Acta Derm Venereol (Stockh) 1985; 65: 472–478

Induction of Formaldehyde Contact Sensitivity: Dose Response Relationship in the Guinea Pig Maximization Test

KLAUS E. ANDERSEN,[1] ANDERS BOMAN,[2] AAGE VØLUND,[3] and JAN E. WAHLBERG[2]

[1]*Department of Dermatology, Gentofte Hospital, Hellerup, Denmark,*
[2]*Department of Occupational Dermatology, National Board of Occupational Safety and Health and Karolinska Hospital, Stockholm, Sweden, and* [3]*Novo Research Institute, Bagsværd, Denmark*

Andersen KE, Boman A, Vølund A, Wahlberg JE. Induction of formaldehyde contact sensitivity: Dose response relationship in the guinea pig maximization test. Acta Derm Venereol (Stockh) 1985; 65: 472–478.

The sensitizing potential of aqueous formaldehyde was evaluated with the guinea pig maximization test (GPMT) in two laboratories (Copenhagen and Stockholm) using different guinea pig strains. Six intradermal (0.01%–3%), and 6 topical (0.5%–20%) concentrations were used for induction, and formaldehyde 1% and 0.1% was used for challenge. The incidence of contact sensitivity depended on the intradermal, but not on the topical induction dose. Statistical analyses showed a non-monotonous (non-linear) dose response relationship. The estimated maximal sensitization rate in Copenhagen was 80% after intradermal induction with 0.65% formaldehyde; in Stockholm it was 84% after induction with 0.34%. The data from the two laboratories could be described by parallel displaced dose response curves suggesting that the guinea pig strain used in Stockholm was significantly more susceptible to formaldehyde than the strain used in Copenhagen. The EC_{50} (formaldehyde concentration at which 50% of the guinea pigs were sensitized) at the 72 h scoring and a 1% challenge concentration, was 0.061% in Copenhagen and 0.024% in Stockholm. *Key words: Allergic contact dermatitis; Predictive test.* (Received March 14, 1985.)

K. E. Andersen, Dermatology Clinic, Algade 33, DK-4000 Roskilde, Denmark.

Formaldehyde is a ubiquitous and potent sensitizer. About 5% of the cosmetic formulations registered with the Food and Drug Administration contain formaldehyde (1). Exposure may occur inadvertently as about 80 trade names and synonyms are used in the marketing of formaldehyde releasing compounds (2).

Diagnostic patch tests carried out at St John's Hospital, London, showed that the incidence of positive reactions to formaldehyde 2% in water varied from 0.7 to 1.4% from 1971 to 1975 (3). Guinea pig allergy tests with formaldehyde have shown different sensitization rates, probably due to the use of different guinea pig strains, and test procedures (4–7).

Guinea pig maximization tests (GPMT) with formaldehyde sensitized 50% of the guinea pigs in Copenhagen and 95% in Stockholm, in spite of a higher formaldehyde concentration used for the intradermal induction in Copenhagen (4). This difference led us to design a comparative dose response study of formaldehyde sensitization in the two laboratories using the same test (GPMT), and the same batch of formaldehyde.

Presented in part at the 5th Workshop of the European Research Group on Experimental Contact Dermatitis, Strasbourg, November 8–9, 1984.

MATERIALS AND METHODS

Animals

Outbred albino female guinea pigs weighing between 350 to 450 g at receipt were housed in plastic cages. The animals in Copenhagen were Ssc:AI. from Statens Seruminstitut (Copenhagen, Denmark); Dunkin-Hartley strain guinea pigs in Stockholm were obtained form J. A. Sahlin (Malmö, Sweden). The animals were kept on a 12-h photoperiod at a room temperature of 20–30°C, a relative humidity of 45–75% with food and water available *ad libitum* (in Copenhagen: rabbit pellets Superfos Korn, Denmark and water with vitamin C added; in Stockholm: Ewos Avels-tillväxtfoder, Ewos, Södertälje, Sweden). As bedding was used Hahnflock H-9 (Hahn & Co., Bredenbek, Kronsburg, West Germany) and Torrax (ALAB Laboratorietjänst, Sollentuna, Sweden) in Copenhagen and Stockholm, respectively. The animals were allowed to adapt for one week before testing. Hair was removed by clipping and shaving.

Chemicals

The formaldehyde dilutions were prepared in Copenhagen from a 20% (w/v) aqueous formaldehyde solution (Ph. Nord. 63) and distributed to Stockholm. The content in the test preparations was determined using the fluorometric method of Wilson (8).

Freund's complete adjuvant (FCA), Difco Laboratories, Detroit, Michigan, USA.

Guinea pig maximization test

The procedure described by Magnusson & Kligman (9) was followed. This includes intradermal induction with FCA on day 0 and topical induction on day 7 followed by challenge patch tests on day 21.

Eighteen groups of 6 animals and 12 sham-treated controls were used in each laboratory. Filter paper mounted on Leukoflex® (Beiersdorf AG) or Blenderm® (3M) was used for the topical induction; Finn Chambers (Epitest Ltd) on Scanpor® (Norgesplaster AS) were used for the challenge. Induction concentrations ranged from 0.01% to 3% intradermally, and from 0.5% to 20% topically. The challenge concentrations were 1% and 0.1%. The challenge reactions were read blindly after 48 h and 72 h. The following grading scale was used: 0 = no visible reaction; 1 = discrete or patchy erythema; 2 = moderate and confluent erythema; 3 = intense erythema and swelling (9). A grade 1 reaction was not regarded as a positive challenge. The number of sensitized animals (grade 2 or 3) in each group was used in the statistical analysis.

Statistical methods

The 4-parameter logistic dose response model (10), widely applied to describe binary biological responses as function of dose x, is given by: $P(x)=p_0+(p_1-p_0)/(1+\exp(a+b\log x))$, where $P(x)$ is the response probability, p_0 the background and p_1 the maximal response probability, respectively. The a and b parameters determine the shape and position of the sigmoid response curve as it varies from p_0 to p_1 when the dose x varies from zero to infinity. This model has been generalized to describe non-monotonous dose response relations in several dose variables. For that purpose the exponent $(a+b\log x)$ is replaced by a second degree polynominal in more than one log-dose variable. With the two variables representing intradermal (x_1) and topical concentrations (x_2) the polynomial is: $(a+b_1\log x_1+b_2\log x_2+c_1(\log x_1)^2+c_2(\log x_2)^2+d(\log x_1)\log x_2)$. When this polynomial is used, it may not be necessary to include a maximal response probability parameter p_1, since the polynomial itself can define a maximal response probability. Therefore, without any appreciable loss of generality, p_1 is replaced by 1. This model was fitted to the data shown in Table I and the corresponding 48 h readings by means of the iterative (Newton-Raphson) maximum likelihood estimation method, and the goodness of fit was evaluated by the asymptotic likelihood ratio test (10).

RESULTS

Table I shows the results for each group. Among the control animals 1 in Copenhagen and 2 in Stockholm were positive at the 48 h reading, possibly of irritant nature.

Statistical analyses where d, c_1, c_2, b_1 and b_2 successively were put equal to zero found that besides the constant a only b_1 and c_1, representing the non-monotonous dependence of the response on the intradermal concentration, were needed in the model. This suggested that the topical dose had no significant effects. The subsequent comparisons between the 2 laboratories were then carried out on the combined results at each intradermal dose

Table I. *Guinea pig maximization tests*

The frequency of sensitized animals in each group after challenge with formaldehyde 1% in water. The 72 h readings are shown

Induction Concentration (%v/v)		Challenge result Number pos./number tested guinea pig strain	
Intradermal Day 0	Topical Day 7	Ssc:AL	Dunkin-Hartley
0.01	0.5	0/6	2/6
0.01	2.0	0/6	1/6
0.01	10.0	0/6	2/6
0.03	1.0	2/6	5/6
0.03	5.0	1/6	4/6
0.03	20.0	2/6	2/6
0.1	0.5	4/6	6/6
0.1	2.0	4/6	3/6
0.1	10.0	4/6	5/6
0.3	1.0	4/6	5/6
0.3	5.0	6/6	4/6
0.3	20.0	4/6	6/6
1.0	0.5	4/6	6/6
1.0	2.0	4/6	5/6
1.0	10.0	5/6	4/6
3.0	1.0	5/6	3/6
3.0	5.0	3/6	5/6
3.0	20.0	4/6	4/6
Controls		0/12	1/12

level. Parallel dose response relations were found using the likelihood methods. The responses from the Dunkin-Hartley guinea pigs were displaced relative to the results with the Ssc:AL guinea pigs along the log dose axis or along the response axis. The best fit was obtained by a combination of both kinds of displacements. This gave a significantly better fit than using the same model for both data sets ($p=0.05$ and $p<0.05$ for 48 h and 72 h readings, respectively) suggesting that the Dunkin-Hartley strain was significantly more sensitive to formaldehyde than the strain from Statens Seruminstitut, Copenhagen.

Fig. 1 shows the observed response rates and the best fitting curves for the 72 h data. The maximal response rates and corresponding formaldehyde induction concentrations, half the maximal concentration (which can sensitize 50% of the maximal number of animals susceptible to this allergen), and EC_{50} (concentration at which contact sensitivity was seen in 50% of the population) were calculated from the fitted models. The results are in Table II and illustrated in Fig. 1.

The 1% challenge reactions at 48 h (not shown) were slightly higher than those at 72 h. Similar non-monotonous logistic curves could be fitted to the 48 h data, and the calculations based hereof are in Table II.

The number of positive animals following the 0.1% challenge were too small to allow a reliable dose response analysis.

Later control analysis of the formaldehyde solutions used for induction showed that one of two nominal 2% solutions used in Stockholm only contained 0.4% formaldehyde. This error could also have affected the 1% FCA/formaldehyde mixture used for intradermal induction. Repeated dose response analyses after omission of the dubious data at 2%

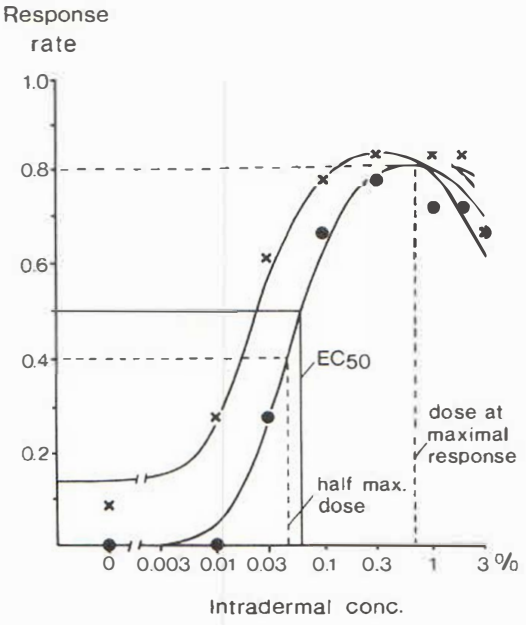

Fig. 1. Formaldehyde sensitivity in the guinea pig maximization test. Response rates (72 h) and best fitting logistic curves (●–●, Copenhagen; ×–×, Stockholm). The maximal response, the concentration at the half maximal response and the EC_{50} for the Copenhagen results are shown. The fitted curves are given by the equations: $P(x) = 1/(1+\exp(-1.42+0.225\ \log x+0.262(\log x)^2))$ for the Copenhagen data and for the Stockholm data. $P(x) = 0.14+0.86/(1+\exp(-1.42+0.225(0.657+\log x)+0.262(0.657+\log x)^2))$.

topical and 1% intradermal concentrations showed essentially unchanged results. The conclusions are thus independent of the above mentioned reservations.

DISCUSSION

The non-monotonous dose response relationship found in this study fits with the concept that the level of contact sensitivity is determined by a balance between activated effector and suppressor cells, and the balance is influenced by the dose given (11). This pattern of response has been reported for dinitro-chlorobenzene (DNCB) (12), sultones (13), p-nitrobenzyl compounds (14), and partially for chlorocresol (15).

Table II. *Guinea pig maximization tests*

Results of 1% formaldehyde challenge based on the best fitting response curves

	Copenhagen Ssc:AL		Stockholm Dunkin-Hartley	
	48 h (%)	72 h (%)	48 h (%)	72 h (%)
Maximal response rate[a]	81	80	86	84
Intradermal conc. at maximal response	0.46	0.65	0.45	0.34
Half max. conc[b]	0.023	0.046	0.015	0.019
EC_{50}[c]	0.032	0.061	0.017	0.024

[a] Percentage of positive animals.
[b] Half. max. concentration is the formaldehyde conc. which can sensitize 50% of the maximal number of guinea pigs susceptible to this allergen.
[c] EC_{50} is the formaldehyde conc. which can sensitize 50% of the test animals.

The 1% formaldehyde previously used for intradermal induction in Copenhagen may have produced an excess activation of suppressor cells, whereas the 0.1% concentration used in Stockholm may have been closer to the dose giving maximal contact sensitivity (4). Marzulli & Maguire (7) reported an even lower formaldehyde sensitization rate (5 of 28 animals), when they used a 5% (v/v) formalin concentration (2% formaldehyde) in saline for induction in the GPMT. Magnusson & Kligman (9) studied the effect of dose and FCA separately. This may explain why they did not observe the "overload" phenomenon.

The "overload" effect seems not to be limited to the use of FCA in the sensitization procedure. However, the qualitative aspects of contact sensitivity are changed by the use of FCA (16). From a physico-chemical evaluation of sultones and p-nitrobenzyl compounds as allergens, the "overload" effect was not related to the use of FCA (17). Lagrange et al. (18) found an optimal dose of sheep red blood cells (SRBC) which in the absence of an adjuvant, produced maximum delayed-type hypersensitivity in mice. Increasing the dose of SRBC reduced the reactivity. The cell-mediated immune response in mice sensitized to 2,4-dinitro-1-fluorobenzene (DNFB) showed maximum sensitivity after a total induction dose of 0.25 mg DNFB. A further dose increase yielded a progressive decline in contact sensitivity (19). On the contrary dose response studies of formaldehyde and Kathon® biocide sensitivity in guinea pigs without the use of FCA showed no "overload" effect. Increased doses resulted in higher response rates (20, 21). Topical application were used for induction in both studies.

The parallel displacement of the dose response curves in the two laboratories (Fig. 1) suggests that the Swedish Dunkin-Hartley strain was more sensitive to lower doses of formaldehyde than the Ssc:AL strain. This is in agreement with our previous data (4) and with the recognition of genetic factors as important parameters in guinea pig allergy tests (22). Dose response studies using moderately potent allergens may provide an appropriate measure (EC_{50}) to classify the sensitivity of a guinea pig strain.

A difference in reading between the two laboratories cannot be excluded. However, the inter-laboratory reading variation is presumably small due to frequent cooperation between the authors (4). Further tests involving exchange of animals are desirable.

The lack of significance of the topical induction for the formaldehyde sensitization rate indicates that the allergy test may be simplified as suggested by Goodwin et al. (23), who developed the single injection adjuvant technique (SIAT). This differs from the GPMT by omission of topical induction. Using 19 human contact sensitizers, they found the SIAT sufficiently similar in sensitivity to that of the GPMT. They recommended the use of the SIAT due to its practical advantages and shorter test period. However, for other allergens the topical induction patch test may be important for sensitization, i.e. for chlorocresol (15).

The present results support the comments by Roberts & Williams (17), where they recommend that "a true maximization test would have to involve finding the induction dose which gives a maximum score by means of a series of tests". The choice for a substance of one moderately irritating concentration for induction may be fortuitous in relation to the concentration giving maximum score. It may improve the value of guinea pig tests if a dose response relationship is built into the procedure as in the open epicutaneous test (24). This is possible without essentially increasing the number of animals employed in a single test. Regarding statistical evaluation, the reduced number of animals in each group is balanced by the increased number of groups. Although the differences observed are small, the techniques of analysis are of considerable significance for this assay. Before assuming that the non-monotonous type of response in allergic contact dermatitis is a general biologic principle, additional compounds must also be so studied.

ACKNOWLEDGEMENTS

We appreciate the technical asisstance of Tove Schneider, Kenneth Krogh, Eva Tiedemann, Gunnel Hagelthorn, Einar Kartus, Rolf Stridde and the staff at the animal facility, the Panum Institute, Faculty of Medicine, University of Copenhagen, and the National Board of Occupational Safety and Health, Solna, Sweden.

The study was supported by the Danish Medical Research Council, the Danish National Agency for Environmental Protection, Liv Bryhn's Fund, and the Swedish Work Health Fund.

REFERENCES

1. Maibach HI, Akerson JM, Marzulli FN, Wenninger J, Greif M, Hjorth N, Andersen KE, Wilkinson DS. Test concentrations and vehicles for dermatological testing of cosmetic ingredients. Contact Dermatitis 1980; 6: 369–404.
2. Fiedler HP. Formaldehyd-Abspalter. Dermatosen 1983; 31: 187–189.
3. Cronin E. Contact dermatitis. Edinburgh: Churchill Livingstone, 1980; 789.
4. Andersen KE, Boman A, Hamann K, Wahlberg JE. Guinea pig maximization test with formaldehyde releasers. Results from two laboratories. Contact Dermatitis 1984; 10: 257–266.
5. Guillot JP, Gonnet JF, Clement C, Faccini JM. Comparative study of methods chosen by the Association française de normalisation (AFNOR) for evaluating sensitizing potential in the albino guinea pig. Fd Chem Toxicol 1983; 21: 795–805.
6. Maibach HI. Formaldehyde: effects on animal and human skin. In: Gibson JE, ed. Formaldehyde toxicity. New York: Hemisphere, 1983: 166–174.
7. Marzulli FN, Maguire HC. Validation of guinea pig tests for skin hypersensitivity. In: Maibach HI, Marzulli FN, eds. Dermatotoxicology. 2nd ed. Washington DC: Hemisphere, 1983: 237–250.
8. Wilson CH. Fluorometric determination of formaldehyde in cosmetic products. J Soc Cosmet Chem 1974; 25: 67–71.
9. Magnusson B, Kligman AM. Allergic contact dermatitis in the guinea pig. Springfield, Ill.: Charles C. Thomas, 1970.
10. Finney DJ. Statistical method in biological assay. 3rd ed. London: Charles Griffin, 1978.
11. Macher E, Chase MW. Studies on the sensitization of animals with simple chemical compounds. XII. The influence of excision of allergenic depots on onset of delayed hypersensitivity and tolerance. J Exp Med 169; 129: 103–121.
12. Buehler EV, Griffith JF. Experimental skin sensitization in the guinea pig and man. In: Animal models in dermatology. Maibach HI, ed. Edinburgh: Churchill Livingstone, 1975: 56–66.
13. Ritz HL, Connor DS, Sauter ED. Contact sensitization of guinea pigs with unsaturated and halogenated sultones. Contact Dermatitis 1975; 1: 349–358.
14. Roberts DW, Goodwin BJF, Williams DL, Jones K, Johnson AW, Alderson JCE. Correlations between skin sensitization potential and chemical reactivity for p-nitrobenzyl compounds. Fd Chem Toxicol 1983; 21: 811–813.
15. Andersen KE, Hamann K. How sensitizing is chlorocresol? Allergy tests in guinea pigs versus the clinical experience. Contact Dermatitis 1984; 11: 11–20.
16. Polak L, Scheper RJ. In vitro DNA synthesis in lymphocytes from guinea pigs epicutaneously sensitized with DNCB. J Invest Dermatol 1981; 76: 133–136.
17. Roberts DW, Williams DL. The derivation of quantitative correlations between skin sensitization and physico-chemical parameters for alkylating agents, and their application to experimental data for sultones. J Theor Biol 1982; 99: 807–825.
18. Lagrange PN, Machaness GB, Miller TE. Influence of dose and route of antigen injection on the immunologic induction of T-cells. J Exp Med 1974; 139: 528–542.
19. Sy MS, Miller SD, Claman HN. Immune suppression with supraoptimal doses of antigen in contact sensitivity. I. Demonstration of suppressor cells and their sensitivity to cyclophosphamide. J Immunol 1977; 119: 240–244.
20. Lee HK, Alarie Y, Karol MH. Induction of formaldehyde sensitivity in guinea pigs. Toxicol Appl Pharmacol 1984; 75: 147–155.
21. Chan PK, Baldwin RC, Parsons RD, Moss JN, Stiratelli R, Smith JM, Hayes AW. Kathon biocide: manifestation of delayed contact dermatitis in guinea pigs is dependent on the concentration for induction and challenge. J Invest Dermatol 1983; 81: 409–411.
22. Parker D, Sommer G, Turk JL. Variation in guinea pig responsiveness. Cell Immunol 1975; 18: 233–238.

23. Goodwin BFJ, Crewel RWR, Johnson AW. A comparison of three guinea pig sensitization procedures for the detection of 19 reported human contact sensitizers. Contact Dermatitis 1981; 7: 248–258.
24. Klecak G. Identification of contact allergens: predictive tests in animals. In: Marzulli FN, Maibach HI, eds., Dermatotoxicology. 2nd, ed. Washington DC: Hemisphere 1983: 193–236.