

Sensitizing potential of acetaldehyde and formaldehyde using a modified cumulative contact enhancement test (CCET)

MARGARETA BERGH AND ANN-THERESE KARLBERG

Department of Occupational Medicine, Dermatology Division, National Institute for Working Life, Solna, Sweden

The contact allergenic activity of acetaldehyde was investigated with a modified cumulative contact enhancement test (CCET) method in guinea pigs. Possible cross-reactivity between acetaldehyde and formaldehyde was also studied. In contrast to the original CCET protocol, we used sham-treated controls and the chemicals were tested with closed epicutaneous application at 1st challenge. The suitability of the method was verified with formaldehyde and the results were comparable with those previously found with the guinea pig maximization test (GPMT). For the 1st time, acetaldehyde was shown to be a contact allergen in predictive tests. No cross-reactivity was observed between acetaldehyde and formaldehyde. Acetaldehyde seems to be a rare sensitizer in man. However, its allergenic activity should be considered, since it might be present as an impurity in ethoxylated surfactants. As the CCET protocol involves topical induction and challenge, we regard the modified version as well suited to evaluation of the contact allergenic potential of chemicals.

Key words: acetaldehyde; allergic contact dermatitis; cross-reactivity; cumulative contact enhancement test (CCET); formaldehyde; guinea pig sensitization; surfactants; topical sensitization. © Munksgaard, 1999.

Accepted for publication 23 October 1999

In our studies on the allergenic activity of ethoxylated nonionic surfactants, we found that peroxides, formaldehyde and a series of ethoxylated aldehydes and formates were formed due to autoxidation of an ethoxylated alcohol (Fig. 1) (1–4). Another low-molecular aldehyde, acetaldehyde, was detected in our study on the ethoxylated nonionic surfactant Tween® 80 (sorbitan mono-oleate) (Fig. 1) (1). Acetaldehyde was present in Tween® 80 of technical quality as received. The amount gradually decreased at air exposure in our laboratory when mimicking normal storage and handling. The highest concentration of acetaldehyde (0.030%, 300 ppm) was quantified in a newly opened bottle of Tween® 80 (1). Acetaldehyde can be formed as an impurity in the industrial production of Tween® 80 (5). It has also been detected in alcohol ethoxylates of technical quality as an impurity, probably due to oxidation of the alcohol used in the production process (6).

Acetaldehyde seems to be a rare sensitizer in man. We have found only 1 case report on allergic contact dermatitis from acetaldehyde (7). A textile worker who developed allergic contact dermatitis

when exposed to dimethoxane in an aqueous system is described. Patch testing with this compound and its possible degradation products demonstrated sensitization to acetaldehyde and crotonaldehyde. Contact urticaria caused by acetaldehyde has been described in case reports (8, 9). The use of acetaldehyde in industrial production accounts for the major exposure. Due to explosive hazards, it is commonly handled in closed systems in industry and exposure is not apt to be continuous or heavy. The formation of acetaldehyde and formaldehyde in soluble oils used as cutting fluids for steel grinding was investigated as part of a study on symptoms in exposed workers (10). No causal association between complaints and acetaldehyde in the cutting fluids was established. Positive patch-test reactions in workers exposed to a fatty acid ester from a cutting fluid have been reported (11). The fatty acid ester is described as a reaction mixture based among others on polyethylene glycol, which is the type of substance that our group has investigated in the studies on ethoxylated surfactants (1–4). Whether acetaldehyde or formaldehyde was present in the product is not reported.

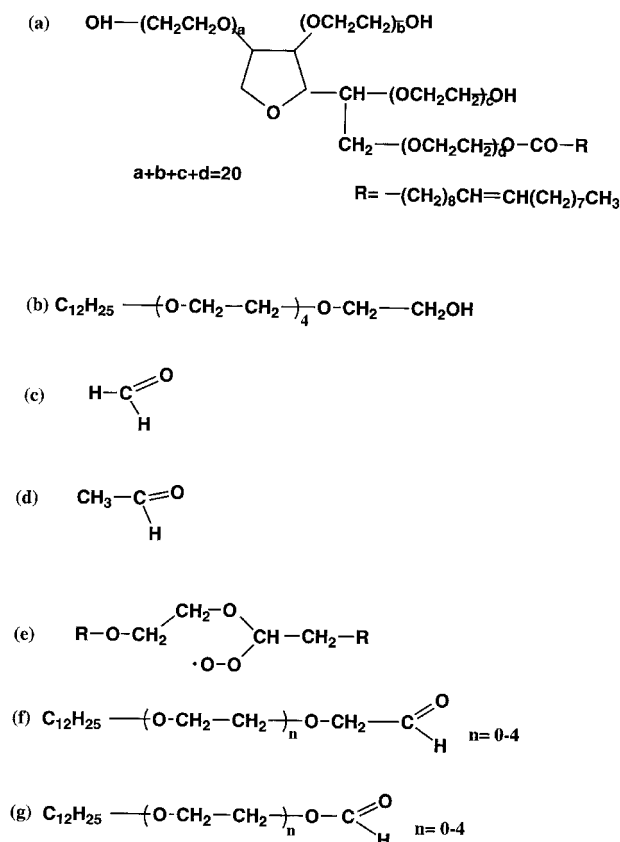


Fig. 1. The chemical structures of investigated ethoxylated non-ionic surfactants: (a) Tween[®] 80 with about 20 ethyleneoxy groups per molecule and (b) pentaethylene glycol mono *n*-dodecyl ether $\text{C}_{12}\text{H}_{25}(\text{OCH}_2\text{CH}_2)_5\text{OH}$ (C_{12}E_5). Compounds identified in these surfactants: (c) formaldehyde, (d) acetaldehyde, (e) peroxides, (f) ethoxylated aldehydes and (g) ethoxylated formates.

In the updated version (1992) of OECD guideline 406 for Testing of Chemicals (12), 2 guinea pig methods are given in preference to others to provide information on the sensitization potential of a chemical. These are the guinea pig maximization test (GPMT) (13) and the Buehler test (14, 15). In previous studies, we found the cumulative contact enhancement test (CCET) (16), in a modified version, to be a valuable alternative to the officially recommended predictive methods (Fig. 2; Table 1). According to the original CCET method, the induction procedure consists of 4 closed epicutaneous applications and 2 injections of 0.1 ml Freund's complete adjuvant (FCA), which are administered to enhance the sensitivity of the method. Open testing is performed at 1st challenge, and if negative, closed challenge testing is performed 1 week later. The controls are non-treated except for the FCA injections. In our modified version, we use sham-treated controls and the chemicals are challenge tested with patch

test technique. According to our experience, we find closed application more reliable than open application (17). In the original description of the CCET method (16), formaldehyde was used as one of the substances to validate the method. However, the original method was found to be rather insensitive regarding formaldehyde compared to the GPMT method (18), since a high concentration was required to get a response.

The aim of the present study was to investigate the contact allergenic potential of acetaldehyde. Formaldehyde was tested in an initial experiment with our modified CCET method to investigate the suitability of this method. We also wanted to study possible cross-reactivity between formaldehyde and acetaldehyde.

Materials and Methods

Chemicals

Formaldehyde stabilized with 10% methanol determined to 41.4% iodometrically (25) and acetaldehyde p.a. (>99.5%) were obtained from Riedel de Haen. Physiological saline (NaCl 9 g/l) was obtained from Kabi-Pharmacia, Stockholm, Sweden and Freund's complete adjuvant (FCA) from Difco, Detroit, Michigan, USA. Octaethylene glycol mono *n*-dodecyl ether, $\text{C}_{12}\text{H}_{25}(\text{OCH}_2\text{CH}_2)_8\text{OH}$ (CAS RN 3055-98-9) (referred to as C_{12}E_8) was purchased from Nikko Chemicals CO., Ltd., Tokyo, Japan. The purity was stated to be 98% by the producer, which was confirmed with gas chromatography (GC) analysis. Tween[®] 80 (Tween 80, polysorbate 80, polyoxyethylene 20 sorbitan monooleate) of technical quality was obtained from Zeneca, Macclesfield, UK.

Induction

Day 0, 2, 9

Exposed

Application of 0.2 ml test material in vehicle, epidermal occlusion for 24 h



Controls

Application of 0.2 ml vehicle, epidermal occlusion for 24 h



Challenge

21

Patch testing on the flanks



Patch testing on the flanks



Fig. 2. The cumulative contact enhancement test (CCET) in a modified version with sham-treated controls and closed challenge testing.

Table 1. Sensitizing capacity in guinea pigs of some chemicals using a modified CCET method with closed challenge testing and sham-treated controls

Cemicals	No. animals exp.+contr.	Induction conc.	Max. challenge conc.	Min. eliciting conc.	(Exp./p Contr.)	Ref.
maleopimaric acid ^{a)}	14+14	0.18 ^{b)} (7.2) ^{c)} in pet.	0.09 ^{b)} (3.6) ^{c)}	0.04 ^{b)} (0.001) ^{c)}	<0.001	(19)
maleopimaric acid ^{a)}	15+15	0.03 (1.2) in pet.	0.18 (7.2)	0.4 (0.01)	<0.001	(19)
maleopimaric acid ^{a)}	15+15	0.01 (0.4) in pet.	0.18 (7.2)	0.4 (0.01)	<0.05	(19)
maleopimaric acid	15+15	0.18 (7.2) in pet.	0.18 (7.2)	0.024 (0.0006)	<0.001	(20)
fumaropimaric acid	15+15	0.18 (7.5) in pet.	0.18 (7.5)	n.f.	n.s.	(20)
glyceryl triabietate	15+15	0.088 (8.3) in pet.	0.088 (8.3)	n.f.	n.s.	(21)
glyceryl 1-monoabietate	14+15	0.088 (3.3) in pet.	0.088 (3.3)	0.029 (1.1)	<0.05	(22)
β -epoxyabietate	15+15	0.15 (5.0) in pet.	0.15 (5.0)	0.03 (1.0)	<0.001	(23)
ethoxylated aldehyde	15+15	10 (0.25) in aq.	0.25 (10)	0.025 (1.0)	<0.01	(3)
ethoxylated aldehyde ^{a)}	15+12	0.25 (10) in aq.	0.25 (10)	0.025 (1.0)	<0.01	(3)
3-carene ^{a)}	15+10	3.7 (50) in o.o.	0.73 (10)	0.073 (1.0)	<0.05	(24)
gum rosin	10+10	(20) in pet.	(10)	n.t.	<0.001	(21)
gum rosin	15+15	(20) in pet.	(10)	n.t.	<0.01	(22)

^{a)}No adjuvant treatment was undertaken. ^{b)}mmol/g. ^{c)}%. n.f.=not found. n.s.=not significant. n.t.=not tested.

Sensitization experiments

Female Dunkin-Hartley guinea pigs from AB Sahlins Försöksdjursfarm, Malmö, Sweden (experiment 1) and Møllegård Breeding & Research Center A/S, Skensved, Denmark (experiment 2) were used in the study. The animals' average weight was 300–350 g. The diet was a standard diet from Beeky, North Humberside, UK, and water ad libitum. At induction the animals received 4 closed epicutaneous applications on the shaved upper back on days 0, 2, 7 and 9. The amount given at each application was about 200 mg on a 2×4 cm area. 2 intradermal injections of 0.1 ml FCA (not w/o emulsified) were given on day 7 in the same skin region (16). The controls were sham-treated and closed challenge testing was performed on day 21 on the shaved flanks of the animals using Finn Chambers[®] (Epitest, Helsinki, Finland) covered with Scanpor[®] and Acrylastic[®] (Beiersdorf AG, Hamburg, FRG) bandage. Approximately 15 mg test material was applied on each patch. The patches were removed after 24 h and assessment of the reactions was made at 48 and 72 h after start of exposure. The minimum criterion for a positive reaction was confluent erythema. The study was approved by the ethics committee.

Experiment no. 1. The exposed group ($n=15$) was treated with formaldehyde 5.0% w/w (1.7×10^{-3} mol/g) in saline and the control group ($n=15$) was exposed to saline. All animals were challenged with formaldehyde at 3 concentrations 0.03, 0.10 and 0.30% w/w (2.5×10^{-5} , 3.3×10^{-4} and 1.0×10^{-3} mol/g) in saline. Saline was tested as a vehicle control. The animals were also tested with air-exposed Tween[®] 80 12.5% in water containing 0.025 % (250 ppm) formaldehyde and undetectable amounts of acetaldehyde. The water solution of Tween[®] 80 was stirred 1 h, 4× a day for 12 months

in daylight at room temperature (20–22°C) at our laboratory, mimicking what we considered normal handling in laboratories and factories (1). The content of formaldehyde and acetaldehyde was quantified using high performance liquid chromatography (HPLC) analysis after derivatization of the low-molecular aldehydes with 2,4-dinitrophenylhydrazine (1).

Experiment no. 2. Group 1 ($n=15$) was induced with formaldehyde 5.0% w/w (1.7×10^{-3} mol/g) in saline. Group 2 ($n=15$) was induced with acetaldehyde 15% w/w (3.3×10^{-3} mol/g) in saline. A control group ($n=15$) received saline alone. All animals were challenged with formaldehyde at the concentrations 0.10 and 0.30% w/w (3.3×10^{-4} and 1.0×10^{-3} mol/g) in saline, and with acetaldehyde at the concentrations 2.5, 5.0 and 10% w/w (5.6×10^{-4} , 1.1×10^{-3} and 2.2×10^{-3} mol/g) in saline. The animals were also tested with Tween[®] 80 12.5% in water containing 0.025% acetaldehyde and 0.0005% formaldehyde, and with a sample of this water solution stored under pure oxygen for 24 h at our laboratory (1). After oxygen exposure the content of formaldehyde was 0.01% (100 ppm) and of acetaldehyde 0.001% (10 ppm).

Pretests. Untreated guinea pigs were tested with formaldehyde (10–30% w/w) and acetaldehyde (15–45% w/w) in saline to define the lowest irritating concentrations for induction. FCA-treated guinea pigs were tested with formaldehyde (0.01–5.0% w/w) and acetaldehyde (0.44–15% w/w) in saline to define the highest non-irritating concentrations for challenge. The test concentrations of the Tween[®] 80 solutions were selected according to previous experience (1).

Rechallenge. The animals were rechallenged 78 days after start of the experiment with formaldehyde at the concentrations 0.005, 0.10, 0.30 and 1.0% w/w (1.7×10^{-6} , 3.3×10^{-5} , 1.0×10^{-4} and

Table 2. Results of sensitization studies on formaldehyde in guinea pigs using the CCET in a modified version

Guinea pigs	No. animals with positive reactions ^{a)}				
	formaldehyde			ox. Tween 80 ^{b)}	
	0.30%	0.10%	0.030%	12.5%	saline
exposed ^{c)} (n=15)					
48 h	8 ^{d)}	1	2	0	0
72 h	6 ^{d)}	1	1	0	0
controls (n=15)					
48 h	0	0	2	1	0
72 h	0	0	0	2	0

^{a)} The figures are the number of animals with confluent reactions 48 and 72 h after application of test materials.

^{b)} Air exposed Tween 80 12.5% in water containing 0.025% (250 ppm) formaldehyde. The solution had been exposed to air for 12 months when a sample was taken out for the challenge testing.

^{c)} Induction: 5.0% (1.7 mmol/g) formaldehyde in saline (% w/w).

^{d)} Significantly different from the controls, $p < 0.01$.

3.3×10^{-4} mol/g) in saline, with acetaldehyde at the concentrations 0.03 and 2.5 % w/w (6.8×10^{-6} and 5.6×10^{-4} mol/g) in saline and with a sample taken from a 10% water solution of the ethoxylated alcohol, C₁₂E₈. The water solution of C₁₂E₈ was handled as described above for 5 weeks in daylight at room temperature at our laboratory, mimicking what we considered normal handling in laboratories and factories (2). HPLC analysis revealed a content of 0.005% (50 ppm) formaldehyde and 0.0005% (5.0 ppm) acetaldehyde. New sham-treated controls (n=15) were used in the rechallenge test. The irritation from the water solution

of C₁₂E₈ was studied in a pretest on 3 FCA-treated guinea pigs.

Statistical method

The number of positive test reactions in the exposed animals was compared to the number in control animals using Fisher's exact test (26). A p -value < 0.05 was considered statistically significant.

Results

Allergenic activity of formaldehyde

Significant reactivity ($p < 0.01$) was seen to 0.30% (3000 ppm) formaldehyde in experiment no. 1 (Table 2). No positive reactions to air-exposed Tween[®] 80 solution containing 0.025% (250 ppm) formaldehyde were seen.

Allergenic activity of formaldehyde and acetaldehyde

Both formaldehyde and acetaldehyde showed significant sensitizing capacity ($p < 0.01$) and a clear dose-response relationship was noticed (Table 3). No cross-reactivity was observed between formaldehyde and acetaldehyde. No significant reactions were seen to Tween[®] 80 solution, containing 0.03% (300 ppm) acetaldehyde, nor to air-exposed Tween[®] 80 solution, containing 0.01% (100 ppm) formaldehyde, in any of the groups.

At rechallenge 78 days after start of the experi-

Table 3. Results of sensitization studies on formaldehyde and acetaldehyde in guinea pigs using the CCET in a modified version. 1st challenge (Day 21)

Guinea pigs	No. animals with positive reactions ^{a)}							
	formaldehyde		acetaldehyde			Tween 80 ^{b)}	ox. Tween 80 ^{c)}	saline
	0.30%	0.10%	10%	5.0%	2.5%	12.5%	12.5%	
group 1 ^{d)} (n=15)								
48 h	6 ^{e)}	3	0	0	0	2	0	0
72 h	11 ^{f)}	8 ^{g)}	0	0	0	0	2	0
group 2 ^{h)} (n=15)								
48 h	0	1	13 ^{f)}	7 ^{e)}	4 ^{e)}	0	0	0
72 h	0	0	13 ^{f)}	9 ^{f)}	5 ^{e)}	0	1	0
controls (n=15)								
48 h	1	0	0	1	0	0	0	1
72 h	0	0	0	0	0	1	1	0

^{a)} The figures are the number of animals with positive reactions 48 and 72 h after application of test material.

^{b)} Tween 80 12.5% in water containing 0.025% acetaldehyde. Taken from the producers vessel and diluted to 12.5%.

^{c)} Oxidized Tween 80 12.5% in water containing 0.010% (100 ppm) formaldehyde and 0.001% (10 ppm) acetaldehyde. The solution had been exposed to pure oxygen for 24 days when a sample was taken out for the challenge testing.

^{d)} Induction: 5.0% (1.7 mmol/g) formaldehyde in saline (% w/w).

^{e)} Significantly different from the controls, $p < 0.05$.

^{f)} Significantly different from the controls, $p < 0.001$.

^{g)} Significantly different from the controls, $p < 0.01$.

^{h)} Induction: 10% (2.3 mmol/g) of acetaldehyde in saline (% w/w).

Table 4. Rechallenge 78 days after start of experiment no. 2; new sham-treated controls were used

Guinea pigs	No. animals with positive reactions ^{a)}							
	formaldehyde				acetaldehyde		C ₁₂ E ₈ ^{b)}	saline
	1.0%	0.30%	0.10%	0.005%	2.5%	0.03%	10%	
group 1 ^{c)} (n=15)								
48 h	5 ^{d)}	2	0	0	0	0	0	0
72 h	9 ^{e)}	6 ^{f)}	0	0	1	0	1	0
group 2 ^{g)} (n=15)								
48 h	1	0	0	0	0	0	0	0
72 h	0	0	0	0	0	0	0	0
new controls (n=15)								
48 h	1	0	0	0	2	0	0	0
72 h	0	0	0	0	1	0	1	0

a) The figures are the number of animals with positive reactions 48 and 72 h after application of test materials.

b) Air exposed C₁₂E₈ 10% in water containing 0.0050% (50 ppm) formaldehyde and 0.0005% (5 ppm) acetaldehyde. The solution had been exposed to air for 5 weeks when a sample was taken for the challenge testing.

c) Induction: 5.0% (1.7 mmol/g) formaldehyde in saline (% w/w).

d) Significantly different from the controls, $p < 0.05$.

e) Significantly different from the controls, $p < 0.001$.

f) Significantly different from the controls, $p < 0.01$.

g) Induction: 10% (2.3 mmol/g) of acetaldehyde in saline (% w/w).

ment, significant reactions were seen to 1.0 and 0.30% formaldehyde in group 1, while no significant reactions were seen to acetaldehyde (2.5 and 0.03%) in group 2. The air-exposed water solution of the ethoxylated alcohol, C₁₂E₈, containing 0.005% (50 ppm) formaldehyde and 0.0005% (5 ppm) acetaldehyde, gave no significant response (Table 4).

Discussion

Acetaldehyde was for the 1st time shown to be a contact allergen in a predictive test in guinea pigs (Table 3). The allergenic potential and the cross-reactivity pattern of acetaldehyde and formaldehyde were investigated according to a test method with epidermal induction and challenge, the cumulative contact enhancement test (CCET) method (16) using sham-treated controls and closed challenge with Finn Chambers[®] (Fig. 2). The suitability of the method was verified with formaldehyde in the 1st experiment (Table 2). Formaldehyde and acetaldehyde were detected in ethoxylated non-ionic surfactants in our previous studies (1–4). Ethoxylated non-ionic surfactants are widely used in household and industrial detergents and as processing aids in a variety of applications (27). Also many topical pharmaceutical formulations and cosmetics contain them (28, 29).

The eliciting capacity of different samples of ethoxylated nonionic surfactants, handled in a way that mimicked normal handling and storage, was investigated in the animals sensitized to formaldehyde and acetaldehyde. No response was obtained to these samples (Tables 2–4). The content of acet-

aldehyde and formaldehyde in the tested products was too low, as can be seen from the results obtained for the pure compounds tested in corresponding concentrations. Acetaldehyde seems to be a rare sensitizer in man. However, it might be a common impurity in ethoxylated surfactants not previously considered. Regarding formaldehyde, its contact allergenic effect is well-known (30). Formaldehyde is incorporated into a large variety of products, e.g., from formaldehyde donors used as preservatives in soluble oils for cutting fluids and in skin care products. Additional formaldehyde from ethoxylated surfactants and emulsifiers in such products can increase the concentration of formaldehyde, and thus increase the risk of allergic contact dermatitis in exposed persons.

No cross-reactivity was observed between formaldehyde, and acetaldehyde (Tables 3–4, Fig. 1). Although the functional group in formaldehyde and acetaldehyde is the same, the additional methylene group in acetaldehyde makes a considerable difference in structure between these 2 low-molecular compounds.

In predictive tests, higher concentrations of acetaldehyde were needed for induction and elicitation compared to those of formaldehyde. Furthermore, a difference in irritancy was seen in the pretests. Formaldehyde gave irritant reactions at concentrations of 3% (1.0×10^{-2} mol/g) and above. Acetaldehyde, on the other hand, gave no irritation when tested at a concentration of 10% (2.2×10^{-2} mol/g). The difference in response observed between acetaldehyde and formaldehyde (Table 3) could be due to a difference in the chemical reactivity, since the carbonyl group of formaldehyde

exhibits a higher electrophilic reactivity than most other aldehydes, acetaldehyde included. The observed difference in response might also be due to differences in penetration and metabolism (31). Formaldehyde is thought to diffuse rapidly through the stratum corneum to enter the lower layers of epidermis. Although a small portion may be metabolized or absorbed as unchanged formaldehyde, distributed or excreted, most remains in the epidermis as reversible or irreversible complexes with proteins (31). No studies on animals or humans concerning the skin absorption of acetaldehyde are available, to the best of our knowledge (32). Endogenous acetaldehyde results from metabolism of ingested ethanol. Accumulation of acetaldehyde in the skin at higher concentrations, due to delayed metabolism of acetaldehyde on consumption of ethanol, was discussed for individuals with low activity of aldehyde dehydrogenase (33). The metabolism of ethanol to acetaldehyde by alcohol dehydrogenase in the skin has been discussed in terms of observed cross-reactivity between ethanol and acetaldehyde, when aqueous ethanol solutions were shown to induce allergic reactivity (9, 34). Reports of contact urticaria caused by ethanol consider the possibility that the observed reactions are provoked by aldehydes (34, 35).

Using the modified CCET protocol, the response rate of formaldehyde for the challenge concentration 0.30% was 40% in experiment 1 (Table 2) and 75% in experiment 2 (Table 3). The observed difference in response rate was not statistically significant. In GPMT studies on formaldehyde, a variation was seen in the response rate which was mainly considered to be due to different strains of guinea pigs used in the parallel experiments (18). In the present investigation, the same strain of outbred animals was used but from different suppliers. Tsuchiya et al. (16) reported a 40% response rate at a challenge concentration of 10% formaldehyde, when tested according to the original version of CCET, while no response was seen to 1 and 0.2% formaldehyde at 1st challenge. The results from a 2nd closed challenge test were not published. The recommended open challenge may explain the lower response rate of formaldehyde. In our modified version, an occlusive patch test is used at 1st challenge, which ensures contact with the skin and the test substance for a longer exposure time. This is especially important for volatile compounds such as formaldehyde. In the present study, the reactivity had decreased in both the formaldehyde and the acetaldehyde sensitized groups when rechallenge was performed on day 78. This could be due to the long time period without boosting, between the 2 challenge tests.

The GPMT protocol prescribes both intrader-

mal and epidermal induction. In a dose-response relationship study of formaldehyde with this method, the incidence of contact sensitivity was found to depend on the intradermal, but not on the epidermal, induction dose (18). In the present investigation, we demonstrated that epicutaneous induction alone gave a response of the same magnitude as the maximal response found in the GPMT study. Published results from earlier experiences in our laboratory with the modified CCET method are presented in Table 1. In some of these studies (3, 19), addition of FCA did not increase the response rate. In a study (36) at our laboratory on the sensitizing potential of 1,2-dibromo-2,4-dicyanobutane (the active ingredient in Euxyl® K400), a GPMT failed to detect the allergen, while a positive response was obtained with the modified CCET method using a dose-response protocol. Previous GPMT studies also failed to show the allergenic activity of 1,2-dibromo-2,4-dicyanobutane (37). The preservative Euxyl® K400 is described as a sensitizer in man (38).

As the CCET protocol involves epicutaneous induction and challenge, we consider the modified version to be well suited to the evaluation of chemicals expected to come in contact with human skin. No comparison has been performed with the Buehler test (14, 15), which only prescribes epicutaneous administration. However, the longer application times for the CCET (4×24 h) compared to those for the Buehler test (3×6 h) should increase the possibility of detecting weak allergens, as also the use of FCA if necessary. Further evaluation of the method is in progress at our laboratory.

Conclusion

Acetaldehyde was for the 1st time shown to be a contact allergen in predictive tests using a modified version of the CCET method. No cross-reactivity was observed between formaldehyde and acetaldehyde. The response rate of formaldehyde as shown by the modified CCET method was equal to that previously found in GPMT studies. As the CCET protocol involves epicutaneous induction and challenge, we regard the modified version as well suited to evaluation of the contact allergenic potential of chemicals.

Acknowledgements

We thank Gunnel Hagelthorn for her skilful assistance with the animal testing and Professor Jan E. Wahlberg for constructive criticism of the manuscript. Financial support from the Swedish Council for Work Life Research is gratefully acknowledged.

References

- Bergh M, Magnusson K, Nilsson J L G, Karlberg A-T. Contact allergenic activity of Tween® 80 before and after air exposure. *Contact Dermatitis* 1997; 37: 9–18.
- Bergh M, Magnusson K, Nilsson J L G, Karlberg A-T. Formation of formaldehyde and peroxides by air oxidation of high purity polyoxyethylene surfactants. *Contact Dermatitis* 1998; 19: 14–20.
- Bergh M, Shao L P, Hagelthorn G, Gäfvert E, Nilsson J L G, Karlberg A-T. Contact allergens from surfactants. Atmospheric oxidation of polyoxyethylene alcohols, formation of ethoxylated aldehydes and their allergenic activity. *J. Pharm. Sci.* 1998; 87: 276–282.
- Bergh M, Shao L P, Magnusson K, Gäfvert E, Nilsson J L G, Karlberg A-T. Atmospheric oxidation of ethoxylated surfactants. Identification of ethoxylated formates as oxidation products and study of their contact allergenic activity. Manuscript submitted for publication.
- Chafetz L, Hong W-H, Tsilifonis D C, Taylor A K, Philip J. Decrease in the rate of capsule dissolution due to formaldehyde from Polysorbate 80. *J Pharm Sci* 1980; 73: 1186–1187.
- Evetts S, Kovalski A, Levin M, Stafford M. High-temperature stability of alcohol ethoxylates. *JOACS* 1995; 72: 811–816.
- Shmunis E, Kempton R J. Allergic contact dermatitis to dimethoxane in a spin finish. *Contact Dermatitis* 1980; 6: 421–424.
- Wilkin J K, Fortner G. Ethnic contact urticaria to alcohol. *Contact Dermatitis* 1985; 12: 118–120.
- Rilliet A, Hunziker N, Brun R. Alcohol contact urticaria syndrome (immediate-type hypersensitivity). *Dermatologica* 1980; 161: 361–364.
- Järvholm B, Ljungkvist G, Lavenius B, Rodin N, Peterson C. Acetic aldehyde and formaldehyde in cutting fluids and their relation to irritant symptoms. *Ann Occup Hyg* 1995; 39: 591–601.
- Niklasson B, Björkner B, Sundberg K. Contact allergy to a fatty acid ester component of cutting fluids. *Contact Dermatitis* 1993; 28: 265–267.
- OECD (1992) *Organisation for Economic Cooperation and Development Guidelines for the testing of chemicals: 406, Skin sensitisation*. Adopted 17 July 1992.
- Magnusson B, Kligman A M. *Allergic contact dermatitis in the guinea pig*. Springfield: Thomas, 1970.
- Buehler, E V. Delayed contact hypersensitivity in the guinea pig. *Archs Derm* 1965; 91: 171–177.
- Ritz H L, Buehler E V. Planning, conduct, and interpretation of guinea pig sensitization patch tests. In: Drill V A, Lazar P. (eds): *Current concept in cutaneous toxicity*. Academic Press: New York; 1980 p. 25.
- Tsuchiya S, Kondo M, Okamoto K, Takase Y. The cumulative contact enhancement test. In: Andersen K E, Maibach H I. (eds): *Contact Allergy Test in Guinea Pigs*. *Curr Probl Derm.* 1985; 14: 208–219.
- Boman A, Karlberg A-T, Wahlberg J E. Experiences with Freund's complete adjuvant test (FCAT) when screening for contact allergens in colophony. *Contact Dermatitis* 1988; 18: 25–29.
- Andersen K E, Boman A, Vølund A, Wahlberg J E. Induction of formaldehyde contact sensitivity. A dose-response relationship in the guinea pig maximization test. *Acta Dermato-venereol* 1985; 65: 472–478.
- Karlberg A-T, Gäfvert E, Hagelthorn G, Nilsson J L G. Maleopimaric acid – a potent sensitizer in modified rosin. *Contact Dermatitis* 1990; 22: 193–201.
- Gäfvert E, Shao L P, Karlberg A-T, Nilsson U, Nilsson J L G. Maleopimaric acid – a contact allergen in fumaric acid modified rosin used for paper size. *Nordic Pulp & Paper Res J.* 1995; 10: 139–144.
- Shao L P, Gäfvert E, Karlberg A-T, Nilsson U, Nilsson J L G. The allergenicity of glycerol esters and other esters of rosin (colophony). *Contact Dermatitis* 1993; 27: 229–234.
- Gäfvert E, Shao L P, Karlberg A-T, Nilsson U, Nilsson J L G. Allergenicity of rosin (colophony) esters (II). Glyceryl monoabietate identified as contact allergen. *Contact Dermatitis* 1994; 31: 11–17.
- Gäfvert E, Shao L P, Karlberg A-T, Nilsson U, Nilsson J L G. Contact allergy to resin acid hydroperoxides. Hapten binding via free radicals and epoxides. *Chem Res Tox* 1994; 7: 260–266.
- Låstbom L, Boman A, Camner P, Ryrfeldt Å. Does airway responsiveness increase after skin sensitisation to 3-carene: a study in isolated guinea pig lungs. *Toxicology* 1998; 125: 59–66.
- Commission Directive 90/207/EEC. In: *Official Journal of European Communities*, no. L 108, 28/04/90, p. 0092.
- Gad S C, Weil C S. *Statistics and experimental design for toxicologists*. New Jersey: Telford Press, 1988: 54–55.
- Modler F, Willhalm R, Yoshida Y. Surfactants, household detergents and their raw material. In *CEH (Chemical Economics Handbook)*. Stanford Research Institute, 1994: 583.8001A–583.8003J.
- Effendy I, Maibach H I. Surfactants and experimental irritant contact dermatitis. *Contact Dermatitis* 1995; 33: 217–225.
- Attwood D, Florence, A T. *Surfactant systems; their Chemistry, pharmacy and biology*. London: Chapman & Hall, 1983.
- Andersen K E, Burrows D, White I R. Allergens from the standard series. In: Rycroft R J G, Menné T, Frosch P J. (eds): *Textbook of contact dermatitis*, 2nd edition. Berlin, Heidelberg, New York: Springer-Verlag; 1995: 438–440.
- Jeffcoat A R. *Percutaneous penetration of formaldehyde. Final report (July 1981–July 1983)*. Research Triangle Park, North Carolina, Research Triangle Institute, p.59.
- Maibach H I. Formaldehyde: effects on animal and human skin. In: Gibson J E (ed.): *Formaldehyde toxicity*. New York: Hemisphere; 1983: 166–174.
- Higuichi S, Muramatsu T, Saito M, Maruyama K, Kono H. Ethanol patch test for low K_m aldehyde dehydrogenase deficiency. *The Lancet* 1987; 14: 629.
- Fregert S, Hakanson R, Rorsman H, Övrum P. Dermatitis from alcohols. *J Allergy* 1963; 34: 404–408.
- Slotts J, Ely J. W. Induction of human skin sensitization to ethanol. *J Invest Dermatol* 1977; 69: 219–222.
- Wahlkvist H, Montelius J, Boman A, Wahlberg J E. The sensitizing potency of dibromo-2,4-dicyanobutane (BCB). The active substance in Euxyl® K400, in humans and in predictive animal studies. *4th Congress of the European Society of Contact Dermatitis* Helsinki, Finland, 8–11 July 1998.
- Bruze M, Gruvberger B, Agrup G. Sensitization studies in the guinea pig with the active ingredients of Euxyl® K400. *Contact Dermatitis* 1988; 18: 27–39.
- De Groot A C, Van Ginkel C J W, Weijland J W. Methylbromoglutaronitrile (Euxyl K 400): An important “new” allergen in cosmetics. *J Am Acad Dermatol* 1996; 35: 743–747.

Address:

Margareta Bergh
 Department of Occupational Medicine, Dermatology Division
 National Institute for Working Life
 S-17184 Solna
 Sweden