combination is too rare to provide an adequate study group. We assume that there is no peculiar antigen distribution in the husbands of these women. We also made no attempt to type the children of these couples, primarily because of their age. Such information would obviously be of great interest.

Antigodies against lymphocytes or monocytes occur in most women with HG. Most of the women in our study were 1–5 years beyond their last pregnancy at the time antibody reactivity was measured. Since anti-HLA antibodies decrease with time, it is likely that an even higher percentage of our patients would have had anti-HLA antibodies if studied at the time of parturition.

HLA-A, B, and C antigens are present on essentially all nucleated cells, including skin cells (keratinocytes). Only about 4% of epidermal cells express HLA-DR antigens (Langerhans cells). There is no evidence that any of these antigens are represented within the BMZ. Further, extensive absorptions of Reunala’s case with HLA-B8 positive lymphocytes failed to alter BMZ staining by the patient’s serum. Thus, anti-HLA antibodies are unlikely to play a direct role in the fixation of C at the dermal-epidermal junction in patients with HG.

It is possible that anti-HLA antibodies exert their effect on the development of HG indirectly by influencing some aspect of immune regulation. It is also possible that the production of anti-HLA antibodies, although more common in patients with HG, has no relationship to the pathogenesis of this disease. The production of these antibodies may be stimulated by the same factors that induce anti-BMZ antibody production. Further research into the factors that influence production of anti-BMZ and anti-HLA antibodies in this group of patients may provide insight into the fundamental abnormality of HG.

The authors wish to thank Gabriel Nunez, M.D. for his help and expertise.

REFERENCES


Kathon Biocide: Manifestation of Delayed Contact Dermatitis in Guinea Pigs Is Dependent on the Concentration for Induction and Challenge

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The potential of Kathon biocide, an aqueous solution containing, as active ingredients (a.i.), a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (14.4% a.i.), to produce delayed contact dermatitis, a sensitization response, was evaluated in outbred Hartley guinea pigs by a modified Buehler’s occluded epicutanous patch technique. The relationship of the response as a function of induction/elicitation concentrations was investigated. Groups of guinea pigs received 9 induction doses of the biocide, 3 times a week, at concentrations ranging from 25–2000 ppm a.i. These guinea pigs were challenged with the biocide at concentrations ranging from 20–2000 ppm a.i., and the application sites were scored for erythema 24 and 48 h after the challenge. The incidence of delayed contact dermatitis in induced guinea pigs was dependent on both the induction and challenge concentrations. The EC50 (concentration at which delayed contact dermatitis was seen in 50% of the population) for induction at a challenge concentration of 2000 ppm a.i., a nonirritating concentration, was estimated to be 88 ppm a.i. with a slope of
3.47 probits/unit log concentration. The EC50 for elicitation at an induction concentration of 100 ppm a.i. was estimated to be 429 ppm a.i. with a slope of 2.74 probits/unit log concentration. These data demonstrate that for Kathon biocide, there is an induction/elicitation concentration dependency for delayed contact dermatitis response, and there is a "no response concentration" zone where the biocide can be used without concern for clinically significant delayed contact dermatitis. In comparison with a previous study, these data also suggest that the number of induction doses may be an important factor in demonstrating the sensitization potential of a chemical.

Kathon biocide,* a widely used, broad-spectrum antimicrobial agent, contains, as active ingredients (a.i.), a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one in an approximate ratio of 3:1, respectively, with MgCl2 (9%) and Mg(NO3)2 (16%) present as stabilizers. The biocide is an effective preservative for toiletries, cosmetics, and household cleaning products. It is also used in some heavy industrial applications such as cooling-tower water, metal-working fluid, and latex emulsions.

A variety of industrial chemicals, cosmetic fragrances, and therapeutic agents, as well as several naturally occurring substances such as poison ivy, can cause delayed contact dermatitis in animals and humans [1-3]. These sensitizers fall into diversified chemical categories ranging from simple inorganic metals such as nickel to complex organic chemicals. Determination of the potential for a chemical to cause delayed contact dermatitis is often needed for the development of a risk assessment process to protect workers and consumers. Since cutaneous contact with products containing Kathon biocide is a primary route of exposure, the capacity of Kathon biocide to produce delayed contact dermatitis was investigated in guinea pigs.

Beuth et al. reported that no incidence of delayed contact dermatitis was observed in guinea pigs even at induction and elicitation concentrations as high as 1500 ppm a.i. when the biocide was applied once a week for 3 weeks. Parsons‡ reported that one of the active ingredients of Kathon biocide, 2-methyl-4-isothiazolin-3-one, induced delayed contact dermatitis at 16,000 ppm a.i., but no response was noted when these induced guinea pigs were challenged at 1600 ppm a.i. A concentration-dependent delayed contact dermatitis response to the biocide was also reported in humans [4]. Therefore, another objective of this study was to characterize the induction/elicitation concentration response relationship for Kathon biocide in guinea pigs.

MATERIALS AND METHODS

Test Substance and Animals

An aqueous solution of Kathon biocide (commercial sample) containing 14.4% a.i. was used for preparing various aqueous dilutions. All final concentrations were confirmed by high-pressure liquid chromatography analysis.

Outbred Hartley guinea pigs weighing 200-300 g (Charles River Breeding Laboratories, Kingston, New York) were individually housed in cages with wire bottoms in an airconditioned room with controls set to maintain 20-22°C, 45-55% relative humidity, and a 12-h light cycle. Standard laboratory guinea pig chow (Ralston Purina Co., Richmond, Indiana) and water were available ad libitum except during exposure (induction or challenge dosing). The animals were quarantined for at least 7 days prior to the study.

Primary Irritation

A range-finding irritation test was conducted with 4 naïve guinea pigs to determine the highest nonirritating concentration of Kathon biocide. Four concentrations were applied in a patch to the closely clipped backs of the guinea pigs. The method of application and the erythema scoring system were the same as described in the assessment of delayed contact dermatitis.

Assessment of Delayed Contact Dermatitis

A modified technique described by Ritz and Buehler [5] was employed to assess delayed contact dermatitis. Nine induction doses, each consisting of 0.4 ml of the appropriate aqueous dilution of Kathon biocide, were applied under cover to the clipped backs of guinea pigs for 3-6 h periods per week for 3 consecutive weeks. The patch was occluded with a rubber "dental dam," and animals were placed in a restrainer during each of the exposures. The application site was washed with water after the exposure. The treated guinea pigs were challenged with 0.4 ml of Kathon biocide by means of an occluded patch, 12-15 days after the last induction dose. The challenge concentrations ranged from 20-2000 ppm a.i. Noninduced naïve guinea pigs were also challenged with Kathon biocide in the same manner and at the same concentrations for comparison to erythema due to primary irritation.

Approximately 24 h after the challenge exposure, the backs of the guinea pigs were depilated with Neet lotion hair remover. Two to five hours after depilation, the guinea pigs were scored for erythema reactions according to the system listed in Table I. Erythema reactions of grade 1 or greater were considered positive responses, indicative of sensitization in animals that received the induction dose.

EC50 (concentration at which delayed contact dermatitis was seen in 50% of the population) values for induction or elicitation of delayed contact dermatitis were estimated by probit analysis as described by Finney [6].

RESULTS

The highest nonirritating concentration for Kathon biocide was 2000 ppm a.i. All concentrations used in the induction or challenge phase of the study were equal to or less than 2000 ppm a.i.

The incidences of delayed contact dermatitis are listed in Table II. No erythema was observed in the noninduced naïve control guinea pigs. The incidence of delayed contact dermatitis was related to the induction concentration; 20/20, 10/10, 9/15, 2/15, and 1/20 guinea pigs induced with 2000, 1000, 500, 100, 50, and 25 ppm a.i., respectively, responded to a challenge concentration of 2000 ppm a.i. Kathon biocide. The incidence of delayed contact dermatitis was also dependent on the elicitation concentration. At an induction concentration of 1000 ppm a.i., 4/5, 3/5, 3/15, and 0/20 guinea pigs responded when challenged with 1000, 500, 200, and 50 ppm a.i., respectively. A "no response concentration" zone which is encompassed by induction and elicitation concentrations of 2000 and 20 ppm, 1000 and 50 ppm, 500 and 100 ppm, 50 and 100 ppm, and 25 and 200 ppm is suggested by the data in Table II. These concentrations are above the recommended final use concentration range of Kathon biocide. The induction/elicitation concentration dependency of delayed contact dermatitis response is illustrated in a 3-dimensional graph (Fig 1) resulting from fitting the sensitization response vs induction or elicitation

<table>
<thead>
<tr>
<th>TABLE I. Erythema scoring system</th>
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<tr>
<td>Erythema reactions</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>No reaction</td>
</tr>
<tr>
<td>Slight patchy erythema</td>
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<tr>
<td>Moderate erythema</td>
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<tr>
<td>Severe erythema</td>
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* Kathon is a Rohm and Haas Company registered trademark. The active ingredients described in this report are commercially available from Rohm and Haas Co. under the names Kathon 886 MW, Kathon WT, Kathon LX, and Kathon CG.
concentrations to a logistic model: \( \log (p/1-p) = A + B_1 \) (induction concentration) + \( B_2 \) (elicitation concentration) + \( B_3 \) (induction concentration) (elicitation concentration), where \( p \) = probability of response, and \( A = -4.069618 \), \( B_1 = 0.000128 \), \( B_2 = 0.000919 \), and \( B_3 = 0.000007 \).

The calculated \( EC_{50} \) for induction in guinea pigs challenged with 2000 ppm a.i. of Kathon biocide was 88 ppm a.i. with 95% confidence limits of 66–145 ppm a.i. and a slope of 3.47 probits/unit log concentration. The calculated \( EC_{50} \) for elicitation in guinea pigs induced with 1000 ppm a.i. of Kathon biocide was 429 ppm a.i. with 95% confidence limits of 272–995 ppm a.i. and a slope of 2.75 probits/unit log concentration.

**DISCUSSION**

The dose-response relationship for a particular toxic response is useful information in assessing the risk of a chemical. Therefore, it is important to conduct sensitization studies with several concentrations of the test substance to evaluate this relationship. Sensitization reactions have been considered by some as lacking a dose-response relationship [7]; however, Marzulli and Maibach [8] have shown a dose-dependent response for the induction of delayed contact dermatitis in humans for several sensitizers including mafenide, benzoic acid, bronopol, \( p \)-phenylenediamine, formalin, and glutaraldehyde. They also demonstrated that the delayed contact dermatitis response with 2-chlorobenzylidene malononitrile and 2-chloroacetophenone was related to the challenge concentration. Our data suggest that the potential of Kathon biocide to cause delayed contact dermatitis is dependent on both the induction concentration and the challenge concentration. These data support the conclusion reported by Marzulli and Maibach [8]. Although the mechanism of the dependency of delayed contact dermatitis on the induction concentration may be different from that of the challenge concentration, the clinical significance is the same, i.e., the manifestation of a sensitization reaction.

Therefore, both concentrations should be taken into consideration in any risk assessment.

Beuthe† reported that no incidence of delayed contact dermatitis was observed in Kathon biocide-treated guinea pigs even at induction and challenge concentrations as high as 1500 ppm a.i. when only 3 induction doses (1 dose per week for 3 weeks) were employed. In our study, however, 9 induction doses at lower concentrations (e.g., 1000 ppm a.i., 3 doses per week for 3 weeks) resulted in sensitization in guinea pigs. These data demonstrate that by topical application in guinea pigs, the number of induction doses may be an important factor in demonstrating the sensitization characteristic of a chemical.

On the basis of these results, there is an induction/elicitation concentration dependency for delayed contact dermatitis response for Kathon biocide, and there is a “no response concentration” zone where the biocide can be used without concern for clinically significant delayed contact dermatitis.

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**REFERENCES**