	Semi-occlusive; 4 hrs	Erythema: 2/2/2 Oedema: 1.33/1.33/1.67	1	Test substance identification not contained in report but provided by sponsor retrospectively
TEA-based EQ 91955-81-2 90% active, 10% IPA [Henkel, 1998bhh]	Rabbit, SPF albino; 0/3	0.083 ml/cm^2; 36.63% in sesame oil	Semi-occlusive; 4 hrs	Erythema: 2/2.50/2.67 Oedema: 3.00/2.67/2.67	1	Test substance identification not contained in report but provided by sponsor retrospectively
TEA-based EQ 91995-81-1 [CECA, 1999ahh]	Rabbit, NZW; 3/0	22.5% suspension in water		Not an irritant	4	Study details not available
TEA-based EQ 91995-81-2 [Henkel, 1999ahh]	Rabbit, SPF albino; 0/3	0.5 g/patch moistened with water	Semi-occlusive; 4 hrs	Erythema: 1.67/1.67/2.33 Oedema: 1.00/1.00/1.00	1	Test substance identification not contained in report but provided by sponsor retrospectively
TEA-based EQ 91032-11-0 85% active; 15% IPA "hardened" [Clariant, 2002ahh]	Rabbit, Crl: KBL (NZW) BR;	0.083 ml/cm^2; paste with water	Semi-occlusive; 4 hrs	Erythema: 0/0/0 Oedema: 0/0/0	1	

Substance	Species M/F	Amount applied; Concentration	Exposure Condition	Mean Irritation Scores	Validity	Comments
	0/3					
TEA- based EQ 91995-81-2 90% active, 10% IPA [Clariant, 2002bhh]	Rabbit, Crl: KBL (NZW) BR; 0/3	0.083 ml/cm^2	Semi-occlusive; 4 hrs	Erythema: 2.67/2.67/2.00 Oedema: 2.67/2.67/2.00	1	
TEA-based EQ CAS-No: N/A 53-58% active [Stepan, 2002bhh]	Rabbit, NZW; 3	0.5 g/cm^2	Occlusive; 4 hrs	On intact skin Erythema: 0.00/0.00/0.00 Oedema: 0.00/0.00/0.00 On abraded sites Erythema: 0.00/0.00/0.00 Edema: 0.00/0.00/0.00	4	Summary of test report available, test conditions not fully specified; incomplete test substance characterisation

The available animal skin irritation data do not provide a coherent picture about the skin irritation potential of the various types and forms of esterquats. Depending on type and concentration of esterquat tested and the study design, irritation responses varied from mild to moderate irritation.

TEA-based esterquats with an active level > 30% applied undiluted under occlusive or semi-occlusive conditions to rabbit skin may produce a mild irritation not justifying a skin irritation classification according to Directive 67/548/EEC (Degussa, 1992bhh; Kao, 1993ahh; Henkel, 1999ahh; Clariant, 2002ahh; Stepan, 2002bhh) or a moderate degree of irritation justifying an R38 classification for skin irritancy according to the Dangerous Substance Directive 67/548/EEC (Stepan, 1988chh; CECA, 1991chh; Henkel, 1991chh; Stepan, 1991bhh; Kao, 1995ahh; Henkel, 1998bhh; Clariant, 2002bhh). At concentrations < 30%, the TEA-based esterquats did not produce an irritation response that would justify a classification as R38. The available skin irritation studies on HEQ- and MDEA-based esterquat did not reveal a cause of concern. Both types of esterquats applied neat to rabbit skin for 4 hours under semi-occluded conditions resulted only in a very mild irritation response (Unilever, 1992ehh; Procter & Gamble, 1993bhh).

The lack of detailed test substance information for most of the studies renders it difficult to unequivocally relate the observed irritation responses to a specific chemical characteristic of the TEA-based esterquat. The available information suggests that, in addition to the testing conditions (*e.g.*, occluded versus semi-occluded), the skin irritation potential may be driven by the presence of unsaturated fatty acids, as characterised by the iodine number of the feedstock, in the esterquat. In two independent studies of similar design, the skin irritation potential of two TEA-based esterquats of similar carbon chain length distribution but of different saturation degrees were evaluated (Clariant, 2002ahh; Clariant, 2002bhh). While under the same testing conditions, the esterquat produced from a saturated fatty acid feedstock (characterised by an iodine number of < 2) did not result in any irritation, the esterquat with a higher content of unsaturated fatty acids (characterised by an iodine number of 35-45) produced a moderate level of irritation (see Table 21). Other known differences of the test substance relates to a slightly higher active level of the test substance from the unsaturated feedstock or the application of the substance in either moistened solid or form, but these minor differences are not expected to explain the observed differences in responses.

4.2.1.3.2 Human data

The skin irritation potential of the various types of esterquats has also been investigated in a range of good quality human patch test studies. Overall, under the conditions chosen in the various investigations, the esterquats showed a very favourable human skin irritation profile. If at all, only transient and fully reversible erythema was observed.

The following Table 22 provides a summary and evaluation of available skin irritation studies in humans.

Table 22: Skin irritation testing in human volunteers

Substance	Panellists	Concentration (vehicle)	Exposure Conditions	Effect	Validity	Comments
TEA-based EQ 91032-11-0 77% active [Henkel, 1994chh]	20	10% active substance	30 minutes Open application	No response indicative of irritation observed in any of the panellists	4	Only summary of test report available, test conditions not fully specified; incomplete test substance characterisation
TEA-based EQ 91032-11-0 90% active; 10% IPA [Henkel, 1994dhh]	20	10% active substance	30 minutes Open application	No skin reaction in 10/20 Mild redness in 2/20 panellists disappearing 30 minutes post application	4	Only summary of test report available, test conditions not fully specified; incomplete test substance characterisation
TEA-based EQ 91032-11-0 80% active; 20% PPG [Henkel, 1998chh1]	20	70µl of 1% and 5% aqueous solutions; buffered to pH = 4	24 hrs occluded (Finn Chamber)	Slight erythema and desquamation. Mean sum scores[1] 1%: E: 0.2; Oe: 2.15 Mean sum scores 5%: E: 0.4; Oe: 2.45 Mean sum score water control: E: 0.33, Oe: 1.28	1	Test substance identification not contained in report but provided by sponsor retrospectively
TEA-based EQ 84643-53-8 [Henkel, 1998chh2]	20	70µl of 5% aqueous solution, buffered to pH = 4	24 hrs occluded (Finn Chamber)	Slight erythema and desquamation. Mean sum scores 5%: E: 0.2; Oe: 1.55 Mean sum score water control: E: 0.33, Oe: 1.28	1	Test substance identification not contained in report but provided by sponsor retrospectively
TEA-based EQ 91032-11-0 80% active; 20%	20	70µl of 1% actives in water	24 hrs occluded (Finn	Mean sum scores[1]: E: 0; Oe: 0	1	Test substance identification not contained in report but

[1] Scoring scale according to Frosch P.J., Kligman A.M. (1979). J Am Acad Dermatol. 1:35-41.

Substance	Panellists	Concentration (vehicle)	Exposure Conditions	Effect	Validity	Comments
PPG [Henkel, 1994ehh]			Chamber)			provided by sponsor retrospectively
TEA-based EQ 91995-81-2 90% active; 10% IPA [Henkel, 1992ahh]	20	70µl of 0.1%, 1%, 2% or 10% aqueous solutions	24 hrs occluded (Finn Chamber)	Mean sum scores 0.1%[1]: E: 0.05; Oe: 0 Mean sum scores 1%: E: 0.15; Oe: 0 Mean sum scores 2%: E: 0; Oe: 0 Mean sum scores 5%: E: 0.05; Oe: 0 Mean sum scores 10%: E: 0.1; Oe: 0	1	Test substance identification not contained in report but provided by sponsor retrospectively
TEA-based EQ 91995-81-2 90% active; 10% IPA [Henkel, 1991dhh]	20	5%, 10%, 20% and 50% solutions	30 minutes Open application	No response indicative of irritation observed in any of the panellists	4	Summary of test report available, test conditions not fully specified; incomplete test substance characterisation
TEA-based EQ 91995-81-2 85.4% active; balance H_2O/IPA [Procter & Gamble, 1998bhh]	12	0.4 ml/patch Applied undiluted	4 hrs Semi-occlusive, single patch,	No response indicative of irritation observed in any of the panellists	2	
TEA-based EQ 91995-81-2 84.9% active; balance H_2O/IPA [Procter & Gamble, 1998ahh & 1998bhh]	12	0.4 ml/patch Applied undiluted	4 hrs Semi-occlusive, single patch,	No response indicative of irritation observed in any of the panellists	2	

In 30-minute open application studies, aqueous solutions of up to 50% TEA-based esterquat did not result, apart from a quickly disappearing slight erythema in two subjects shortly after application, in any evidence of a skin irritation response (Henkel, 1991dhh; Henkel, 1994chh; Henkel, 1994dhh).

Following 24 hrs exposure to aqueous solutions of up to 10% TEA-based esterquats only mild and transient irritation indicated by the occurrence of erythema and/or oedema was observed. In 4 hrs semi-occluded patch tests, TEA-based esterquats did not cause any visible skin irritation under the conditions chosen by the study investigators (Procter & Gamble, 1998ahh; Procter & Gamble, 1998bhh). The latter finding is further supported by additional studies with neat application of fabric conditioner formulations with up to 60% of a TEA-based esterquat which did not reveal any evidence of irritation in the test panellists (Procter & Gamble, 1998bhh).

Conclusions

Esterquats were found to be mildly to moderately irritating to rabbit skin. The degree of irritation was dependant on the type of esterquat, the exposure time and patch conditions as well as the concentration of the test material applied to the animals' skin. While HEQ- or MDEA-based esterquats only resulted in a mild irritation response, TEA-based esterquats could cause a moderate level of irritation when applied at concentrations larger than 30% under occluded or semi-occluded conditions. There is some information suggesting a correlation of the irritation potential of TEA-based esterquats with the degree of unsaturated fatty acids.

In human open application tests, reflecting more realistically the type of exposure humans are experiencing when using esterquat-containing fabric conditioners, showed a very favourable skin compatibility profile indicated by an absence of a skin irritation response. Even under more stressed exposure conditions such as 4 hrs or 24-hrs patch tests, the exposure to TEA-based esterquats in concentrations up to 10% resulted in only mild and transient irritation.

4.2.1.4 Eye irritation

The potential of TEA-, HEQ- and MDEA-based esterquats to cause eye irritation in experimental animals has been evaluated on the basis of 20 largely good quality and OECD/EC guideline compliant rabbit eye irritation studies.

The *in vivo* eye irritation studies were conducted with concentrations ranging from 5% to neat product application containing approximately 80-90% of the active test substance (balance to 100% typically isopropanol). The application volumes were largely 0.1 ml for liquid test substances or 0.1 mg for solid test substances (Stepan, 1983chh; Stepan, 1988bhh; Stepan, 1990ahh; Stepan, 1991chh; Degussa, 1993ahh, Henkel, 1993ahh; Kao, 1994ahh; Kao, 1994bhh; Kao, 1995ahh, Kao, 1995bhh; Kao, 1996ahh; Henkel, 1998dhh; CECA, 1999bhh; Stepan, 2002ahh; Evonik, 2008ahh). Few studies were conducted according to the so-called 'low volume eye irritation test' protocol which considers the application of 0.01ml or 0.01mg of the test substance (Degussa, 1994ahh; Degussa, 1994bhh; Procter & Gamble, 1997ahh; Unilever, 1990dhh; Procter & Gamble, 1993chh).

While the available studies are different with regard to the type of test substances and application volumes, the majority of the studies were rated as 'reliable without restriction (Klimisch score 1) or 'reliable with restriction' (Klimisch score 2). For those studies which were rated with a Klimisch score 2, the characterisation of the test substance were either incomplete or missing. For those few studies rated with a Klimisch score of 4, the documentation provided by the study sponsors were incomplete and did not allow reproduction of the study conduct and its result.

The following Table 23 provides a summary of the available studies investigating the eye irritation potential of esterquats in experimental animals.

Table 23: Eye irritation in animals (rabbits)

Substance	Species M/F	Amount applied; Concentration	Exposure Conditions	Mean irritation score (per animal: 24/48/72hrs)	Validity	Comments
TEA-based EQ CAS-No: N/A	Rabbit, NZW;	0.1 ml; applied		Corneal opacity: 0/0/0/0/0/0 Iris lesions: 0/0/0/0/0/0	4	Test substance not identified

Substance	Species M/F	Amount applied; Concentration	Exposure Conditions	Mean irritation score (per animal: 24/48/72hrs)	Validity	Comments
[Stepan, 1983chh]	4/2	undiluted		Redness of conjunctiva: 1.33/1.33/0.33/0.66/1.00/1.33 Chemosis: 0.66/0.33/0.33/0.33/0.66/0.66		nor characterised
TEA-based EQ 91995-81-2 [Stepan, 1988bhh]	Rabbit, NZW; 6/0	0.1 ml; 10% aqueous solution of test substance		Corneal opacity: 0/0/0/0/0/0 Iris lesions: 0/0/0/0/0/0 Redness of conjunctiva: 0/0.33/0/0/0/0 Chemosis: 0/0.33/0/0 /0.33/0.33	2	Incomplete test substance characterisation
TEA-based EQ 91995-81-2 20% active, 80% water [Stepan, 1990ahh]	Rabbit, Rabbit, NZW; 3/0	0.1 ml; applied undiluted		Corneal opacity: 0/0/0 Iris lesions: 0/0/0 Redness of conjunctiva: 0/0.3/0 Chemosis: 0/0/0	2	Incomplete test substance characterisation
TEA-based EQ 157905-74-3 [Stepan, 1991chh]	Rabbit, NZW; 3/0	0.1 ml; applied undiluted		Corneal opacity: 0.7/0.3/1.7 Iris lesions: 0.0/0.0/1.0 Redness of conjunctiva: 1.7/1.0/2.0 Chemosis: 2.0/1.3/3.0	2	Incomplete test substance characterisation
TEA-based EQ 91995-81-2 90% active, 10% IPA [Degussa, 1992chh]	Rabbit, NZW; 6	0.1 ml; applied undiluted		Corneal opacity: 0 Iris lesions: 0.66 Redness of conjunctiva: 1.66 Chemosis: 1.00	3	Due to severity of symptoms, 4 animals did not complete the study
TEA-based EQ 91995-81-2 5% active [Degussa, 1993ahh]	Rabbit, NZW; 3/3	0.1 ml		Corneal opacity: 0/0/0/0/0/0 Iris lesions: 0/0/0/0/0/0 Redness of conjunctiva: 0/0/0/0/0/0 Chemosis: 0/0/0/0/0/0	1	Test substance identification not contained in report but provided by sponsor retrospectively
TEA-based EQ 93334-15-7 90% active, 10% IPA 35%-40% unsaturated FA C18:1 and C18:2 [Henkel, 1993ahh]	Rabbit, Kleinrussen, Chbb: HM; 3/0	100 mg; applied undiluted	Thorough rinsing of eyes after 24 hrs	Corneal opacity: 0/0/0 Iris lesions: 0/0/0 Redness of conjunctiva: 1.66/1.66/1.00 Chemosis: 1.66/2.0/1.0	1	Test substance identification not contained in report but provided by sponsor retrospectively
TEA-based EQ 91995-81-2 90% active, 10% IPA [Degussa 1994ahh]	Rabbit, NZW; 3/0	10 mg; applied undiluted		Corneal opacity: 0/0/0 Iris lesions: 0/0/0 Redness of conjunctiva: 0/0/0.7 Chemosis: 0/0/0	2	Test substance was applied in a low-volume (0.01 ml) instead of 0.1 ml (rationale provided) as mandated by OECD TG 405
TEA-based EQ 91995-81-2 80.5% active; 10.5% IPA; 8-9% RP [Degussa,	Rabbit, NZW; 3/0	10 mg; applied undiluted		Corneal opacity: 0/0/0 Iris lesions: 0/0/0 Redness of conjunctiva: 0.7/0.3/0.3 Chemosis: 0/0/0	2	Test substance was applied in a low-volume (0.01 ml) instead of 0.1 ml (rationale

Substance	Species M/F	Amount applied; Concentration	Exposure Conditions	Mean irritation score (per animal: 24/48/72hrs)	Validity	Comments
1994bhh]						provided) as mandated by OECD TG 405
TEA-based EQ 94095-35-9 90% active; 10% IPA [KAO, 1994ahh]	Rabbit, NZW; 3/0	0.1 ml; applied undiluted		Corneal opacity: 1/2/1.33 Iris lesions: 0/2/1 Redness of conjunctiva: 3/3/3 Chemosis: 3.33/4/4	1/2	Incomplete test substance characterisation
TEA-based EQ 91995-81-2 22% active; 2.4% IPA [KAO, 1994bhh]	Rabbit, NZW; 3/0	0.1 ml; applied undiluted pH = 2.86		Corneal opacity: 0/0/0 Iris lesions: 0/0/0 Redness of conjunctiva: 0.66/0.33/0 Chemosis: 0.66/0/0	1	Test substance identification not contained in report but provided by sponsor retrospectively
TEA-based EQ 91995-81-2 85% active; 14.6% IPA [KAO, 1995bhh]	Rabbit, NZW; 3/0	0.1 ml; applied undiluted		Corneal opacity: 0.66/0.33/1.00 Iris lesions: 0.66/0.00/0.33 Redness of conjunctiva: 1.66/1.33/2.00 Chemosis: 1.33/1.00/2.00	1	Test substance identification not contained in report but provided by sponsor retrospectively
TEA-based EQ 91995-81-2 90% active; 10% IPA [KAO, 1995chh]	Rabbit, NZW; 1/2	0.1 ml; applied undiluted		Corneal opacity: 0/0/0 Iris lesions: 0/0/0 Redness of conjunctiva: 2/0.33/1.67 Chemosis: 2.00/0.33/1.67	1	Test substance identification not contained in report but provided by sponsor retrospectively
TEA-based EQ 91995-81-2 85% active; 15% IPA [KAO, 1996ahh]	Rabbit, NZW; 3/0	0.1 ml; applied undiluted		Corneal opacity: 0.33/0.66/0.33 Iris lesions: 0/0/0 Redness of conjunctiva: 1.33/1.33/1.33 Chemosis: 1/1.33/0.66	1	Test substance identification not contained in report but provided by sponsor retrospectively
TEA-based EQ 91995-81-2 [Procter & Gamble, 1997ahh]	Rabbit, NZW; 1/2	0.01 ml; applied undiluted		Corneal opacity: 0/0/0 Iris lesions: 0/0/0 Redness of conjunctiva: 0.33/0/0.33 Chemosis: 0/0/0	2	Test substance was applied in a low-volume (0.01 ml) instead of 0.1 ml (rationale provided) as mandated by OECD TG 405
TEA-based EQ 91955-81-2 90% active, 10% IPA [Henkel, 1998dhh]	Rabbit, SPF Albino; 0/1	0.1 ml; 36.63% in sesame oil		Corneal opacity: 2.0 Iris lesions: 1.0 Redness of conjunctiva: 3.0 Chemosis: 4.0	3	Investigative study; not sufficient animals to derive conclusions
TEA-based EQ CAS-No: N/A [CECA, 1999bhh]	Rabbit, NZW; 3/1	22%		Not irritating to eyes	4	Study details not available
TEA-based EQ 91995-81-2 [Stepan,	Rabbit, NZW; 3/0	0.1 ml; applied undiluted		Corneal opacity: 0/0/0 Iris lesions: 0/0/0 Redness of conjunctiva:	4	Limited reporting and incomplete test

Substance	Species M/F	Amount applied; Concentration	Exposure Conditions	Mean irritation score (per animal: 24/48/72hrs)	Validity	Comments
2002ahh]				0.33/0/0.33 Chemosis: 0/0/0		substance identification and characterisation
TEA-based EQ 157905-74-3 100% active; no solvent [Evonik, 2008ahh]	Rabbit, NZW; 1/2	0.1 ml; applied undiluted	eyes were rinsed with 0.1% saline 1 hr after instillation	Corneal opacity: 0/0/0 Iris lesions: 0.33/0.33/0.33 Redness of conjunctiva: 1/1/1 Chemosis: 0.33/0/0.33	1	
HEQ-based EQ 19467-38-0 82% active [Unilever, 1990dhh]	Rabbit, NZW; 3	100 mg; applied undiluted		Corneal opacity: 0/0/0 Iris lesions: : 0/0/0 Redness of conjunctiva: : 0/0/0 Chemosis: : 0/0/0	2	Incomplete test substance characterisation
	1	50 mg; applied undiluted		Corneal opacity: 0 Iris lesions: : 0 Redness of conjunctiva: : 0 Chemosis: : 0	3	Investigative study; not sufficient animals to derive conclusions
	1	10 mg; applied undiluted		Corneal opacity: 0 Iris lesions: : 0 Redness of conjunctiva: : 0 Chemosis: : 0	3	Investigative study; not sufficient animals to derive conclusions
MDEA-based EQ 67846-68-8 [Procter & Gamble, 1993chh]	Rabbit, NZW; 1/2	0.01ml; applied undiluted		Corneal opacity: 0/0/0 Iris lesions: 0/0/0 Redness of conjunctiva: 0/0/0 Chemosis: 0/0/0	2	Test substance was applied in a low-volume (0.01 ml) instead of 0.1 ml (rationale provided) as mandated by OECD TG 405

The observed eye irritation potential of the examined esterquats is somewhat similar to their skin irritation response in the sense that the available information does not provide a coherent picture that would allow associating an observed response with a single factor such as concentration, solvent content or chemical or physical characteristics specific to the respective test substance. Only HEQ- or MDEA-based esterquats did not reveal any evidence of an eye irritation response if applied undiluted to the rabbit eyes.

At application volumes of 0.1ml or 0.1mg, aqueous solutions of TEA-based esterquat with active concentrations less than 30% are only mildly irritating to eyes. The responses that have been typically observed at these active levels are related to conjunctival redness or chemosis. Corneal damage or iritis was not observed.

With increasing the active content at the same application volume, TEA-based esterquats do exert the potential to cause eye irritation also at the level of cornea and iris. At active levels larger than 80% TEA-based esterquat, 2 out 5 studies rated with Klimisch score 1 or 2 revealed a level of eye irritation sufficiently high to require classification for eye irritation (R36) according to Directive 67/548/EEC (Stepan, 1991chh; KAO, 1994ahh). One of the test materials is characterised by a high level of

unsaturated fatty acids in the fatty acid chain (> 50%) and contained in addition to the esterquat 10% isopropanol. The 2nd material also contained 10% isopropanol, but no further information on the fatty acid chain was available. Exposure to either of these materials led to effects in cornea, iris and conjunctivae with conjunctival redness and/or chemosis triggering the need for R36 classification. While the other 3 studies did not indicate the need for R36 classification of the respective test materials, irritation effects in cornea, iris and/or conjunctivae were also seen in these studies (Kao, 1995bhh; Kao, 1995chh, Kao, 1996ahh). These test materials contained levels of 10-15% isopropanol and a percentage of unsaturated fatty acids in the fatty acid chain of up to 50%.

In a more recent investigation, the eye irritation potential of isopropanol-free TEA-based esterquat was investigated in an *in vitro* HET-CAM assay as well as in an *in vivo* study in rabbits (Evonik, 2007ahh; Evonik, 2008ahh). Except for the solvent content, the test substance is structurally comparable to the material used in an earlier rabbit eye irritation study which revealed severe irritation responses in individual animals preventing the completion of the study for animal welfare reasons (Degussa, 1992chh). In the HET-CAM assay, an accepted alternative method to the Draize test for the identification of severely irritating substances, the treatment with the test substance resulted in the appearance of vascular injection on the CAM of 4 eggs 5 minutes post application. No other effect was detected after treatment and a total of 0.67 scores (of 21 scores possible) were determined. According to the irritation index developed for the HET-CAM in analogy to the Draize irritation test, the test substance would be considered as not irritating. In the subsequent eye irritation study in rabbits, only slight ocular reactions were observed in the conjunctiva and iris 24 hours following exposure. Conjunctival redness remained 72 hrs after exposure and were completely cleared at study termination 7 days after study start. No corneal damage was observed in any of the animals at any time point. This investigation underlines the influence of the solvent isopropanol in the test material and one could assume that the irritation response could be related to the inherent irritation potential of isopropanol itself or the increased bioavailability of the esterquat for tissue reaction in the presence of isopropanol. However, other factors such as the physico-chemical characteristics of the test substance such as its granularity leading for example to mechanical irritation should also be considered in this context.

At application volumes of 0.01ml or 0.01mg, the TEA-based esterquats caused only a mild irritation response, which was limited to redness of the conjunctivae.

Conclusion

In conclusion, there is no evidence suggesting that HEQ- or MDEA-based esterquats have the potential to cause eye irritation upon accidental eye exposure. Available eye irritation studies with neat test materials in rabbits did not reveal any evidence of an eye irritation response in experimental animals.

With regard to the eye irritation potential of TEA-based esterquat, the available information provides a less coherent picture. While the information indicates that at application volumes of 0.1 ml and concentrations larger than 80% active esterquat irritation responses at corneal, iris and/or conjunctivae level are observable in experimental animals, in the majority of the studies the responses are slight to moderate and the eyes of the treated animals returned to normal a few days after exposure. The degree of irritation is concentration dependent as dilutions in water result in proportionally lower level of irritation.

There is some evidence that for certain types of TEA-based esterquats, the presence of the solvent isopropanol or a proportionally high content of unsaturated fatty acids in the TEA-based esterquat may amplify the eye irritation response. However, these observations are currently only supported by a small number of studies. The overall data set is not yet sufficient to draw firm conclusions for all TEA-based esterquats. Moreover, other factors such as pH or the granularity of the solid material require further consideration in the assessment.

5.2.1.5 Skin Sensitization

The skin sensitisation potential of TEA-, HEQ-, and MDEA-based esterquats has been evaluated on the basis of a total of 30 studies that were provided by manufacturers and users of these materials. The studies can be broken down as follows:

- 22 studies on TEA-based esterquats
    - 7 Guinea pig maximisation tests
    - 9 Buehler tests
    - 1 Human maximisation test
    - 3 Human repeat insult patch tests
    - 2 Human diagnostic patch tests
- 6 studies on HEQ-based esterquats
    - 2 Guinea pig maximisation tests
    - 1 Mouse local lymph node assay
    - 1 Human maximisation test
    - 2 Human diagnostic patch tests
- 2 studies on MDEA-based esterquats
    - 2 Human repeat insult patch tests

For all 30 skin sensitisation studies, the full study reports were available and subsequently reviewed and summarised. Information on the chemical structure, source of raw materials and other types of process-related residues or impurities were available at a variable degree of detail for most of test substances.

The original study reports were evaluated according to the Klimisch criteria (Klimisch *et al.*, 1997) and summarised in form of robust data summaries. The skin sensitisation potential was evaluated using a weight of the evidence approach by taking into account the quality, reliability, relevance and adequacy of the data.

5.2.1.5.1 Animal data: Guinea Pig Maximization Tests (GPMT)

Table 24 summarises the results of a total of 9 GPMT, 7 with TEA-based esterquats and 2 GPMT with HEQ-based esterquats.

Table 24: Results obtained in the guinea pig maximisation test

Substance	Strain M/F	Induction intradermal (%)	Induction epidermal (2%)	Challenge epidermal (%)	Results	Conclusion	Relia-bility
TEA-based EQ	Dunkin	1% in	25% in	10% in	Reaction in 5%	Not a	1

Substance	Strain M/F	Induction intradermal (%)	Induction epidermal (2%)	Challenge epidermal (%)	Results	Conclusion	Relia-bility
91995-81-2 [CECA, 1991dhh]	Hartley 15/15	Paraffin	Water	Paraffin	of treated animals	sensitizer	
TEA-based EQ 93334-15-7 [Degussa, 1992dhh]	Pirbright White 10/10	5% in Water	25% in Water	10% in Water	No reaction in treated animals	Not a sensitizer	2
TEA-based EQ 94095-35-9 [KAO, 1997bhh]	Dunkin Hartley 10/10	1% in Saline	20% in Water	1% in Water	Reaction in 15% of treated animals at first challenge	Not a sensitizer	1
TEA-based EQ (tallow) 91995-81-2 [Henkel, 1990bhh]	Pirbright white 0/20	0.5% in Paraffin	5% in Paraffin	2% in Paraffin	Reaction in 95% of treated animals but high level of irritation at challenge	Not a sensitizer	3
DEEDMAC 97158-31-1 [Akzo Nobel, 1994ahh]	Hima-layan 20*	5% in Water	50% in Water	50%, 25%, 10% in Water	Reaction in 5% of treated animals	Not a sensitizer	1
HEQ-based EQ 19467-38-0 [Unilever, 1995ahh]	Dunkin Hartley 20./20	10% in Alembicol	25% in Petrolatum	7.5 & 3.5% in Petrolatum	Reaction in 10% of treated animals	Not a sensitizer	1
TEA-based EQ 91995-81-2 [Unilever, 1990ehh]	Dunkin Hartley 4/6	0.05% in Saline	25% in Saline	1% in Saline	Reaction in 60% of treated animals at 1st challenge and 30% at 2nd challenge	Sensitizer	2
TEA-based EQ (tallow) 91995-81-2 [Unilever, 1990fhh]	Dunkin Hartley 10/10	0.05% in Saline	25% in Saline	1% in Saline	Mild reaction in 40% of treated animals at 1st challenge, not maintained at 48 hr reading	No conclusions	3

* No information on number of males and females

The majority of the studies were judged to be of good quality and reliability. The studies were either in full compliance or generally, but not totally compliant with OECD guideline 406 for the guinea pig maximisation test.

Two out of a total of nine GPMT were rated as 'not reliable' (Klimisch rating 3). Although broadly compliant with the OECD testing guidelines, the limited reporting of one study (Unilever, 1990fhh) did not allow the findings and conclusions of the investigators to be reproduced. The second study (Henkel, 1990bhh) demonstrated a too high level of irritation in test substance and control groups throughout the induction and challenge phases. Any skin sensitisation response may have been masked by irritation.

Considering only Klimisch 1 or 2 rated studies, one study was positive and 7 studies were negative for skin sensitisation as defined by Directive 67/548/EEC. Some weak responses indicative of skin

sensitisation (but below threshold for skin sensitisation classification within the meaning of Directive 67/548/EEC) were also seen throughout most of the studies that were considered negative. The interpretation of results of the study which was evaluated to be positive for skin sensitisation was complicated through occurrence of a relatively high level irritation throughout the induction and challenge phase.

5.2.1.5.2 Buehler tests

The potential of esterquats to induce a skin sensitisation reaction in guinea pigs was further evaluated in a total of 9 studies according to the Buehler protocol. All 9 studies were conducted with TEA-based esterquats. Three of these 9 studies were rated as 'not reliable' (Klimisch 3) or 'not assignable' (Klimisch 4). While broadly compliant with the testing guidelines, a high level of irritation was observed in two studies in test substance and control animals (Henkel, 1995bhh; Kao, 1989chh). The third study did not include a negative control and hence, the results could not be interpreted (Stepan, 1983dhh).

From a total of 6 studies which were rated as reliable without (Klimisch 1) or with restrictions (Klimisch 2), four studies were negative and two studies positive for skin sensitisation as defined by Directive 67/548/EEC. One of the studies which were evaluated as positive was considered borderline as an inconsistent pattern of response was observed. However, the net rate of positive responses in the animals at the 1st and 2nd challenge with the 3% challenge concentration was consistently above 15% and therefore, according to the criteria of Directive 67/548/EEC, considered positive.

Similar to that observed in the GPMT's, relatively high and variable level of irritation throughout induction and challenge were seen in the Buehler tests in the test substance as well as in the control groups, rendering the interpretation of the study results more difficult.

Table 26 provides a summary and overview of the available Buehler tests including their reliability rating.

Table 25: Buehler tests

Substance	Strain M/F	Induction concentration	Challenge concentration	Result	Conclusion	Validity
TEA-based EQ (unsaturated) 91995-81-2 [Henkel, 1994fhh]	Pirbright White 0/20	60%, 50%, 25% in Water	10% in Water; 3% in Water at 2nd challenge	Reaction in at least 15% of treated animals at 1st challenge	Sensitizer	1
TEA-based EQ 91032-11-0 [Henkel, 1994ghh]	Pirbright White 0/20	12.5% in Saline	3% in Saline	Reaction in at least 15% of treated animals at 2nd challenge	Sensitizer	2
TEA-based EQ (hardened) 91995-81-2 [Clariant, 2002chh]	Pilbright White 0/20	100% , moistened	100%, moistened	No reaction in treated animals	Not a sensitizer	1
TEA-based EQ 68921-27-7 [Clariant, 2004]	Himalayan spotted 0/20	25% in Water	1% in Water	No reaction in treated animals	Not a sensitizer	1
TEA-based EQ 93334-15-7 [KAO, 1989dhh]	Dunkin Hartley 0/20	100% Moistened	100%	No reaction in treated animals	Not a sensitizer	2
TEA-based EQ (tallow) 91995-81-2 [Henkel, 1992ehh]	Pirbright White 0/20	15% in Saline	5% in Saline	Irritation not sensitization in treated animals	Not a sensitizer	2
TEA-based EQ 91995-81-2 [Henkel, 1995bhh]	Dunkin Hartley 0/20	75%,75%,15% in Water	25%; 5% in Water; 15% in Water at 2nd challenge	Severe irritation in treated and controls animals, results cannot be interpreted	No conclusion	3
TEA-based EQ 93334-15-7 [KAO, 1989chh]	Dunkin Hartley 0/20	100% Moistened	50% in Water; 25% in Water at 2nd challenge	Severe irritation in treated and controls animals, may have masked potential sensitization effects	No conclusion	3
TEA-based EQ 68921-27-7 [Stepan, 1983dhh]	Albino Hartley 0/10	10% in Water	10% in Water	Slight to no reaction in treated animals	No conclusion	4

5.2.1.5.3 Local Lymph Node Assay

An HEQ-based esterquat was evaluated in a mouse local lymph node assay. The study was consistent with OECD guideline 429 and evaluated as reliable without restriction (Klimisch 1).

In this study CBA/Ca mice were treated by topical application on the dorsum of each ear of 25 µl of 0, 5, 10 and 25% test substance in olive oil once daily for three consecutive days. Each mouse received daily topical application of the test substance on the dorsum of both ears for 3 consecutive days.

The test substance did not elicit a positive sensitisation responses at any of the concentrations tested. The highest recorded stimulation index was 1.1 at the 10% concentration. The mice showed no visible signs of toxicity to HEQ throughout the study.

5.2.1.5.4 Human data

Table 27 provides an overview and summary of available skin sensitisation studies according to Stott's human repeated insult patch test and Kligman's human maximisation test protocol.

Table 26: Human Repeated Insult Patch Tests (HRIPT) and Human Maximisation Tests (HMT)

Substance	Study Type	Subjects (compl.)	Induction Concentration	Challenge Concentration	Result	Validity
TEA-based EQ 91032-11-0 [Henkel, 1995ahh]	HRIPT	88	0.5%, 1%, 2% (w/v) in Water	0.5%, 1%, 2% (w/v) in water	Not a sensitiser	1
TEA-based EQ 91032-11-0 [Henkel, 1998ehh]	HRIPT	95	2% (w/v) in Water	2% (w/v) in Water	Not a sensitiser	1
MDEA-based EQ 67846-68-8 [Procter & Gamble, 1986ahh]	HRIPT	84	1.5% (w/v) in Water	1.5% (w/v) in Water	Not a sensitiser	2
MDEA-based EQ 67846-68-8 [Procter & Gamble, 1993dhh]	HRIPT	95	2% (w/v) in Water	2% (w/v) in Water	Not a sensitiser	2
TEA-based EQ in fabric softener formulation 91995-81-2 [Procter & Gamble, 1999ahh]	HRIPT	93	$\pm$ 0.5% (w/v) in Water	$\pm$ 0.5% (w/v) in Water	Not a sensitiser	2
HEQ-based EQ 19467-38-0 [Unilever, 1994ahh]	HMT	25	15% in H_2O + 1% SLS pre-treatment	15% in H_2O	Not a sensitiser	1
TEA-based EQ 91995-81-2 [Unilever, 1994bhh]	HMT	25	15% in H_2O + 1% SLS pre-treatment	15% in H_2O	Not a sensitiser	1

All studies were considered to be of good quality and rated either with Klimisch score 1 or 2.

The absence of significant skin sensitisation potential of TEA-, HEQ-, or MDEA-based esterquats was confirmed in a total of 7 human volunteer studies, 5 studies according to HRIPT and 2 studies according to the human maximisation test protocol.

In all studies, a low percentage of subjects showed low-grade signs of skin irritation during or after induction. One study involving a fabric softener formulation containing a TEA-based esterquat at 28% caused reactions indicative of skin sensitisation at challenge in 2 volunteers, but a re-challenge did not confirm that the original challenge response were allergic in nature.

In summary, in none of the 7 studies repeated exposures to the various types of esterquats induced a skin sensitisation response in humans.

5.2.1.5.5 Additional human data

The incidence of skin sensitisation to HEQ and TEA based quats in patients attending dermatology clinics was evaluated. In two independent studies, patients attending St John's Institute of Dermatology, London, UK (185 patients) and the Contact Dermatitis Clinic at Leuven University Hospital, Belgium (56 patients) for evaluation of their dermatitis, not considered to be related specifically to exposure to fabric conditioner, were patch-tested as part of the normal clinical diagnostic process to evaluate possible sensitisation to HEQ and TEA based esterquats. (Unilever 1994chh; Unilever 1995bhh).

A preliminary study was performed to identify appropriate concentrations for diagnostic patch testing. At a concentration of 2% (w/v), neither test material was associated with skin irritation reactions whereas 5% (w/v) was identified as a concentration of test material on the irritant threshold. For the main study, each material was thus prepared at a concentration of 2% (w/v) and/or 5% (w/v) in petrolatum and was applied to patients' back in 8mm Finn Chambers under occlusion for 48 hours. Patch sites were assessed immediately after patch removal and again after a further 48 hours. Any reactions were scored according to the standard international contact dermatitis research group scale (Fregert S., 1981).

In the main study at the St John's Institute for Dermatology, no inflammatory skin reactions were observed with either HEQ- or TEA-based esterquats at the sub-irritant concentration of 2%. At the irritant threshold concentration 31 out of the 185 patients had reactions to TEA-based esterquats. Only 12 of these reactions were present at the key scoring 48 hours after patch removal. Furthermore, all of the reactions were equivocal or weak and in each case were judged by the clinician to be irritant in nature and unrelated to the patients' eczema. Two individuals had reactions to HEQ-based esterquats, only one of which was present at the 48 hour reading. These reactions were weak and were considered by the clinician to be irritant in nature. Out of the 56 patients in the study at the Leuven University hospital, five patients had reactions to TEA-based esterquats and none to HEQ-based esterquats. The reactions to the TEA-based esterquat were judged by the clinicians as weak or equivocal and to be irritant in nature.

Table 28 summarises the outcome of the human diagnostic patch testing with TEA- and HEQ based esterquats.

Table 27: **Human Diagnostic Patch Tests**

	Substance	Patients	Patch Concentration	Result	Validity
Study 1: [Unilever, 1994chh]	HEQ-based EQ 19467-38-0	185	2% and 5% in petrolatum	No reactions indicative of skin sensitisation	2
	TEA-based EQ 91995-81-2	185	2% and 5% in petrolatum	No reactions indicative of skin sensitisation	
Study 2: [Unilever, 1995bhh]	HEQ-based EQ 19467-38-0	56	5% in petrolatum	No reactions indicative of skin sensitisation	2
	TEA-based EQ 91995-81-2	56	5% in petrolatum	No reactions indicative of skin sensitisation	

5.2.1.5.6 Case reports

A literature search was conducted to identify human case reports of skin sensitisation to esterquats. Databases and search terms are described in Annex 6. Not a single case of documented skin sensitization to any of the esterquats was found. While in general absence of evidence of effects should not be construed as evidence of absence of effects, it should be noted that the use of esterquats in fabric conditioners and hair conditioners is widespread and a significant proportion of the population must have been exposed to them with no documented cases of allergic contact dermatitis arising This allergy has not escaped detection due to lack of knowledge, because the medical community is well aware of the composition of softeners due to occasional skin problems associated with fabric and hair softeners. In general, these are immediate-type reactions (e.g. urticaria or itching), and other components of the commercial product may be involved, but sensitization to esterquats has never been implicated. Under these conditions, the absence of any case reports contributes significantly to the weight of evidence.

5.2.1.5.7 Weight of evidence

This analysis considered a total of 30 studies that investigated the skin sensitisation potential of TEA-, HEQ-, or MDEA-based esterquats.

The original study reports of all studies as well as associated test substance characterisation reports or information were reviewed and the quality of each was evaluated according to the so-called Klimisch criteria. Twenty five (25) studies obtained a Klimisch rating of 1 (‘reliable without restrictions’) or 2 (‘reliable with restrictions’). These are composed of 7 guinea pig maximisation test (GPMT), 6 guinea pig tests according to the Buehler protocol (BT), 1 mouse local lymph node assay (LLNA), 5 human repeat insult patch tests (HRIPT), 2 human maximisation test (HMT) and 2 human diagnostic patch tests (HDPT). Five studies (2 GPMT; 3 BT) were evaluated to be ‘not reliable’ and thus are not considered in the following weight of the evidence analysis.

None of the 11 studies involving exposure of human volunteers to TEA-, HEQ-, or MDEA-based esterquats were considered to induce a skin sensitisation response in humans. One study involving a fabric softener formulation containing a TEA-based esterquat caused reactions indicative of skin

sensitisation at challenge in 2 volunteers, but a re-challenge did not confirm that the original challenge response were allergic in nature. All studies involved esterquat exposures that either reflect or are higher than exposures consumers would experience when using esterquats in a fabric softener context.

The skin sensitisation potential of HEQ-based esterquats was evaluated on the basis of a total of 3 animal studies, 2 GPMT and 1 LLNA, which were considered to be 'reliable without restriction' (Klimisch rating 1). While the HEQ-based esterquats showed some weak skin sensitisation responses in the GPMT, this response was clearly below the threshold that would trigger a classification for skin sensitisation according to the criteria of Directive 67/548/EEC. In the LLNA, the HEQ-based esterquat did not increase the stimulation index above 3. No animal data were available as part of this investigation for MDEA-based esterquats.

This review evaluated the skin sensitisation potential of TEA-based esterquats on the basis of a total of 11 animal studies, 5 GPMT and 6 BT. There was equivocal evidence for TEA-based esterquats to cause skin sensitisation in experimental animals. Three studies (1 GPMT, 2 BT) were considered positive and 8 studies (4 GPMT, 4 BT) were negative for skin sensitisation as defined by Directive 67/548/EEC. Some weak skin responses indicative of a sensitisation response were seen in most of the studies. However, the interpretation of the study results, especially those which were assessed to be positive, was rendered more difficult by variable levels of irritation throughout the induction phase which sometimes led to a reduction of the induction concentration during the induction phase. It cannot be excluded that these changes may have impacted the study outcome. Likewise, two studies considered negative displayed also a variable level of irritation at challenge and it could not be excluded that in these cases the irritation observed also in the control groups may have masked potential skin sensitisation responses. The results obtained in the various guinea pig assays must be also seen in context of their overall accuracy of 72% relative to human sensitisation data (Dean J.H., 2001).

Conclusion

There is no information suggesting HEQ- or MDEA-based esterquats to have skin sensitisation potential in animals or humans.

Taking all evidence from available human and animal data together, the weight of the evidence suggests that also TEA-based esterquats do not represent a skin sensitisation hazard to humans. This is supported by Rodriguez *et al.* who reported that esterquat containing liquid fabric softener formulations and softener treated fabrics were tested at concentrations ranging from 2 to 30% in more than 4,000 individuals over a 20-year period. No sensitisation was observed in any of the subjects (Rodriguez C. *et al.*, 1994). This assessment takes further into account that:

- There is no evidence that TEA-based esterquats cause skin sensitisation in humans based on the outcome of 3 human repeat insult patch tests and 1 human maximisation test.
- There is no evidence from 2 independent human diagnostic patch tests among more than 200 contact dermatitis patients that existing exposures to a TEA-based esterquat has induced skin sensitisation. The expert dermatologists involved in the study rated the few reactions observed as irritant in nature;
- The skin sensitisation response in animal studies is generally weak or equivocal.

- The ability of chemicals to penetrate the human skin is a pre-requisite to cause a skin sensitisation response. The relatively high molecular weight and available physico-chemical information suggest that the dermal penetration of TEA-based esterquats can be considered to be very low (TGD, 2003). Skin penetration can be enhanced by certain testing or skin conditions (e.g., skin with reduced skin barrier function). In this context it is of note that under normal and foreseeable use conditions esterquat softened fabrics did not cause any skin irritation effects on infant, sensitive or adult skin (Rodriguez C. *et al.*, 1994; Pierard G.E. *et al.,* 1994a; Pierard G.E. *et al.*, 1994b; Hermans J.F. *et al.*, 2001). These studies actually suggested a beneficial effect of softened fabrics by reducing frictional effects on the skin relative to non-treated fabric as determined by visual skin grading for redness, dryness and smoothness and instrumental measurements (i.e., capacitance, trans-epidermal water loss, and colorimetry). These studies indicate that the prolonged skin contact with softened fabrics does not compromise the skin barrier function and therefore is not expected to increase the dermal penetration of esterquats under use conditions.

4.2.1.6 Repeated dose toxicity

4.2.1.6.1 Subacute and subchronic oral toxicity

The repeated oral dose toxicity of TEA-, HEQ- and MDEA-based esterquats has been evaluated in two 28-day oral gavage, one 28-day dietary, two 90-day oral gavage and one 90-day drinking water study.

In a recent OECD guideline 407 compliant 28-day oral gavage study, a TEA-based esterquat was given at doses of 0, 80, 240, or 800 mg/kg bw/day active substance by oral gavage (Degussa, 2005ahh). No mortality, morbidity or significant changes of any of the investigated parameters were noted in any of the observed groups. Macroscopic post mortem examination and histopathology revealed no test item-related changes in the esterquat treated animals. Under the conditions of the study, the NOAEL was determined to be larger than 800 mg/kg bw/day of the active esterquats which reflects a NOAEL of 1,000 mg/kg bw/day of the test substance (*i.e.*, test substance contains > 80% TEA-based esterquat).

In another OECD guideline 407 compliant 28-day dietary study, Wistar rats were fed with a diet containing 0%, 0.008%, 0.04%, 0.2% or 1% on an HEQ-based esterquat (Unilever, 1992ahh). A final confirmation of the concentrations in the food was not feasible due to limitations of the analytical methods. Neither mortality nor significant toxicity was observed in the experimental animals as a result of the treatment. Probably as a result of increased food consumption, the body weights as well as the absolute weights of spleen, kidney and liver were increased at study termination in the female rats of the 1% dose group. These changes were, however, not associated with any histological change. There were minor changes in blood biochemistry which neither resulted in any concurrent histopathological changes. The histological examination including also the reproductive organs did not reveal any treatment related abnormalities. Subsequently, a NOAEL can be established for 1% dose group which reflects an exposure of approximately 1,000 mg/kg bw/day.

The MDEA-based esterquat DEEDMAC was also investigated in a 28-day oral gavage study in rats at doses of 10, 100 or 1,000 mg/kg bw/day (Unilever, 1997ahh). Group sizes were five per sex for the 10 and 100 mg/kg bw/day dose groups and 10 animals per sex in the 1,000 mg/kg bw/day and the two control (water; dose vehicle 1% isopropanol) group. Half of the rats of the two controls and the high

dose group were terminated after 4 weeks of treatment, the remainder being maintained for a 4-week recovery period. Neither mortality nor any clinical signs or changes related to body weight, food/water consumption, haematology or organ weights which were observed or attributable to the treatment of the animals. In males receiving 1,000 mg/kg bw/day, there was an indication of suppression in arousal processes. After the 4-week recovery, changes in activity were still apparent. There was no evidence of adverse effects neither at lower dosages nor in females receiving 1,000 mg/kg bw/day. While these findings may be indicative of some alteration in the functioning of the nervous system, the absence of any pathological change limit its toxicological importance. The male high dose group showed increased liver enzyme levels which did not go along with any notable effects on liver weight or microscopic pathology. Hence, these changes were considered adaptive in nature and not indicative of toxicity. In conclusion, on the basis of this study a dose of 1,000 mg/kg bw/day can be established for DEEDMAC as the NOEL for female rats and NOAEL for male rats. The latter considers the minor effect on male behaviour at this dose. The respective NOEL for male rats can be established at 100 mg/kg bw/day.

The subchronic toxicity of a TEA-based esterquat was evaluated in OECD guideline and GLP conform 90-day oral gavage study (Henkel, 1991ahh) at dose levels of 0, 100, 300 or 1000 mg/kg bw/day. Each group considered 10 animals per sex. For the control as well as the high dose group, 5 animals of each sex were used as recovery groups to determine the reversibility of any potential effects. The interpretation of this study is somewhat hampered due the occurrence of a bacterial infection in all dose groups. Some of the macroscopical or microscopical findings were directly related by the study investigators to this infection. Apart from these effects, animals of the high dose groups displayed potentially substance related increases of blood liver enzyme, signs of gastric irritation and regressive epithelial changes in the urine bladder. However, an interaction with the bacterial infection cannot be entirely excluded. The histological examination including also the reproductive organs did not reveal any treatment related abnormalities. On the basis of this study, a NOEL of 300 mg/kg bw/day for the investigated TEA-based esterquat can be derived.

The MDEA-based esterquat DEEDMAC was further investigated in a 90-day oral gavage study (Procter & Gamble, 1994ahh). In this study, rats with group sizes of 15 animals per sex were gavaged with doses of 0, 10, 100 or 500 mg/kg/day. Nor mortality, morbidity or significant changes of any of the investigated parameters were noted in any of the dose groups. Macroscopic post mortem examination and histopathology including reproductive organs did not reveal test substance related changes in any of the treated animals. Thus, the high dose level of 500 mg/kg bw/day was considered the NOEL for DEEDMAC on the basis of this study.

Finally, a 90-day study investigated the subchronic toxicity of TEA-based esterquat (i.e., 85% in IPA) in rats (Colgate-Palmolive, 1991ahh). In this study, the test material was given in drinking water in concentrations of 0, 0.01%, 0.32% and 1.6% v/v. Combining male and female dose ranges, this equals an active substance intake of about 0, 80-190, 247-703 and 1840-3860 mg/kg/day respectively. The only noticeable effects in this study were in the male top dose group, diarrhea going along with a weight loss, a slightly increased kidney- to-bodyweight ratio without any concurrent histopathological effects and some functional effects of mild dehydration. The histological examination which included also the reproductive organs did not reveal any abnormalities. On the basis of this study, a NOEL of 247-703 mg/kg bw/day was established.

Table 29 summarises the available 28-day and 90-day repeated dose toxicity studies discussed in this Chapter.

Table 28: **Repeated oral dose toxicity studies**

Substance	Species M/F	Dose (mg/kg bw/d)	NOAEL (mg/kg bw/d)	Validity	Comments
28-day subacute oral toxicity					
TEA-based EQ 157905-74-3 80% active [Degussa, 2005ahh]	Rat, Crl: CD; 5/5 per group	0, 80, 240, 800 (corrected); 0, 100, 300, 1000 (nominal); gavage	800	1	
HEQ-based EQ CAS-No: N/A 83.2% active [Unilever, 1992ahh]	Rat, Wistar; 10/10 per group	6.5, 33.0, 159.7, 820.1 (corrected); 7.8, 39.6, 191.9, 985.7 (nominal); (active in diet)	820	2	Study conducted closely follows OECD TG 407. However, the following deviations from OECD TG were observed: 1. One of the mandatory organ weight (epididymis) not recorded 2. Dietary test substance conc., stability & homogeneity could not be measured 3. Dose selection rationale not provided (highest dose level producing no appreciable toxicity)
MDEA-based EQ CAS-No: N/A [Unilever, 1997ahh]	Rat, Crl CD BR VAF+; 5/5 (in 10 & 100 mg/kg dose group); 10/10 (in 1000 mg/kg dose group)	0, 10, 100, 1000; (gavage)	1000	1	
90-day subchronic oral toxicity					
TEA-based EQ 93334-15-7 [Henkel, 1991ahh]	Rat, Sprague-Dawley; 10/10 per group	0, 100, 300, 1000; oral (gavage)	300	2	Incomplete test substance characterisation; occurance of bacterial infection in all dose groups hampers the interpretation of the study results
MDEA-based EQ CAS-No: N/A [Procter & Gamble, 1994ahh]	Rat, Crl CD BR; 15/15 per group	0, 1, 10, 500; (gavage)	500	2	Comparable to OECD TG 408. However, the following deviations were observed: 1. All the mandatory organs were not weighed at necropsy 2. Detailed neurobehavioural screening not done 3. Dose selection rationale improper (as the highest tested dose shows no effect)
TEA-based EQ CAS-No: N/A [Colgate-Palmolive, 1991ahh]	Rat, Sprague Dawley-derived outbred albino; 10/10 per group	0, 80-190, 287-703, 1,840-3,800 (drinking water)	~ 247-703 mg/kg bw/d	4	Study details not available for review

Dose was corrected for active content if this information was available; in cases where the active level was not provided, the nominal test substance concentration was provided.

Conclusion

The subacute and subchronic toxicity of TEA-, HEQ-, and MDEA-based esterquats was investigated in a total of 6 good quality and generally guideline compliant studies and coherently revealed a low order of systemic toxicity.

No major clinical or histopathological effects were observed in any of the studies even at the top dose levels. Any findings at the top dose levels were generally considered as mild and adaptive in nature. In a single 90-day study, local effects were observed at the top dose in the forestomach and the urinary bladder. These effects may have been the result of high local concentrations with little relevance to lower dosages. The established NOELs ranged from 100 to700 mg/kg bw/day. It should, however, be noted that the differences in NOEL are possibly due to different dosing procedures and regimes and the spacing of the dose levels rather than real differences in toxicity. The NOEL of 100 mg/kg in the 28 day study with DEEDMAC, in which the next higher dose level was 1000 mg/kg, is offset by the NOEL of 500 mg/kg found in the 90-day study with the same substance. Therefore the NOEL of 300 mg/kg in the 90-day study with a TEA-based esterquat is the lowest NOEL from subacute and subchronic studies and therefore will be used for risk assessment purposes.

4.2.1.7 Neurotoxicity

In addition to the existing subacute and subchronic toxicity studies which partly included neurobehavioural examinations, the potential neurotoxicity of an HEQ-based esterquat was evaluated in a 13-week oral neurotoxicity study (Unilever, 1993bhh). In this study, four groups of rats (10 animals per sex) were given by oral gavage doses of 0, 10, 100, and 1,000 mg/kg bw/day. Prior to the start of the dosing regime and during the 4^{th}, 8^{th}, 13^{th} week of the study all the animals were observed as part of a functional observational battery (FOB) and motor activity was monitored using an automated apparatus. In this study, the HEQ-based esterquat did not produce any signs of neurotoxicity during the routine observation of clinical signs. During the neurotoxicity screen (FOB), there were no changes which were considered to represent neurotoxicity. It was concluded that that the investigated HEQ-based esterquat was not neurotoxic to the rat after oral dosing for 13 weeks at levels up to 1,000 mg/kg bw/day.

Table 29: **Neurotoxicity studies**

Substance	Species M/F	Dose, Route (mg/kg bw/d)	NOAEL (mg/kg bw/d)	Validity	Comments
HEQ-based EQ CAS-No: N/A [Unilever, 1993bhh]	Rat, Sprague-Dawley; 10/10 per group	10, 100, 1000; (gavage)	1000	2	Comparable to OECD TG 408. However, the following deviations were observed: 1. The screening battery tests were not performed as frequently as suggested by the TG. 2. Ophthalmological observations were not performed 3. Selection of dose was not according to the TG

Dose was corrected for active content if this information was available; in cases where the active level was not provided, the nominal test substance concentration was provided.

4.2.1.8 Genetic toxicity

4.2.1.8.1 Bacterial mutagenicity

A total of ten good quality and largely guideline compliant bacterial mutagenicity studies are available with TEA-, HEQ- and MDEA-based esterquats. All studies provide a coherent picture in that the investigated esterquats did not induce reverse mutations in the presence or absence of a metabolic activation system in the so-called Ames test.

Table 32 provides a summary of the available bacterial mutagenicity studies with esterquats.

Table 30: **Bacterial mutagenicity**

Substance	Bacterial Strain	S9 mix	Top dose (µg/plate); S9+/S9-	Result S9+ /S9-	Validity	Comments
TEA-based EQ 91995-81-2 90% active, 10% IPA [Henkel, 1989ahh]	TA 1535, 1537, 1538, 98, 100	Aroclor-1254	5000 / 5000	-/-	1	E.coli WP2/S. typhimurium 102 not considered
TEA-based EQ 91995-81-2 [Degussa, 1993bhh]	TA 1535, 1537, 1538, 98, 100	Aroclor-1254	5000 / 5000	-/-	2	Following deviations were observed from OECD TG 471: 1. Positive control used in presence of S9, alone is not sufficient. 2. E.coli WP2/S. typhimurium 102 not used. 3. No historical control data
TEA-based EQ 91032-11-0 77% active [Henkel, 1994ahh]	TA 1535, 1537, 1538, 98, 100	Aroclor-1254	5000 / 5000	-/-	1	E.coli WP2/S. typhimurium 102 not considered
TEA-based EQ 91995-81-2 [Kao, 1996chh]	TA 1535, 1537, 1538, 98, 100	Aroclor-1254	5000 / 5000	-/-	4	Study details not available
TEA-based EQ 91032-11-0 80% active [Henkel, 1998ahh]	TA 1535, 1537, 98, 100	Phenobarbital/β-naphthoflavone	5000 / 5000	-/-	2	Following deviations were observed from OECD TG 471: 1. Positive control used in presence of S9, alone is not sufficient. 2. E.coli WP2/S. typhimurium 102 not used. 3. No historical control data
HEQ-based EQ 19467-38-0 89.9% active [Unilever, 1989ahh]	TA 1535, 1537, 98, 100	Aroclor-1254	5000 / 150	-/-	2	Incomplete study report (absence of rationale for dose selection, assessment criteria). Following

						deviations were observed from OECD TG 471: 1. Positive control used in presence of S9, alone is not sufficient. 2. E.coli WP2/S. typhimurium 102 not used. 3. No historical control data
HEQ-based EQ 19467-38-0 [Unilever, 1990ahh]	TA 1535, 1537, 98, 100	Aroclor-1254	5000 / 5000	-/-	2	Following deviations were observed from OECD TG 471: 1. E.coli WP2/S. typhimurium 102 not used. 2. Positive control used in presence of S9, alone is not sufficient. 3. No historical control data P.S. No additional clarification provided on effect of precipitation
HEQ-based EQ 19467-38-0 84% active [Unilever, 1993chh]	TA 1535, 1537, 1538, 98, 100, E Coli WP2 uvrA	Aroclor-1254	5000 / 5000	-/-	1	Following deviations were observed from OECD TG 471: 1. Positive control used in presence of S9, alone is not sufficient.
MDEA-based EQ CAS-No: N/A [Unilever, 1997bhh]	TA 1535, 1537, 98, 100	Aroclor-1254	5000 / 5000	-/-	2	Following deviations were observed from OECD TG 471: 1. E.coli WP2/S. typhimurium 102 not used. 2. Positive control used in presence of S9, alone is not sufficient. 3. No historical control data P.S. No additional clarification provided on effect of precipitation

4.2.1.8.2 Non-bacterial *in vitro* genotoxicity studies

The potential of esterquats to cause mutagenicity or clastogenicity in non-bacterial test systems was evaluated in four further studies. Table 33 provides a summary of existing studies.

Table 31: Mammalian cell mutation and clastogenicity tests

Substance	Type of test	S9 mix	Top dose (µg/ml); S9+/S9-	Result S9+ /S9-	Validity	Concerns
TEA-based EQ 157905-74-3 100% active [Degussa, 2004bhh]	Chromosome aberration test in Chinese hamster V79 cells	Phenobarbital/β-naphthoflavone	5000 µg/ml	-/-	1	
HEQ-based EQ, 84.2% active, 13.3% free fatty acid & 3.6% amine hydrochloride [Unilever, 1992bhh]	CHO/HRTP Locus Assay	Aroclor-1254	1000/1000 µg/ml	-/-	1	
MDEA-based EQ 67846-68-8 77.9% active; 22.1% acetone [Procter & Gamble, 1996ahh]	Forward Mutation Assay in mouse lymphoma L578Y cells	Aroclor-1254	550/150 µg/ml	-/-	1	Historical control data not provided
MDEA-based EQ CAS-No: N/A [Unilever, 1997chh]	Chromosomal aberration in cultured human lymphocytes	Aroclor- 1254	100/100 µg/ml	-/-	1	

The potential of a TEA-based esterquat to cause chromosomal aberrations was investigated in Chinese Hamster V79 cells. No effects were observed in the presence or absence of a metabolic activation system (Degussa, 2004bhh).

A mammalian gene mutation test conducted with an HEQ-based esterquat in the CHO/HRTP Locus Assay in the presence and absence of metabolic activation did not show any evidence for mutation (Unilever, 1992bhh). Moreover DEEDMAC, neither showed any clastogenic activity in a chromosomal aberration test in cultured human lymphocytes did with and without S9 mix (Unilever, 1997chh) nor a mutagenicity in a forward mutation assay with mouse lymphoma cells (Procter & Gamble, 1996ahh).

4.2.1.8.3 *In vivo* genotoxicity studies

The potential of TEA-based esterquats to cause chromosomal abnormalities at a dose of 5,000 mg/kg *in viv*o was investigated in a well conducted mouse micronucleus test. The test substance which was shown to have reached the bone marrow did not cause any chromosomal damage under the study conditions.

Table 32: In vivo genotoxicity tests

Substance	Species (M/F)	Type of test	Dose (oral)	Result	Validity	Concern
TEA-based EQ 91995-81-2 90% active, 10% IPA [Henkel, 1990ahh]	Mice, Albino (CFW 1); 6/6	Micronucleus assay	5000 mg/kg	negative	1	Historical control data not presented

Conclusion

There is a range of short-term good quality and guideline compliant *in vitro* and *in vivo* tests available covering all types of esterquats. In none of the studies was there any evidence that TEA-. HEQ-, or MDEA-based esterquats have genotoxic properties.

4.2.1.9 Developmental and reproductive toxicity

4.2.1.9.1 Embryotoxicity/teratogenicity

The potential embryotoxicity/teratogenicity of esterquats has been evaluated in two reliable OECD 414 guideline compliant prenatal developmental toxicity studies.

In an investigation with the MDEA-based esterquat DEEDMAC, rats were orally dosed by gavage with 0, 50, 250 or 1000 mg/kg bw/day (Procter & Gamble, 1992ahh). The controls received pH-adjusted water (i.e., pH = 2.5) at identical volumes. Each group consisted of 25 mated female rats. The test substance was administered once daily from day 6 to day 15 post mating. Females were sacrificed on day 21 and the foetuses were removed by Caesarean section. Up to and including the highest dose level of 1,000 mg/kg bw/day, there were no effects on the maternal or foetal organism. In the 1,000 mg/kg bw/day dose group a slight, statistically significant increased post-implantation loss was noted. Although these post-implantation losses were within the normal range of historical control data for this strain of rats, the study investigators considered this observation to be a slight test substance related effect. The investigators supported their view on the basis of the additional observation that two females which were excluded from the statistical analysis for test substance unrelated biological reasons displayed total post-implantation losses. External-, visceral or skeletal examinations of the foetuses did not indicate test article related abnormal findings. The mean foetal body weights and the sex ratios of the foetuses were similar in all groups.

The authors of this HERA report do not share the original study investigator's view that the slightly statistically significantly increased post-implantation losses were test substance related. The original study report for the embryotoxicity study with DEEDMAC provides the contract laboratory specific range of historical control data for post-implantation losses for this strain of rat of 4.8% to 11.6% of implantation sites. The control group of this specific study had a post-implantation loss of 4.8%, *i.e.*, a rate which is at the lower end of the normal range of control values, and a total post-implantation loss of 8.7% which is below the higher end of control values that have been historically observed by the contract laboratory for this strain of rat. Further, the study investigator's consideration of the two dams which were excluded from statistical analysis in their argumentation for a substance-related effect is inappropriate as these two animals had only 1 or 2 corpora lutea and were therefore not considered fit for reproduction. This effect was not considered test-substance related as ovulation started well before the start of dosing. It is inconsequential to first exclude these animals for biological reasons, but then to use observations made in these two animals to support the hypothesis that potential effects were test substance related.

In conclusion, in this study no effects on maternal reproduction, embryolethality, or developmental effects were observed following maternal exposure to DEEDMAC at doses up to 1,000 mg/kg bw/day. There is no evidence that slight increase in post-implantation losses in the high-dose group is test substance related.

In a 2nd investigation, an HEQ-based esterquat was administered orally by gavage to groups of 25 mated female rats from days 6 to 15 of pregnancy at doses of 0, 100, 300 or 1,000 mg/kg bw/day. On day 21 of gestation, the rats from each treatment group were sacrificed and the foetuses removed by Caesarean section. In conclusion the dosing of up to 1,000 mg/kg bw/day HEQ-based esterquat by gavage did not result in any adverse reaction to treatment. There was no increased embryolethality, foetotoxicity, nor any specific defect in the foetuses which could be attributable to maternal exposure to the HEQ-based esterquat.

Table 35 summarises the key study parameters of the existing teratology studies for esterquats.

Table 33: **Developmental toxicity studies (rats)**

Substance	Species M/F	Dose; Route (mg/kg bw/d)	NOAEL (mg/kg bw/d)	Validity	Concern
HEQ 19467-38-0 [Unilever, 1993ahh]	Rat, Sprague-Dawley; 0/25	100, 300 & 1000; oral (gavage)	1000	1	
MDEA-based EQ 67846-68-8 10% active, 90% water [Procter and Gamble, 1997bhh]	Rat, WIST HanIbm (SPF); 0/25	50, 250 & 1000; oral (gavage)	1000	1	

4.2.1.9.2 Fertility

No one- or two-generation studies with esterquats are available for esterquats (see: Chapter 5.2.1.11 Data Gaps)

4.2.1.10 Carcinogenicity

No chronic studies are available for any of the esterquats. (see: Chapter 5.2.1.11 Data Gaps)

4.2.1.11 Data Gaps

Reproductive toxicity

At the time of review, no studies were identified that specifically addressed the potential effects of esterquats on fertility. However, as summarised in Chapter 5.2.1.6.1, due to the absence of any effects on gonads in subacute or subchronic toxicity studies and the absence of effects on maternal reproduction, embryo lethality or embrytoxicity in teratology studies in rats with doses of up to 1,000 mg/kg bw/day, there is only little concern for possible effects on fertility.

While the need to conduct an additional reproductive toxicity study to complement the existing data and to explore the significance of the post-implantation losses may be considered, from a risk assessment standpoint, there is only little value in conducting such study. As demonstrated in Chapter 5.1.3 and summarised in Table 14, the human exposure to esterquats under normal and foreseeable conditions of use is very low (0.04 mg/kg bw/day) resulting in substantial margin of exposure.

An alternative approach to addressing the lack of specific fertility data from a risk assessment perspective could be the application of the concept of the Toxicological Threshold of Concern (TTC) (Kroes R. *e. al*., 2004). In context of this approach, esterquats would fall into the Cramer class I which are substances that have efficient modes of metabolism and are generally considered to be of low

toxicity (Cramer *et al.*, 1978). For Class I chemicals, the respective TTC level reflecting a daily exposure below there is a low probability of an appreciable risk to human health amounts to 1,800 µg/person/day (Kroes R. *et al.*, 2004). Considering a body weight of 60 kg, the estimated human exposure to esterquats is with 2.4 mg/person/day in the same range of the TTC level.

Carcinogenicity

No carcinogenicity study has been identified for any of the esterquats. However, the data on genotoxicity indicate that these substances are neither mutagenic nor genotoxic. Moreover, the available subchronic studies do not show any specific or non-specific organ damage. In particular, there is no indication of chronic inflammatory state or any other chronic event that is likely to contribute to increased cell turnover, which makes non-genotoxic carcinogenicity highly unlikely.

Considering the low toxicity of esterquats, the absence of genotoxicity or inflammatory reactions as well as the low human exposures, additional testing for carcinogenicity is not deemed necessary at this stage.

4.2.2 Identification of critical endpoints

4.2.2.1 Overview on hazard identification

Esterquats are of low acute oral and dermal toxicity with LD50 values exceeding 2,000 mg/kg bodyweight without any signs of adverse effects at these levels.

In animal experiments, esterquats range from mildly to moderately irritating to skin and eyes. In the case of skin irritation, the degree of the observed irritation response was dependant on the type of esterquat, the exposure time, patch test conditions as well as the concentration of the test substance applied to the skin. While HEQ- or MDEA-based esterquats only resulted in a mild irritation response, TEA-based esterquats could cause a moderate level of irritation when applied at concentrations larger than 30% under occluded or semi-occluded conditions. In humans, however, exposures reflecting more realistically human use conditions, esterquats show a very favourable skin irritation profile. Skin irritation is not to be expected under these conditions.

Likewise, the eye irritation profile of esterquats depends on type of esterquat and concentrations applied. On the basis of the available data there is currently no evidence that HEQ- or MDEA-based esterquats cause eye irritation upon accidental exposure. For TEA-based esterquats, moderate eye irritation potential has been observed in animal experiments at concentrations larger than 80%. There is some evidence that specifically in the rabbit eye irritation test the solvent content as well as a higher proportion of unsaturated fatty acids may amplify the skin irritation response. However, at esterquat concentrations present in consumer products such as those considered in this evaluation, only mild and transient eye irritation can be expected upon accidental eye exposure.

With regard to the potential to cause skin sensitization upon prolonged skin contact, there is currently no information suggesting that HEQ- or MDEA-based esterquats have skin sensitization potential in humans or experimental animals. While TEA-based esterquats have resulted in some weak sensitization responses in a few individual animal studies, the weight of the evidence suggests that also TEA-based esterquats do not represent a skin sensitization hazard to humans. This is supported by specifically designed clinical and market research.

Existing subacute and subchronic toxicity studies with esterquats coherently demonstrated a low level of systemic toxicity of all types of esterquats. No major clinical or histopathological effects were observed in any of the studies even at top dose levels of 1,000 mg/kg bw/day. Minor effects at these top dose levels such as minor changes in blood biochemistry or some minor increase of body or organ weight as a result of increased food consumption were generally considered as mild and adaptive in nature. In a single 90-day rat gavage study, local effects were observed at the top dose of 1,000 mg/kg bw/day in the forestomach and the urinary bladder, but these effects were considered a result of high local exposure with little relevance to lower dosages.

The mutagenic and clastogenic potential of esterquats has been evaluated in a range of *in vitro* and in a single *in vivo* genotoxicity studies. There was no evidence for genotoxic properties of any of the investigated esterquats. Although carcinogenicity studies are not available for esterquats, the absence of genotoxicity or inflammatory responses in repeated dose toxicity studies do no raise any specific concerns with regard to carcinogenicity.

There was further no evidence for esterquats to cause teratogenic effects in specifically designed and guideline compliant prenatal developmental toxicity studies. While there are no studies that specifically addressed reproductive toxicity, the information received from existing repeated dose toxicity studies which did not reveal any treatment related effects on gonads and the absence of any embryotoxicity at doses up to 1,000 mg/kg bw/day suggest only little concern for effects on fertility.

4.2.2.2 Rationale for identification of critical endpoints

Dermal exposure is the main exposure route for consumers and subsequently, dermal effects such as skin irritation and sensitisation as well as long-term systemic toxicity following exposure must be considered for the human health risk assessment of esterquats.

Substantial amount of data are available addressing skin irritation and skin sensitization potential of esterquats solutions and esterquat containing product formulations. With regard to systemic toxicity following dermal exposure, dermal penetration studies in rats have shown that esterquats have only very limited potential to penetrate the skin to become systemically available. While only oral and no dermal repeated toxicity studies are available, the profile of systemic toxicity after oral and dermal administration is assessed to be similar justifying the use of rat oral repeated dose toxicity studies to assess potential human exposure via the dermal route. This assessment takes also into account information from dermal, oral or intravenous ADME type of studies on esterquats but also their main metabolites as presented in Chapter 5.3.1.1

4.2.2.3 Adverse effects related to accidental exposure

The acute oral and dermal toxicity of neat esterquats is considered to be low. Esterquats can be present in liquid fabric conditioner formulations at a maximum of 23% and in fabric conditioner sheets up to 25%. Generally, accidental oral exposure to a surfactant containing formulation such as detergents of fabric conditioner poses only a minor risk of aspiration.

The available information suggests that fabric conditioner formulations containing up to 23% of the esterquat may only be mildly irritating to eyes and not irritating to skin under the conditions of accidental exposure. Other components in the formulation may contribute to these effects. Therefore, in case of accidental eye contact, immediate rinsing with plenty of water is recommended. In animal experiments, this immediate action has been shown to minimize eye irritation effects.

4.2.2.4 Determination of NOAEL or quantitative evaluation of data

As discussed before, the available oral repeated dose toxicity studies provide a coherent picture and demonstrate the low toxicity of esterquats.

For assessing the risk associated with human exposure to esterquat in context of their use in fabric conditioners, it is suggested to take a conservative approach by using the lowest NOAEL of 300 mg/kg bw/day which has been established on the basis of a 90-day oral toxicity study with a TEA-based esterquat.

4.3 Risk Assessment

4.3.1 Margin of Exposure Calculation

The Margin of Exposure (MOE) is the ratio of the No Observed Adverse Effect Level (NOAEL) or an appropriate substitute to the estimated or actual level of human exposure to a substance. A systemic NOAEL for esterquats was determined by using the 90-day oral NOAEL of 300 mg/kg bw/day in the rat.

5.3.1.1 Exposure scenario: Direct skin contact from hand-washing laundry

For calculation of the MOE, the NOAEL of 300 mg/kg bw/day was divided by the daily systemic dose of 0.021 mg/kg bw/day which was estimated for the dermal exposure to esterquats from hand-washed laundry.

$\mathbf{MOE_{sys\ (hand\ laundering)}} = 300/0.021 > \mathbf{14{,}000}$

5.3.1.2 Exposure scenario: Direct skin contact from wearing laundry

The systemic dose from skin exposure to esterquat residues on washed fabric was estimated to be 0.012 mg/kg bw/day. For calculation of the MOE, the NOAEL of 300 mg/kg bw/day was divided by the daily systemic dose of 0.012 mg/kg bw/day which was estimated for the dermal exposure to esterquats resulting from the transfer from treated fabric to the skin.

$\mathbf{MOE_{sys\ (fabric\ wearing)}} = 300/0.012 = \mathbf{25{,}000}$

5.3.1.3 Exposure scenario: Systemic oral exposure in humans

The systemic dose from oral exposure to esterquat residues via drinking water or food was estimated to be 0.0039 mg/kg bw/day. For calculation of the MOE, the NOAEL of 300 mg/kg bw/day was divided by the daily systemic dose of 0.0039 mg/kg bw/day which was estimated for the systemic oral exposure to esterquats via drinking water or food residues.

$\mathbf{MOE_{sys\ (hand\ laundering)}} = 300/0.0039 > \mathbf{76{,}000}$

5.3.1.4 Total consumer exposure

In a worst case scenario, the total systemic consumer exposure to esterquats from hand laundering, fabric wearing and drinking water and food uptake amounts to 0.037 mg/kg bw/day. For calculation of the MOE for total consumer exposure, the NOAEL of 300 mg/kg bw/day was divided by the daily systemic dose of 0.037 mg/kg bw/day.

$\mathbf{MOE_{sys\ (hand\ laundering)}} = 300/0.037 > \mathbf{8{,}000}$

The following Table 36 summarises all possible exposures through the individual paths and resulting total exposure as well as the respective margin of exposures.

Table 34: Overview of possible frequent exposures to esterquats and associated MOE's

Consumer Contact Scenario	Exposure estimate (mg/kg bw/day)	Margin of Exposure
Hand-washing laundry	0.021	> 14,000
Wearing Fabric	0.012	> 25,000
Oral uptake via drinking water or food	0.0039	> 76,000
Total	0.0369	> 8,000

5.3.2 Risk Characterisation

5.3.2.1 Systemic toxicity

Consumers are exposed to esterquats through their use in fabric conditioner. Scenarios relevant to consumer exposure scenarios were identified, quantified and assessed by comparing the estimated systemic exposure values with the systemic NOAEL for esterquats as determined on the basis of a subchronic oral gavage study. The estimated MOE for the systemic dose resulting from the total consumer exposure is 8,100. This MOE calculation reflects the aggregate of all possible exposure scenarios using worst case assumptions, an exposure situation which is unlikely to occur.

Considering the conservatism in the exposure calculation and the assigned systemic NOAEL for esterquats, the determined MOE is certainly large enough to account for the extrapolation from subchronic to chronic exposure, the inter- and intra-species variation and for any inherent uncertainty of the database.

Taking all together, the use of esterquats in fabric conditioners does not raise any safety concerns with regard to systemic toxicity.

5.3.2.2 Local toxicity

Esterquats are not considered to be contact sensitizer and the irritation potential of esterquats is concentration dependent. Under normal use conditions with direct skin contact (*i.e.*, hand-washing laundry, wearing of softened fabric), the consumer is exposed to diluted fabric conditioner solutions or

residues present at low levels in the fabric. At these exposure levels, esterquats are virtually non-irritating to the skin. This has been demonstrated in clinical as well as animal studies.

Esterquats are present in fabric conditioner formulations at levels up to 23%. Accidental eye contact with undiluted formulation is not expected to cause more than mild irritation which is fully reversible shortly after exposure. This assessment is supported by poison control centre data demonstrating that accidental eye contact with fabric conditioner will not result in serious, irreversible eye irritation. Nevertheless, in case of such accidents, the eyes should be rinsed immediately with plenty of water.

5.3.2.3 Acute effects

Accidental ingestion of esterquat containing liquid fabric conditioner is not expected to result in any significant adverse health effects. This assessment is based on available toxicological data demonstrating low acute oral toxicity of esterquats. Neither mortality nor significant toxicity has been observed in animal experiments at doses up to 5 g/kg bodyweight (see section 5.3.1.2) which is significantly higher than the exposure of about 450 mg/kg bodyweight a toddler would experience as a result of an accidental exposure. The latter can be estimated by assuming that a 10kg toddler may be orally exposed to no more than 20 ml of fabric conditioner (HERA, 2005) containing 23% esterquat. National poison control centers have not reported a case of lethal poisoning or severe health effects associated with accidental ingestion of fabric conditioners containing esterquats.

5.4 Summary and Conclusion

Consumers are exposed to esterquats through their presence in fabric conditioners mainly via the dermal route, but to some minor extent also via the oral route. Skin exposure occurs mainly in hand-washed laundry and through esterquats being present on the fabric of laundry treated with fabric conditioner. Consumers are orally exposed to esterquats through residues in drinking water or eating foods that have taken up esterquats through their presence in surface waters. The aggregate exposure of consumers to esterquats has been estimated to be 36.9 µg/kg bw/day.

A substantial amount of toxicological studies demonstrate that esterquats are of low toxicity. Esterquats were found to be mildly to moderately irritating to rabbit skin and eyes. The degree of irritation was concentration dependant as dilutions in water resulted in proportionally lower level of irritation. Local dermal effectst due to skin contact with esterquat containing handwasing solutions or esterquat residues on skin are not of concern because esterquats are neither considered skin sensitizer nor expected to be irritating under in-use conditions. Accidental eye contact with undiluted esterquat containg fabric conditioner formulation may cause mild irritation which is, however fully reversible shortly after exposure. As other components in the fabric conditioner formulation may contribute to these effects, immediate rinsing with plenty of water is recommended and will mitigate any potential eye irritation effects.

With regard to repeated dose toxicity, existing subacute and *subchronic* toxicity studies with esterquats coherently demonstrate a low level of systemic toxicity of all types of esterquats. No major clinical effects were observed in any of the studies, even at dose levels up to 1,000 mg/kg bw/day. There is further no information suggesting that esterquats are genotoxic, mutagenic or toxic to the foetus. Although no carcinogenicity study has been conducted with esterquats yet, the absence of genotoxicity and the overall low toxicity of esterquats do not raise any carcinogenicity concern. Likewise, although no multigeneration studies are available, the absence of any effects on gonads in

well conducted subacute and subchronic toxicity studies, does not raise an immediate concern for a possible effect of esterquats on fertility.

For assessing risks associated with human exposure to esterquats in context of their use in fabric conditioner, a conservative NOAEL of 300 mg/kg bw/day was established on the basis of 90-day oral toxicity study with a TEA-based esterquat. The comparison of the aggregate exposure of 36.9 µg/kg bw/day and the NOAEL results in an MOE of 8,100. Taking into account the conservatism in the exposure calculation and the assigned NOAEL for esterquats, this margin of exposure is considered to be large enough to account for the inherent uncertainty of the database and variability of the database.

In summary, the human health risk assessment has demonstrated that the use of esterquats in fabric conditioners is safe and does not cause concern with regard to consumer use.

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6. CONTRIBUTORS TO THE REPORT

- **Leading company**

  Stepan Europe

- **Consultant companies**

  Akzo Nobel Chemicals Research
  (Department Environmental Chemistry and Regulatory Affairs)

  ToxMinds BVBA
  Product Safety & Regulatory Affairs

- **Other contributors**

  Akzo Nobel Surface Chemistry AB
  CECA Arkema
  Clariant GmbH
  Cognis Deutschland GmbH & Co. KG
  Colgate Palmolive Co
  Evonik Goldschmidt GmbH
  Henkel KGaA
  Kao Corporation S.A.
  Procter & Gamble, Eurocor
  Unilever Home & Personal Care Europe

7. ANNEXES

Annexes are temporarily stored in separate zip file.