The preparation technique involves procedures with compounds in which 8-MOP is easily soluble. This could lead to the extraction of intercalated 8-MOP, but presumably not of covalently bound $^3$H-8-MOP in DNA after the UV A exposure. Since no grains are observed in the smears, extraction is not likely to be of influence. Furthermore, addition of cold 8-MOP to the liquids used for washing cells made no difference.

We checked that $^3$H-8-MOP was stable during the culturing period by examining the amount of $^3$H$_2$O formed. Radioactivity from tritiated water was responsible for <5% of the total radioactivity and from this fact it can be deduced that $^3$H-8-MOP is stable.

Irradiation at the beginning of the culturing period could lead to cell death with consequent loss of these cells.

The lack of grains in mitoses, irradiated just prior to preparation, might be explained by the inability of DNA to react with $^3$H-8-MOP in prophase and metaphase. However, this explanation is not very plausible, since no grains were seen, either in the nucleus or in other dividing stages.

It must be appreciated that the formation of a silver grain (0.25 µm in diameter) (6) needs about 20 disintegrations to occur in the film emulsion used (6). With this sensitivity we could not detect any binding of $^3$H-8-MOP to DNA or other cell structures when using 8-MOP concentrations and light intensities corresponding to the doses given to patients.

REFERENCES

5. Pathak, M. A., Krämer, D. M. & Fitzpatrick, T. B.: The resins used in UV-cured inks are acrylated prepolymers diluted with multifunctional acrylates. The multifunctional acrylates have a dual role in UV-curable coatings or inks. One is to serve as cross-linking sites for the reactive base prepolymer and the other is to function as diluents for the usually more viscous base resins (12). In general, acrylic prepolymers and monomers are relatively transparent and consequently unable to initiate a fast...
polymerization without a photoinitiator, such as benzophenone.

In recent years reports have appeared about dermatitis among those working with UV-curing inks at printing plants or among workers manufacturing UV-curing resins (3, 4, 9, 10, 11, 13).

The advanced technology of using UV-curing inks at printing plants, has recently been introduced in Sweden. We have seen 6 patients with dermatitis after working with UV-curing inks at printing plants in southern Sweden (1).

The manufacturers informed us that the most commonly used inks at the printing plants in question consisted of trimethylol propane triacylate (TMPTA) and diacylate ester of bisphenol A epoxy resin (called epoxy acrylate). The inks also contained a benzophenone as a photoinitiator.

According to the manufacturers the inks were “tested for allergy”. Most commonly the tests performed were the skin irritation test of Draize (2). The “Guinea pig maximization test” (GPM) (6, 7) was performed with some inks as such but not with the constituents separately. The differences between, and performance and assessing of the test methods and results have caused much confusion among the manufacturers and users.

The purpose of the present investigation was to assess, with the “Guinea pig maximization test”, the sensitizing capacity of TMPTA and to confirm the clinical test results (1). It was investigated whether cross-reactions occur with other multifunctional acrylates having similar molecular structures: penta-erythritol triacylate (PETA) and trimethylol propane trimethacrylate (TMPTMA) (Fig. 1). The animals were also tested with acrylic acid, corresponding to the end groups of the tested compounds.

MATERIAL AND METHODS

Induction and challenge was performed in accordance with the original description of the GPM test (5, 6, 7).

Animals. Albinó female guinea pigs, weighing 300-400 grams, were used.

Chemicals. Induction was performed with TMPTA. Challenges were made with TMPTA, TMPTMA, PETA and acrylic acid. The chemicals used were commercial products supplied by the manufacturer and reported to be pure.

Topical irritancy. The topical irritancy of the chemicals was studied with a 24-hour occluded patch test in 6 other animals than those used in the main test. Challenge patch test concentrations were used which did not give any irritant reactions.

Sensitization concentrations. Preliminary investigations were performed to determine the optimal sensitization concentration of TMPTA for intradermal induction in 6 animals without causing systemic toxicity.

Intradermal injections for induction of sensitivity. Three injections of the following mixtures were made in a row on each side of the shoulder region:

0.1 ml Freund’s complete adjuvant (CFA) (Difco Lab., Detroit, Mich.) blended with an equal amount of water.

0.1 ml of TMPTA in an appropriate vehicle and concentration (Table I). 0.1 ml of a mixture containing the test substance in the vehicle and an equal amount of CFA.

Topical application for induction of sensitivity. One week after the injections the same shoulder area was clipped and shaved, after which closed patch exposure to the test substance in a vehicle was performed. The allergen in petrolatum was spread over a 2 by 4 cm patch of Whatman 3MM paper in a thick, even layer. The patch was covered by an overlapping, impermeable plastic adhesive tape (Leukoflex, Beiersdorf AG, Hamburg). This in turn was firmly secured by an elastic adhesive bandage (Acrylastic, Beiersdorf AG, Hamburg). The dressing was left in place for 48 hours. The final concentrations of the test substance for intradermal and topical inductions are given in Table I.

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Table I. Concentrations of acrylates for sensitization and challenge

<table>
<thead>
<tr>
<th>Acrylate</th>
<th>Sensitization</th>
<th>Topical Challenge</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Intradermal (%W/V)</td>
<td>Topical (%W/V)</td>
</tr>
<tr>
<td>TMPTA</td>
<td>1.0 in olive oil</td>
<td>50 in petrol</td>
</tr>
<tr>
<td></td>
<td>0.5 and 0.1 in petrol</td>
<td>1.0 and 0.1 in petrol</td>
</tr>
<tr>
<td>PETA</td>
<td>0.5 in petrol</td>
<td>0.5 in petrol</td>
</tr>
<tr>
<td>TMPTMA</td>
<td>0.5 in petrol</td>
<td>0.5 in petrol</td>
</tr>
<tr>
<td>Acrylic acid</td>
<td>0.5 in petrol</td>
<td>0.5 in petrol</td>
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</tbody>
</table>

Challenge. Two weeks after the second stage of sensitization a 24 hour occluded patch test (Finn-chamber on Scanpor, Norgesplaster A/S, Norway, firmly secured by Acrylastic, Beiersdorf, AG, Hamburg) was performed on the flank after the area had been clipped and shaved. The animals were tested with TMPTA and PETA, both in a concentration of 0.5% and 0.1% in petrolatum (Table I). Three hours prior to the reading, the test site was again shaved with electric razor. Only evident redness and/or swelling was regarded as an allergic response.

Rechallenge. One week after challenge the animals were rechallenged with TMPTA, TMPTMA and acrylic acid, all in a concentration of 0.5% in petrolatum (Table I).

Controls. At the same time as the animals in the experimental groups were sensitized, the control animals in each series were also exposed intradermally to CFA and vehicle, and topically to the vehicle. When the sensitized animals in each series were challenged, control animals were also patch tested with the same acrylates in the same concentrations.

RESULTS
The test results are summarized in Table II. Sixteen (67%) of the 24 animals exposed to TMPTA became sensitized. Even when the test concentration was as low as 0.1%, 6 of the 24 animals (25%) reacted positively. Eighteen of 24 animals (75%) also reacted to PETA in a 0.5% test concentration and 12 (50%) to 0.1%. None of the control animals, tested simultaneously, reacted to any of the compounds.

When the animals were rechallenged one week later, 7 of 24 (29%) reacted to TMPTA, but none to TMPTMA and acrylic acid. However, 10 of the 24 control animals (40%) now reacted positively to TMPTA, but none to the other two compounds (Table III).

DISCUSSION
Clinical experience shows that workers exposed to UV-curing inks at printing plants can become sensitized. Presumably, many workers assume the inks are harmless and therefore fail to take appropriate precautions, as manufacturers have given information that "allergy tests" have been made and have shown that the inks are not "allergenic".

67% of the guinea pigs were sensitized to TMPTA, which can therefore be classified as a strong sensitizer (6, 7).

Of the TMPTA-sensitive animals, 75% also reacted to PETA, suggesting cross-sensitivity between the two substances. PETA has been shown to be a strong allergen (11).

When rechallenged one week later, 40% of the control animals reacted positively to TMPTA. They had been sensitized by only one application of TMPTA at the challenge procedure one week earlier, which also shows the high rate of allergenicity of TMPTA. No cross-reaction occurred between TMPTA and TMPTMA or acrylic acid (Table III).

The results indicate that the whole molecule of TMPTA—and not merely the acrylic parts—acts as an allergen (Fig. 1). It is rather difficult to sensitize guinea pigs to acrylic acid (8).

The methyl groups in the molecule of TMPTMA seem to influence the allergenicity. By using acrylates substituted with methyl groups the allergenic potential might be diminished and could be one way of making less hazardous compounds.
It should be stressed that it is important to perform the guinea pig maximization test of the ingredients in new products before marketing. In view of the results of the present investigation, proper prevention measures should be performed.

REFERENCES


Age Incidence of *Pityrosporum orbiculare* on Human Skin

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Abstract. We have investigated the incidence of the lipophilic fungi *Pityrosporum orbiculare* and *P. ovale* on clinically normal skin in newborn children and in children at the age of 6 months, 1 year, 5 years, 10 years, and 15 years. *P. orbiculare* was absent in children of less than 5 years of age. It was present in the highest incidence (93%) in 15-year-old children, about the same frequency as in adults. The colonization starts during the period when the sebaceous glands become active. *P. ovale* could not be cultured and this may depend on geographical variations in the distribution of these yeasts.

Key words: *Pityrosporum orbiculare; P. ovale; Culturing study; Children*

The two lipophilic yeasts *Pityrosporum orbiculare* and *P. ovale* can be isolated from human skin as apparently true residents (4, 7, 9). *P. ovale* is most often—though not always—found on the scalp of both healthy individuals and patients with seborrhoeic dermatitis (1, 4, 5, 9, 12). *P. ovale* was earlier thought to play some part in the aetiology of seborrhoeic dermatitis, though most workers now believe that seborrhoeic dermatitis is the primary condition (1, 7). *P. ovale* can be cultured not only from the scalp but also from other areas of the skin supplied with sebaceous glands (7, 9).

The other lipophilic member of the genus *Pityrosporum, P. orbiculare*, differs from *P. ovale* mainly in its micromorphology (6), although growth characteristics have been used to distinguish the two species (11). It is now generally accepted that *P. orbiculare* and the dimorphic fungus seen in tinea versicolor are one and the same (4). *P. orbiculare* can be cultured not only from tinea versicolor scales but also from normal-looking skin in healthy adults (4, 5, 9). Some workers believe *P. ovale* and *P. orbiculare* to be identical (8, 10), while others consider them to be different (3, 5, 6). This question has not yet been settled.

*P. ovale* has been cultured from the normal scalp of children but only in small, ill-defined series (12). There are no adequate, published surveys of the