

SOME CHARACTERISTICS OF THE SENSITIZER IN ALKYL ETHOXY SULPHATE

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Abstract. It has been reported that a sample of alkyl ethoxy sulphate (AES) caused contact hypersensitivity amongst consumers in Norway who were exposed to it in a liquid dishwashing product. In our experiments, guinea pigs were sensitized to this AES sample, but AES samples from many other sources did not provoke allergic reactions when tested on the sensitized animals. A sample of AES more recently produced by the makers of the original allergenic material also failed to provoke a reaction in sensitized guinea pigs. Hence, it appears that the sensitizing capacity is not an inherent property of AES, but rather is characteristic of the specific batch of material. Petroleum ether extraction of the sensitizing AES material yielded an extract that sensitized guinea pigs and that elicited positive responses in animals which had been sensitized to the unextracted AES. The residue left after extraction, which amounted to more than 99% of the AES material, did not sensitize guinea pigs and did not give a response when tested in previously sensitized animals. It is concluded that the sensitizing agent is not AES itself, but rather is a foreign substance present at a low level in the offending batch.

A recent outbreak of dermatitis in Norway was associated with the use of a liquid dishwashing composition, and the effect has been attributed to the alkyl ethoxy sulphate which was an important component of the composition (3). The alkyl chains of this AES were predominantly of 12 carbon atoms' length; hence, the material was referred to as lauryl ethoxy sulphate (LES), lauryl alcohol being the trivial name for $C_{12}H_{25}OH$.

Two possible explanations can be advanced for the effects reported, namely (a) the AES was itself a sensitizer, or (b) some other substance present in the AES was a sensitizer. The work reported here was carried out to determine which of these is the correct explanation, and to provide guidance for additional work aimed at identifying

the material and preventing a recurrence of the problem.

From the outset, we suspected that the sensitizing agent was a contaminant, rather than the AES itself. This view was based on our experience with clinical tests in which some 70 000 women were tested with more than 1 500 batches of AES. Careful examination of the subjects' hands after exaggerated exposures to products that contained AES had given no evidence of allergic response. Further support for this view came from marketing experience, for products containing AES had been sold in large quantities in many countries for several years without evidence of sensitization problems.

Consideration of AES manufacturing processes suggests that there are many opportunities for the introduction or formation of contaminants if the processes and raw materials are not adequately controlled. AES has the chemical structure $H-(CH_2)_n-(O-C_2H_4)_m-OSO_3^-M^+$, where m usually has values of two to four, and M^+ is sodium or ammonium. In lauryl ethoxy sulphate, n has values distributed around 12. Manufacture of AES involves the following operations. Fatty alcohol is prepared either from vegetable oil (a "natural" source) or from petroleum hydrocarbons (a "synthetic" source). The alcohol is ethoxylated by the addition of ethylene oxide. The resulting ether-linked alcohol is sulphated with sulphur trioxide or chlorosulphonic acid. The product is neutralised with alkali. Since both ethoxylation and sulphation are highly exothermic, inadequate dissipation of reaction heat may cause discolouration of the product, requiring that it be bleached with hypochlorite or peroxide before use.

MATERIALS

A sample of the AES paste that had been responsible for the outbreak of dermatitis in Norway in 1966 was obtained. This material, known as LES 13-2035, had been in storage for about 6 years. Magnusson (3) reported that he sensitized 22 out of 25 guinea pigs with this material.

An additional sample of AES was obtained from the factory that had produced LES 13-2035.

Seventeen other samples of AES were obtained from different factories of The Procter & Gamble Company and its subsidiaries.

METHODS

Two methods of sensitizing and challenging animals were used. The first method was that of Magnusson & Kligman (4, 5). The animals used for this work were female guinea pigs of the Hartley (Dunkin) strain. Groups of 10 were housed in metal cages with wire mesh floors, and were freely supplied with water and a pelleted, vitamin C-enriched guinea pig ration. Sensitization was induced by intradermal injections in the scapular region of 0.1 ml of solutions containing 5% LES 13-2035 in water and in Freund's complete adjuvant (prepared by blending commercial adjuvant with an equal volume of water). The injections were followed 1 week later by topical applications on the same sites of 0.25 ml of a 25% aqueous solution of LES 13-2035 under closed patches for 48 hours. Challenge applications were made 2 weeks later, using 0.1 ml of a 0.5% test solution under closed patches. The patches were applied to a freshly shorn area on the animals' left flanks for 24 hours. Sites were graded 24, 48, and 72 hours after the patches were removed. Patched sites showing erythema and oedema were scored as positive. The right flanks of sensitized animals were subsequently used for cross-challenges with other materials. Control animals were used in cross-challenge tests in order to distinguish between reactions due to sensitisation and those due to irritation.

The second technique was that of Buehler (1). Guinea pigs of the Hartley (Hamm) strain, and of mixed sexes, were housed in individual stainless steel cages with wire mesh floors. They received water and Purina guinea pig chow *ad libitum*. Sensitisation involved only topical applications. Three successive closed patches were applied to the shorn backs of the animals at 1-week intervals. Each patch contained 0.5 ml of test solution, and was left in place for 4 hours. Two weeks after removal of the third induction patch, challenge patches were applied to the original sites and to fresh sites on the same animals. The patches were kept in place for 24 hours, and the sites were graded 24 and 48 hours after removal. Challenge patches were simultaneously applied to control animals that had not received the induction treatments. Erythematous or oedematous responses in the test animals, greater than those seen in the control animals, were counted as positive reactions.

EXPERIMENTS AND RESULTS

First series. Guinea pigs were sensitized to LES 13-2035, and were then used to screen other samples of AES to see whether they also con-

tained the sensitizer or a cross-reacting substance. Some 120 animals were treated by the Magnusson-Kligman technique. When they were challenged with a 0.5% solution of LES 13-2035, positive reactions were seen in 32 animals. Animals that gave negative reactions were rechallenged 1 week later with a 5% solution, and an additional 53 positive responses were seen; thus the incidence of sensitization was 71%. (Subsequent repetitions of this experiment gave sensitization incidences of only 32 and 36%; the reasons for the lower incidences are not known.)

The sensitized animals were cross-challenged with other AES-containing samples. Each of these was diluted with water so that its concentration of AES was equal to that contained in the 0.5% solution of LES 13-2035 used for initial challenges. One of the materials tested was a recent product run at the Swedish factory that had produced LES 13-2035. Six of the materials were AES samples representing 4 manufacturing units in Great Britain, 3 sources of raw material, and 2 sulphation processes. Two materials were AES samples from two manufacturing units in Europe. Four were AES from 4 manufacturing units in North America, using three sources of raw material and two different sulphation processes. Two of the American samples contained 12-carbon alkyl chains (lauryl groups), while the others had chains of 16 to 18 carbon atoms. Four of the materials used in cross-challenge experiments were samples of dishwashing products obtained from the retail market in Great Britain, and another one was a dishwashing product from Sweden. The dishwashing products represented 5 different batches of AES.

Each of the 18 materials gave negative results when applied to 10 presensitized guinea pigs.

The animals were finally challenged again with LES 13-2035. A substantial incidence of positive reactions showed that the animals had retained their sensitivity during the 3-month course of the experiments.

Second series. An attempt was made to sensitize guinea pigs with other batches of AES, using the Magnusson-Kligman technique. Three different samples of AES were chosen from the 17 Procter & Gamble samples tested in the cross-challenge experiments. Each was applied to 10 animals, according to the procedure used in the first series. No positive reactions were seen.

Third series. It was found by Hansen (2) that a sensitizing agent could be extracted from LES 13-2035 with petroleum ether. We therefore challenged guinea pigs that had been sensitized to LES 13-2035 with petroleum ether extracts and raffinates of that material. Guinea pigs were sensitized and challenged as in the first series of experiments. The extracts and raffinates were so diluted that the applied solutions contained the same concentrations of their respective components as the 0.5% solution of LES 13-2035 used in the original challenges.

Three extraction techniques were used. The first involved only brief contact of LES 13-2035 with petroleum ether in a separatory funnel. The extract so produced gave positive reactions in 3 of 10 animals; the raffinate gave positive reactions in 2 of the 10 animals challenged with it.

The second technique was a continuous liquid/liquid extraction. A 250 g sample of LES 13-2035 was diluted with 375 ml each of ethanol and water, and was extracted continuously for 8 hours with petroleum ether (boiling range 40–60°C). The extract phase, which amounted to about 150 ml, was washed 5 times with 25 ml of 50% aqueous alcohol; its volume was then reduced to 50 ml by evaporation under reduced pressure. This extract also gave positive reactions in 3 of 10 animals; the raffinate gave a positive reaction in 1 of 10 animals.

The third extraction technique differed from the second only in that the extraction time was extended to 168 hours. The extract from this experiment was not tested; the raffinate gave no positive reactions when it was used to challenge 10 guinea pigs previously sensitized to LES 13-2035. This raffinate was also used in an attempt to sensitize 10 guinea pigs by the Magnusson-Kligman technique. No evidence of sensitization was obtained.

The continuous extraction process was carried out on a non-sensitizing AES sample from a different source. Neither the extract nor the raffinate produced an allergenic response when applied to animals that had been sensitized to LES 13-2035.

Fourth series. LES 13-2035 was extracted by the 168-hour technique described above. The extract phase was evaporated to constant weight, and the residue, which amounted to only 0.9% of the LES 13-2035, was diluted to 0.1% in 80%

aqueous ethanol. This solution was then used to sensitize and challenge guinea pigs by the Buehler technique. Two hundred and fifty animals have been so treated in three groups; the incidences of sensitization were 64%, 48%, and 51%. An attempt was made to increase the incidence of sensitization by injecting 0.1 ml of Freund's complete adjuvant at the patch site immediately before application of the first induction patch, but 45 animals treated in this way showed a sensitization rate of only 37%.

The same 168-hour extraction procedure was applied to three of the samples of AES which gave negative cross-challenge results in the first series. The extracts failed to sensitize any of the 20 guinea pigs to which each was applied by the Buehler technique.

Fifth series. Petroleum ether extracts of LES 13-2035 were applied to guinea pigs by the Magnusson-Kligman technique. Of the 25 animals so treated, 20 were sensitized.

Sixth series. The capacity of the Buehler technique to detect the sensitizer before extraction was verified by applying the usual series of three induction patches containing a 25% aqueous solution of LES 13-2035 to 19 guinea pigs. They were challenged at weekly intervals thereafter with increasing concentrations of LES 13-2035. When the concentration was 5%, only 1 animal reacted. With a 10% solution, 5 reacted, and with a 20% solution, positive reactions were seen in 12 animals. No positive reactions were obtained in groups of control animals challenged with similar concentrations.

DISCUSSION

The first series of experiments showed that, despite its having been stored for several years, LES 13-2035 is still able to sensitize guinea pigs. Thus, the sensitizing agent must be a reasonably stable substance. This series also showed that sensitized animals retain their sensitivity for several weeks, at least, and therefore can be used for screening other materials. Since many other batches of AES, representing a variety of manufacturing locations, raw material sources, process conditions, and alkyl chain lengths did not provoke an allergic response when applied to sensitized animals, it is concluded that the sensitizing property is not an inherent property of the

alkyl ethoxy sulphate entity. Although these experiments do not prove that LES 13-2035 is unique, they do demonstrate that it is not typical.

The second series of experiments showed that not all batches of AES have the capacity to induce sensitization.

The third series gave information about the chemical characteristics of the sensitizing material. The AES molecule itself is highly polar, since it contains several ether linkages and terminates in a sulphate salt. Therefore it cannot be extracted to any appreciable extent from an aqueous solution with a non-polar hydrocarbon solvent. Petroleum ether extracted only a very small fraction of material from AES solution, even at a high ratio of extractant to extrahend. This extracted material must be of a predominantly non-polar character. Our experimental results showed that the substance responsible for provoking the allergic response in sensitized animals could be extracted from LES 13-2035 by petroleum ether. Extraction was incomplete after brief contact of LES solution with petroleum ether, but was complete after prolonged contact. The residual AES, after removal of the extractable material by prolonged extraction, was unable either to sensitize guinea pigs or to elicit a reaction from guinea pigs that had already been sensitized to LES 13-2035. This evidence strongly supports the view that the sensitizer is a relatively non-polar substance, chemically distinct from AES.

Negative challenge results with the extract and raffinate from a different AES sample show that the allergic responses produced by extracts of LES 13-2035 were not caused by any artifact of the extraction process.

The fourth series of experiments showed that the extractable material from LES 13-2035 is capable, not only of provoking a response in previously sensitized animals, but also of inducing

sensitization. Hence it must be recognized as containing the sensitizing component of LES 13-2035.

The fourth, fifth and sixth series showed that both the Magnusson-Kligman technique and the Buehler technique can be used to detect the sensitizer in LES 13-2035 and in petroleum ether extracts of that material.

At present, we can only speculate about the nature and source of the active agent. Representatives of several AES manufacturers have formed a committee to establish the cause of the problem. Work is in progress in several laboratories to isolate and identify the allergenic factor, and to define process conditions under which the allergen can and cannot be made.

Preliminary gas chromatographic results indicate that the petroleum ether extract of LES 13-2035 is a very complex mixture, and differs in many respects from the extracts of other batches of AES.

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