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New scientific arguments for regulation of ethylene oxide residues in skin-care products

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Abstract. Ethylene oxide (EO) occurs as a contaminant of skin-care products because current commercial preparations of polyglycol ethers may contain ethylene oxide monomer residues, up to the order of 1 ppm. Using current regulatory worst-case assumptions, the presence of EO in skin-care products might lead to a maximal human daily external ethylene oxide dose of about 2.8 μg, and a consecutive maximal daily absorbed dose of 0.39 μg. Two methods of toxicokinetic analysis have been used to compare this possible EO load by use of skin-care products with the inevitable load of EO which is produced endogenously in the organism. On the basis of a previous assessment of the endogenous production of ethylene and ethylene oxide (Filser et al. 1992) it is inferred that the absorbed EO dose of 0.39 μg is about 1/30 of the unavoidable human endogenous load by endogenous EO. Alternatively, for a second calculation molecular dosimetry data have been used which were based on experimental quantification of the hydroxyethyl adduct of EO to the *N*-terminal valine of hemoglobin (HOEtVal) in rats. If the worst-case assumptions for human EO absorption from skin-care products are transferred to the rat species, the associated internal EO doses are about 1/110 of the internal EO doses which were calculated from the background HOEtVal concentrations observed in untreated animals. The divergence between both lines of calculation is mainly due to differences in HOEtVal background concentrations between man and rat. It is concluded that the additional internal body burden of EO associated with the use of current skin-care products, even under a series of worst-case assumptions, is neglegible compared to the physiological and unavoidable internal EO burden of the organism.

Key words: Ethylene oxide – Polyglycol ethers – Skin-care products – Risk assessment

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Introduction

Ethylene oxide (EO) is used in the manufacture of polyglycol ethers which are ingredients of skin-care products. Specifically, EO is indispensable for the synthesis of non-ionic surfactants. Since EO is recognized as a genotoxic carcinogen and since complete removal of EO monomer from technical polyglycol ether preparations is not possible, the problem of regulation of EO monomer residues in cosmetic products has been raised.

The Scientific Committee on Cosmetology (SCC) of the European Communities has based its safety estimations on the "worst-case" of a maximal daily consumption of 27.6 g skin-care products, containing maximally 10% polyglycol ethers (i.e. 2.8 g). In general, current commercial preparations of polyglycol ethers contain EO monomer concentrations below 1 ppm (μg/g). This is in line with data measured by Kreuzer (1992) showing a range between 1.9 and 34 nmol/cm^3 (0.08–1.5 ppm).

The regulatory handling of genotoxic compounds is generally based on the concept of minimization of exposure. The setting of numerical limits is therefore difficult and in practice largely influenced by political and economic argumentation.

Alternatively, the regulatory case of residues of EO monomer in skin-care products may be based on recent scientific insights into the toxicology and disposition of EO (Bolt et al. 1988; Filser et al. 1992). EO is an intermediate metabolite of ethylene as has been shown in laboratory animals (Ehrenberg et al. 1977; Segerbäck 1983; Filser and Bolt 1984; Maples and Dahl 1993) and in humans (Törnqvist et al. 1989a; Filser et al. 1992). Ethylene is a natural body constituent, the endogenous formation of which has been deduced from its exhalation in untreated rats (Frank et al. 1980; Sagai and Ichinose 1980; Shen et al. 1989) and unexposed humans (Ram Chandra and Spencer 1963; Shen et al. 1989; Filser et al. 1992). Consequently, its metabolite EO must also be regarded as a natural body constituent (Filser et al. 1992).

According to current knowledge, possible endogenous sources of ethylene and, in consequence, of EO are:

(i) lipid peroxidation (Lieberman and Mapson 1964; Frank et al. 1980; Sagai and Ichinose 1980; Törnqvist et al. 1989b);
(ii) oxidation of free methionine (Lieberman et al. 1965; Kessler and Remmer 1990);
(iii) oxidation of hemin in hemoglobin (Clemens et al. 1983; Kessler 1987);
(iv) metabolism of intestinal bacteria (Törnqvist et al. 1989b).

These endogenous sources of ethylene and, in consequence, of EO contribute to a background alkylation (2-hydroxyethylation) of physiological macromolecules such as hemoglobin (Törnqvist et al. 1986, 1989a; Filser et al. 1992; Walker et al. 1992a) and DNA (Föst et al. 1989; Walker et al. 1992b; Cushnir et al. 1993; Bolt and Leutbecher 1993). Therefore, internal exposure caused by exogenous traces of EO can be compared with the background concentrations of EO and its macromolecular adducts. Such a comparison may provide new arguments for regulatory bodies.

Present database

Exogenous loads of low levels of EO (from skin-care products) may be compared with the endogenous EO load by use of toxicokinetic data on EO (Kreuzer 1992), and/or by the quantities of base concentrations of hydroxyethylated macromolecules.

The quantity of the endogenous production of ethylene and the consecutive formation of EO has been estimated for humans. The rate of the endogenous ethylene production was taken as 32 nmol/h, resulting in a body burden for ethylene of 0.44 nmol/kg body weight. The corresponding body burden of EO, resulting from endogenous ethylene production, was 0.17 nmol/kg body weight (Filser et al. 1992).

Exposure of humans to exogenous EO gives rise to increased hydroxyethylation of histidine and of *N*-terminal valine in hemoglobin (e.g. Calleman et al. 1978; Farmer et al. 1986). In non-smokers, not occupationally exposed to EO or ethylene, background concentrations of HOEtVal in hemoglobin in the range between 10 and 106 pmol/g hemoglobin have been reported (Törnqvist et al. 1986, 1989a, 1992; Bailey et al. 1988; Sarto et al. 1991; Filser et al. 1992). Only a part of these HOEtVal background concentrations result from EO as metabolite of endogenous ethylene (about 12 pmol/g hemoglobin; Filser et al. 1992). Another part was contributed to ethylene as an ubiquitous contaminant in the environment from plants, combustion processes and industrial sources (Törnqvist and Ehrenberg 1990; Filser et al. 1992).

There is also a background of 7-(2-hydroxyethyl)-guanine in DNA. In different organs of mice and rats background concentrations of this specific adduct of 2–6 pmol/mg DNA (Walker et al. 1992b), and in human lymphocytes a mean concentration of 8.5 pmol/mg DNA, have been reported (Föst et al. 1989).

Table 1. Maximal skin permeation of EO from different formulations [percent absorption, according to data of Kreuzer (1992) and (*) Kreuzer et al., in preparation]

	Rat	Human
Lutensol	2.7%	1.0%
Oil-in-water emulsion (*)	3.1%	7.8%
Water-in-oil emulsion (*)	9.3%	14 %

Exposure scenario and human toxicokinetic considerations

As mentioned above, current regulatory worst-case assumptions are based on a daily use of about 28 g of skin-care products, containing maximally 2.8 g polyglycol ethers. This is associated with a maximal daily external EO dose of 2.8 μg, considering currently possible EO contents in commercial polyglycol ether preparations of 1 ppm (μg/g).

For estimation of the resulting human internal dose, the percentage of EO monomer that permeates through skin has to be taken into account; this skin permeability differs with the formulation of products. The skin permeation of EO from different formulations has been determined in different sets of experiments in vivo and in vitro (rat, in vivo; rat and human skin, in vitro; Kreuzer 1992, Kreuzer et al., in preparation). Table 1 summarizes the resulting maximal percentages of EO absorption. The highest value was measured from water-in-oil emulsions. The predominant part (the difference to 100%) is evaporated (Kreuzer and Filser 1994). Considering the highest permeation value of 14%, the maximal (worst-case) absorbed EO amount from skin-care products may therefore be 0.39 μg per person and day.

This highest possible external EO dose (caused by the use of skin-care products) is opposed to the body burden of endogenous EO due to the endogenous ethylene production which, according to the calculations of Filser et al. (1992), results in an endogenous body burden of 0.17 nmol EO per kg body weight (see above). This body burden is the average tissue concentration of EO in man which is unavoidable. By means of toxicokinetic calculations (Filser et al. 1992), it can be deduced that it gives rise to a HOEtVal background concentration of about 12 pmol/g hemoglobin (see above).

Following the administration of a distinct dose (D) of a compound (EO) to the body, the rate of elimination (dD/dt) at time point zero ($t_{(0)}$) can be expressed as the product of the average concentration in the body ($y_{(0)}$) at $t_{(0)}$, the body volume (V), and the rate constant of the elimination from the body (k):

$$dD/dt = -y_{(0)} \cdot V \cdot k \quad (1)$$

The average concentration in the body (y) is defined as

$$y = \sum_{i=1}^{i=n} (yi \cdot Vi)/V \quad (2)$$

yi being the average concentration in the organ i or tissue i with the volume Vi, and n being the sum of the number of all organs and tissues.

In the dose range in which the rate of EO elimination from the organism is proportional to y, (first-order elimination) the value of k remains constant and is given by the ratio ln2 divided by the half-life ($t1/2$):

$$dD/dt = -y_{(0)} \cdot V \cdot \ln 2/t1/2 \quad (3)$$

D can be calculated from the integral of the concentration over the time from $t_{(0)}$ to $t_{(\infty)}$:

$$D = (\ln 2/t1/2) \cdot V \cdot \int_0^\infty y dt \quad (4)$$

The integral equals the area under the body concentration-time curve (AUC).

The daily AUC for endogenous EO is given by the product of the exposure time (24 h) with the internal body burden (0.17 nmol/kg body weight; Filser et al. 1992).

Considering the molecular weight of EO (MW = 44), the short half-life of EO in man which was calculated by Filser et al. (1992) from data of Brugnone et al. (1986) to be 42 min (0.70 h), and assuming a human body weight of 70 kg, the internal body burden of EO corresponds to a theoretical daily absorbed EO (D_{dEO}) of 12.5 µg:

$$D_{dEO} = 44/1000 \cdot \ln 2/0.70 \cdot 70 \cdot 0.17 \cdot 24 = 12.5\ [\mu g] \quad (5)$$

This means that the maximal internal EO dose by daily application of skin-care products (0.39 µg) is about 1/30 of the unavoidable endogenous EO load.

Because such a toxicokinetically based calculation for man requires a set of intrinsic assumptions, it is feasible to compare this scenario for man with that in experimental animals where direct data of EO dose-adduct relationships have been well established.

Molecular dosimetry based on animal experiments

An alternative "molecular dosimetry" approach can be based on dose-adduct relationships and established basic adduct concentrations of HOEtVal in hemoglobin.

The most extended experimental data set for the hemoglobin adduct, both for rats and mice, is that of Walker et al. (1992a). These authors reported about "background concentrations" of HOEtVal in rats averaging 42 ± 8 (SEM), and in mice averaging 58 ± 10 (SEM) fmol of this specific adduct/mg globin.

Upon daily exposure (6 h/day; 5 days/week; for 4 weeks) to 3 ppm EO average amounts of 3.5 and 3.4 pmol HOEtVal/mg globin were found in rats and mice, respectively; the corresponding figures for 10 ppm exposure were 11.2 and 11.1 pmol/mg globin. As these figures for both species are very similar, the following calculations are done for rats only.

In rats, hemoglobin life-time (ter) is about 60 days (Berlin 1964). Consequently, after the exposure period of 4 weeks the steady state HOEtVal(st) adduct concentration was not yet reached. The HOEtVal(28) concentration reached after 28 days (4 weeks) can be estimated according to Osterman-Golkar et al. (1976) using the following equation:

$$\mathrm{HOEtVal}(28) = a \cdot t - a \cdot t^2/2/ter \quad (6)$$

The average daily HOEtVal increment is symbolised by (a) and the exposure time by (t): An assumed relative faster removal of HOEtVal containing erythrocytes compared to that of erythrocytes free of adducts (Walker et al. 1992a) is not considered in eq (6). Since $t = ter$ is valid for all exposure times $t \geq ter$, the final steady state HOEtVal(st) concentration can be estimated to be:

$$\mathrm{HOEtVal(st)} = a \cdot ter/2 \quad (7)$$

The last day of the exposure experiment of Walker et al. (1992a), i.e. $t(28)$, can be considered as: $t(28) = ter \cdot 28/60 = 0.467 \cdot ter$, and the share of the corresponding HOEtVal(28) on HOEtVal(st) is obtained from eqs (6) and (7):

$$\mathrm{HOEtVal}(28) \cdot 100/\mathrm{HOEtVal(st)} = [a \cdot 0.467 \cdot ter - a \cdot (0.467 \cdot ter)^2/2/ter] \cdot 100 \cdot 2/(a \cdot ter) = 71.6\% \quad (8)$$

This means that after the exposure period of 4 weeks in the experiment of Walker et al. (1992a) about 72% of the final HOEtVal(st) concentrations were reached. Consequently, a continuous exposure of F344 rats with a mean body weight of 170 g (Walker et al. 1992a) to EO at 3 and 10 ppm (6 h/day; 5 days/week; $\geq$ 60 days) would not lead to the concentrations of 3500 and 11200 (pmol/g Hb) observed after 4 weeks of exposure, but to 4900 and 15600 (pmol/g Hb), respectively. Experimentally obtained adducts increased linear with the EO dose, at least up to 33 ppm exposure (Walker et al. 1992a; Osterman-Golkar et al. 1983). From the HOEtVal concentrations determined by Walker et al. (1992a) it can therefore be deduced that subacute daily exposures of rats (6 h/day; 5 days/week; 4 weeks) to 38 ppb EO would result, after 4 weeks, in an alkylation of hemoglobin equivalent to the observed background value of 42 pmol/mg Hb. The same alkylation concentration would be reached after subchronic (at least 60 days) or chronic exposure (6 h/day; 5 days/week) to 27 ppb ($0.72 \cdot 38$ ppb).

The daily AUC resulting from exposure to 27 ppb (6 h/day; 5days/week) EO in rats of 170 g body weight can be calculated by means of the toxicokinetic data of Filser and Bolt (1984). Since at low concentrations the rates of inhalation and elimination of EO follow first order kinetics (Filser and Bolt 1984, Krishnan et al. 1992) the amounts of EO taken up and eliminated are solely a function of the mean atmospheric EO concentrations over time, regardless of the exposure profile (Bolt et al. 1981). This means that exposure to 27 ppb (6 h/day; 5days/week) is equivalent to a continuous (uninterrupted) EO exposure at a concentration of $27 \cdot 6/24 \cdot 5/7 = 4.8$ ppb. The resulting average steady-state concentration of EO is given by the product of the atmospheric concentration with the "bioaccumulation factor" (body/air, as defined as K'st* by Filser 1992). The value of this factor for one rat of 170 g ($n' = 1$; $V'_2 = 170$ ml assuming a body density of 1) can be calculated using the values given in Filser and Bolt (1984) for the "clearance of EO uptake related to the atmospheric concentration" ($k_{12}V_1$ = 11100 ml/h), the rate constant of the exhalation process ($k_{21} = 0.37\ h^{-1}$) of EO (both parameters normalised for the sum of two rats of 500 g each: $n = 2$; $V_2 = 1000$ ml)

and the rate constant of the metabolic EO elimination (k_{el} = 6.95 h^{-1}) which is independent of the animal size. The corresponding parameters for one rat are computed by means of equations (7), (10) and (13) of Filser (1992) to be k'_{12} $V'_{1(170)}$ = 2700 ml/h and $k'_{21(170)}$ = 0.53 h^{-1}. With equation (18) of Filser (1992) $K'st^*_{(170)}$ is then obtained to be 2700/(0.53 + 6.95)/170 = 2.12 [assuming a body density of 1 and not respecting the endogenous unavoidable EO production rate ($dNpr/dt$ = 0.67 nmol/h, in a rat of 250 g; Denk 1990)]. With this value and the gas molecular volume of about 25 l, the average steady state concentration of EO in a rat exposed to 4.8 ppb EO becomes 4.8 · 2.12/25 = 0.41 nmol/kg body weight.

The AUC per day resulting from continuous exposure to 4.8 ppb is given by the product of the average steady state concentration of EO in the rat with the exposure time (24 h). Considering the molecular weight of EO (MW = 44) and the short half-life of EO in the rat calculated for a rat of 170 g to be $\ln 2/(k_{el} + k_{21(170)})$ = 0.093 h (according to Filser 1992), the daily AUC corresponds to a theoretical daily absorbed EO dose ($D_{dEO(rat\ 170)}$) of 0.549 µg:

$$D_{dEO(rat170)} = 44/1000 \cdot \ln 2/0.093 \cdot 0.17 \cdot 0.41 \cdot 24 = 0.549\ \mu g \quad (9)$$

The worst-case assumption for humans was based on a daily administration of 2.8 µg EO onto the skin surface. According to Conclin (1975), the skin surface can be approximated for different mammalian species by

$$\text{"skin surface"} = 10 \cdot (\text{body weight})^{2/3} \quad (10)$$

which gives a body surface of 1.7 m^2 for a human individual of 70 kg, and of 307 cm^2 for a rat of 170 g. A skin-care product layer containing 2.8 µg EO on a skin surface of 1.7 m^2 is equivalent to 2.8/17 000 · 307 = 0.051 µg on rat skin surface. According to Table 1, a maximal EO absorption through rat skin of 9.3% was determined, equivalent to about 0.005 µg of permeated EO. This means that in the rat the maximal internal EO exposure by daily application of skin-care products is about (0.005/0.549) = 1/110 of the unavoidable internal EO load.

This value is of a comparable order to the value of 1/30, calculated above for humans on the basis of toxicokinetic data. The divergence is mainly caused by the different HOEtVal background concentrations in both species: the value determined by Walker et al. (1992a) for rats is 3.5 times higher than the value calculated for human endogenous HOEtVal.

Conclusions

In many cases, residues of proven carcinogens (labelled "R45" in the European Communities) in environment, food and consumer products are unavoidable. Regulatory actions for such compounds are based on the concept of minimization of exposure, but are largely influenced by political and economical argumentation. From the scientific side, arguments of a quantitative risk estimation are brought in; however, establishing an acceptable residue level for a genotoxic carcinogen inevitably relies on a formal or mutual socio-political agreement on acceptable associated cancer risks. This procedure may lead to cases of overt overregulation if scientific, political and economic arguments are not clearly distinguished.

On this background, the present case of EO residues in skin-care consumer products provides a new line of argumentation. Using two different approaches it could be shown that the internal EO burden associated with the use of current products of this type, even under a series of worst-case assumptions, is very small compared to the physiological and unavoidable internal ethylene oxide burden in the organism.

In general, identification and quantification of natural and unavoidable risks, as opposed to risks by exogenous chemicals, need more to be considered in regulation of potentially toxic chemicals.

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