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Contact allergy to chlorocresol, formaldehyde and other biocides

Guinea Pig Tests and clinical studies

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København s universitet, den 30. april 1986
L. Klinken
dekan

This thesis has together with the following previously published papers been accepted by the Medical Faculty of the University of Copenhagen to be defended in public for the medical doctorate.

Copenhagen, April 30, 1986
L. Klinken
Dean

The present survey is based on the following papers which will be referred to by their preceding roman numerals:

I. Andersen KE, Hamann K. The sensitizing potential of metalworking fluid biocides (phenolic and thiazole compounds) in the guinea pig maximization test in relation to patch test reactivity in eczema patients. Fd Chem Toxic 1984; 22: 655-60.

II. Andersen KE, Hamann K. How sensitizing is chlorocresol? Allergy tests in guinea pigs versus the clinical experience. Contact Dermatitis 1984; 11: 11-20.

III. Andersen KE, Hamann K. Is cytox 3522 (10% methylene-bis-thiocyanate) a human skin sensitizer? Contact Dermatitis 1983; 9: 186-90.

IV. Andersen KE, Boman A, Hamann K, Wahlberg JE. Guinea pig maximization tests with formaldehyde releasers. Results from two laboratories. Contact Dermatitis 1984; 10: 257-66.

V. Andersen KE, Boman A, Vølund Aa, Wahlberg JE. Induction of formaldehyde contact sensitivity: dose-response relationship in the guinea pig maximization test. Acta Derm Venereol 1985; 65: 472-8.

VI. Andersen KE. Sensitivity and subsequent »down regulation« of sensitivity induced by chlorocresol in guinea pigs. Arch Dermatol Res 1985; 277: 84-7.

VII. Andersen KE, Staberg B. Quantitation of contact allergy in guinea pigs by measuring changes in skin blood flow and skin fold thickness. Acta Derm Venereol 1985; 65: 37-42.

VIII. Andersen KE. Guinea pig maximization test: effect of type of Freund's complete adjuvant emulsion and of challenge site location. Derm Beruf Umwelt 1985; 33: 132-6.

IX. Andersen KE, Carlsen L, Egsgaard H, Larsen E. Contact sensitivity and bioavailability of chlorocresol. Contact Dermatitis 1985; 13: 246-51.

PREFACE

The present survey is based on work carried out from 1980 to 1984 at the Department of Dermatology, Gentofte Hospital, Hellerup.

Professor *Niels Hjorth,* head of the Department of Dermatology, inspired me to work on the subject of allergic contact dermatitis and predictive allergy testing. He introduced me to *Professor Howard I. Maibach,* Department of Dermatology, University of California Medical Center, San Francisco, who showed me considerable hospitality and taught me the animal models required for the study. Due to the support from Niels Hjorth and Howard Maibach I was able to hold a one-year research fellowship in San Francisco, where I enjoyed and profited from the inspiring and stimulating research environment provided by *Howard Maibach.* The continuous support and constructive criticism which I received from *Niels Hjorth* and *Howard Maibach* is gratefully acknowledged.

In San Francisco I had the opportunity to meet Professor *Jan E. Wahlberg,* Department of Occupational Dermatology, Karolinska Hospital, Stockholm, Sweden. Since then he has shown interest in my work and without his experience, active participation in some studies, and never failing willingness to offer in depth criticism this thesis would have been more difficult to write.

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Good animal care and technical assistance is of fundamental importance for this type of study. I express my thanks for excellent help to the staff at the Animal Department of the Panum Institute, Faculty of Medicine, University of Copenhagen and the Department of Microbiology, Gentofte Hospital, Hellerup. The expertice guidance of chief veterinary surgeon *Hans J. Skovgaard,* chief animal technician *Rasmus Bredahl,* senior animal technician *Jan Thomassen,* veterinary surgeons *Tove Schneider,* and *Kenneth Krogh* is acknowledged.

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Throughout the study staff nurse *Ella Ploug* and the personnel at the Allergy Laboratory of the Department of Dermatology, Gentofte Hospital, provided invaluable help. They applied and read the patch tests in humans and also manufactured patch test materials for the guinea pig tests. My medical colleagues at the Department of Dermatology, *Jytte Roed-Petersen, Torkil Menné,* and *Ole Clemmensen* contributed valuable discussions and advice.

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Finally I want to thank my family and friends for their patience and support. The burden was not on me, who enjoyed the work, but on my wife *Anne Marie* and our children *Mads, Marie* and *Morten.*

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CONTENTS

INTRODUCTION 5

GUINEA PIG SENSITIZATION ASSAYS 5

WHICH TEST SHOULD BE USED? 5

BIOCIDES 5

AIM OF STUDY 8

GUINEA PIG SENSITIZATION ASSAYS USED IN AUTHOR's STUDIES:
- Guinea pig maximization test 9
- Open epicutaneous test 9
- Cumulative contact enhancement test 10

CLINICAL BIOCIDE ALLERGY AMONG WORKERS AND CONSUMERS RELATED TO THE RESULTS OF GUINEA PIG SENSITIZATION ASSAYS:
- Metal working fluid dermatitis 10
- Results with phenolic compounds 10
- Results with Cytox 3522 11
- Results with formaldehyde and formaldehyde releasers 11

INTERLABORATORY VARIATIONS WITH FORMALDEHYDE AND FORMALDEHYDE RELEASERS 12

FORMALDEHYDE DOSE-RESPONSE RELATIONSHIP 12

SUCCESSIVE CHALLENGES WITH CHLOROCRESOL 13

READING OF TEST REACTIONS 13

OTHER SOURCES OF RESULT VARIATIONS 14
- Patch test technique 14
- Adjuvant emulsion 15
- Effect of vehicle 15

KEY FACTORS IN INTERPRETATION 16

WHAT TO TEST AND NOT TO TEST? 17

CONCLUSIONS 17

SUMMARY IN DANISH 18

REFERENCES 19

INTRODUCTION

New chemicals and products that may come into contact with the skin are introduced daily. Evaluation of allergenicity is needed to reduce the risk of sensitization from occupational and consumer's exposure. The frequency of contact allergy appears from epidemiological investigations. The various estimates differ, however, partly due to variations in methodology. Skin diseases made up about half of the reported industrial diseases in the United States, and contact dermatitis was the most common diagnosis comprising 32% of dermatological occupational diseases in New York (*Johnson et al,* 1979). A nationwide Danish study of permanent disability from skin diseases, proved eczema to be the most frequent cause, affecting 53% of 564 cases. Seventy percent of these were due to nickel and chromate allergy (*Mennè and Bachmann,* 1979). In Denmark, 7% of patients seeking outpatient dermatological care had contact dermatitis (*Christoffersen,* 1984), many of the allergic type. Topical drugs and cosmetics are frequent causes of allergic contact dermatitis (*Andersen and Maibach,* 1983). However, dermatological studies may underestimate the frequency of adverse reactions to cosmetics, mostly because many consumers solve their skin problems themselves by ceasing the use of a suspected product or by other trial-and-error methods (*Eiermann et al,* 1982).

GUINEA PIG SENSITIZATION ASSAYS

Guinea pig sensitization assays are used to assess the sensitizing *potential* of a chemical or a product. The effort is stimulated by a desire to reduce the hazard of exposure to the substances. Economic considerations and an increasing demand for safety tests raised by consumer organizations, unions and governmental agencies, act to increase the need for sensitization assays, as part of the toxicity profile of a chemical or a product.

Sensitization assays started with *Landsteiner and Jacobs* (1935), *Draize* (1959) and *Buehler* (1965). *Magnusson and Kligman* (1969), *Maguire and Chase* (1972), *Maurer et al* (1975) and *Klecak et al* (1977) have contributed to the development of sensitization assays in guinea pigs, the laboratory animal species of choice. Mouse models have been developed, but their predictive value has not yet been established (*Möller,* 1984; *Miller,* 1984; *Stadler and Karol,* 1985).

Several guinea pig assays are available. A schematic presentation of 14 different methods is shown in chronological order in Fig. 1. They have certain features in common: an induction phase, followed by a resting period of about two weeks, and a subsequent challenge phase to prove whether or not sensitization occurred. The technical variations of the methods have been introduced to increase their sensitivity and to make them applicable for testing of different substances. Each test employs 10 to 40 animals, divided into test groups and a control group (*Andersen and Maibach,* 1985).

Guidelines for testing the skin sensitization potential of chemicals in laboratory animals have been harmonized internationally and agreed among the 24 OECD countries (OECD, 1981).

Contact sensitivity in laboratory animals seems to be genetically controlled. By selective breeding (*Chase,* 1952/1953; *Polak et al,* 1968), guinea pig strains have been developed that are refractory or less susceptible to sensitization with some chemicals, and not to others.

WHICH TEST SHOULD BE USED?

There is no simple answer to this question. More studies must be performed, comparing a wide range of chemicals, to establish the efficacy of the different assays in determining the contact sensitization potential and the hazard for humans. Most authors found their own method comparable to other test methods, when examining a range of chemicals (*Fahr et al,* 1976; *Turk and Parker,* 1977; *Maurer et al,* 1978, 1980; *Kero and Hannuksela,* 1980; *Goodwin et al,* 1981; *Guillot et al,* 1983; *Klecak,* 1983; *Marzulli and Maguire,* 1983). Table 1 shows selected comparative test results from the literature. In general, the assays applying Freund's complete adjuvant (FCA) as an immune enhancer are more sensitive than the non-adjuvant methods.

Most guinea pig sensitization assays are performed by industry in contract research laboratories. The price of a sensitization test performed with a single substance varies according to the choice of technique and the number of animals involved. Often 40 animals are used, 20 in the test group and 20 in the control group. As an example, the August-1984 prices for a guinea pig maximization test performed at Scantox Biologisk Laboratorium, DK-4623, Ll. Skensved, Denmark, and Huntingdon Research Center, Huntingdon, England were 15.500 dkr and 16.800 dkr (about 1500 and 1600 $), respectively (*Kallesen,* personal communication, 1984).

BIOCIDES

Biocides, chemicals capable of destroying living organisms, are used as preservatives for aqueous and emulsion systems in industry, household products, cosmetics and topical drugs. The use concentration in the finished products is generally in the range of 0.01%-1%. Owing to their inherent reactivity, many of these chemicals can induce allergic contact dermatitis. An overwhelming number of biocide formulations are marketed under various trade names and synonyms. There is no single source of information regarding the production, import and use of the different biocides in Denmark. This information must be obtained from producers, agents, factories, and governmental registers.

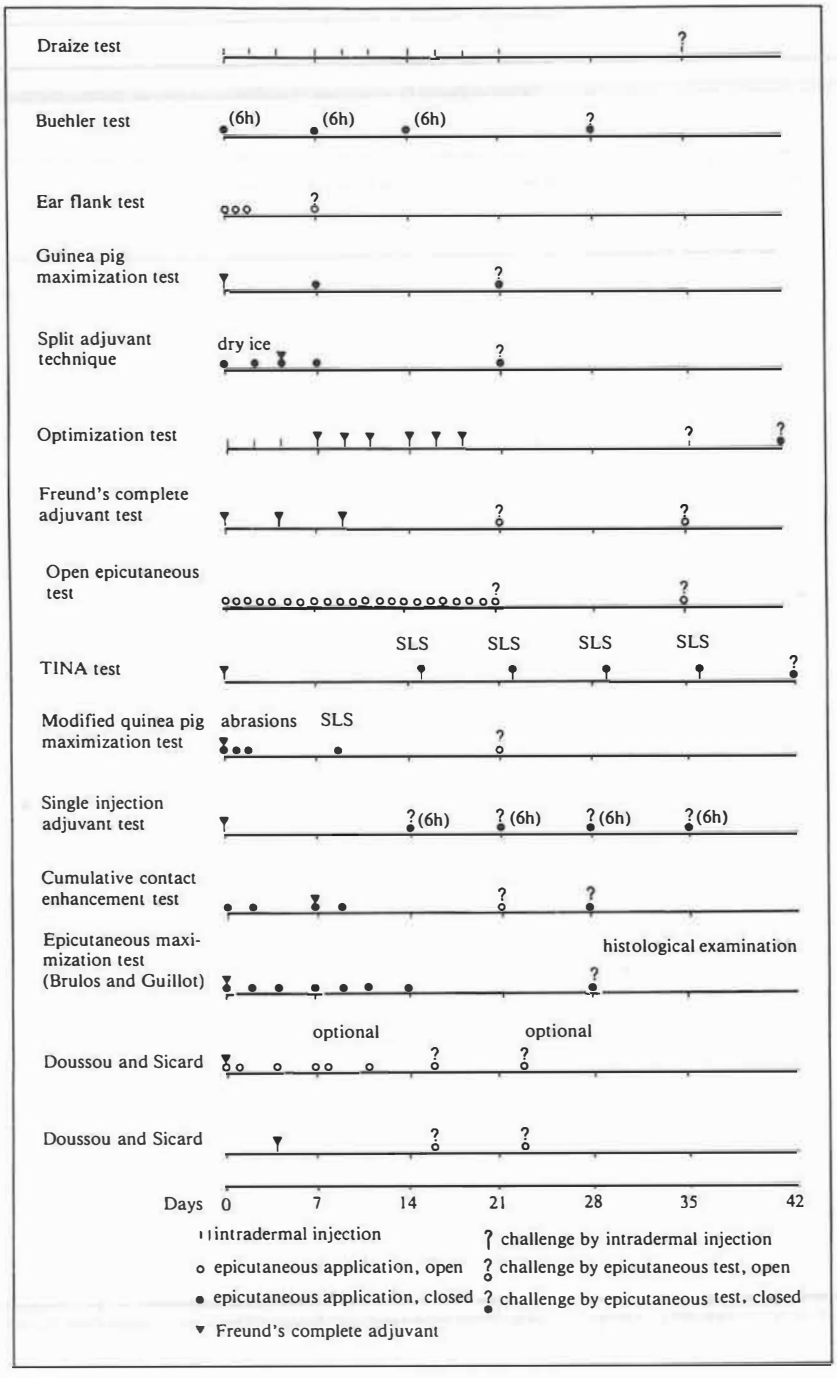


Fig. 1. *For each guinea pig assay the time schedule for induction and challenge is given. The question mark(?) indicates the challenge procedure which determines whether or not the induction treatment succeeded in sensitizing the animal.*

The basis for the present study was metal-working fluid biocides, because these have been incriminated as a frequent cause of occupational allergic contact dermatitis among machinists (*Andersen and Hamann,* 1984a; *Andersen et al,* 1984a)(I & II). Many of them occur also in other water-based industrial, household, medical and cosmetic products. Thus, evaluation of the allergenicity of these biocides is relevant to a wide range of consumers.

The biocides included were selected from metal-working fluids on the Danish market and are listed in Table 2. Only Preventol CMK (chlorocresol) was tested for chemical composition and purity, and for the other biocides information was obtained from the

Table 1. *Comparative results of sensitization assays in guinea pigs*). The percentage of guinea pigs sensitized is shown.*

Test	Chemical					
	Formaldehyde	Benzocaine	Pencillin G	Dihydro-coumarin	Nickel sulphate	MBT**)
Modified Draize test[1, 3]	10%[1], 33%[3]	0%			0%[1]	0%[1]
Optimization test[2]	10-100%	0-90%	33-100%	100%		
Freund's complete adjuvant test[2]	30%	40-50%	67-100%	100%		
Single injection adjuvant test[1]	100%	0%			20%	0%
Buehler test[3]	0%					
Open epicutaneous test[2]	0-38%	38-63%	0-50%	88%		
Epicutaneous maximization test[2]	35-60%	10-45%	50-95%	100%		
Split adjuvant test[2, 3]	0-70%[2], 7%[3]	40-60%[2]	80-100%[2]	100%[2]		
Guinea pig maximization test[1, 2, 3]	100%[1, 2], 18%[3]	0%[1], 60-85%[2]	90-100%[2]	100%	10%	60%
Doussou and Sicard test[2]	0-50%	0%	0-17%	33-75%		

*) Data compiled from: 1) Goodwin et al, 1981. 2) Guillot et al, 1983. 3) Marzulli and Maguire, 1982
For details on techniques and choice of concentration, see references.
**) MBT = mercaptobenzothiazole

Table 2. *Biocides tested for sensitizing potential with the guinea pig maximization test.*

Biocide and supplier	Composition	Field of application
Preventol O extra (Bayer, Leverkusen, FRG)	o-phenylphenol >99.5%	Soluble oils, textiles, glues, detergents, paints, cosmetics, citrus fruits
Preventol ON extra (Bayer)	Sodium 2-phenylphenolate 61.5% Preventol O extra	Soluble oils, textiles, glues, detergents, paper, cosmetics
Preventol L (Bayer)	Mixture of phenols, contains pentachloro-phenol and chlorocresol	Soluble oils, tanning agents, glues
Preventol CMK (Bayer)	Chlorocresol (4-chloro-3-methylphenol) >99.5%	Soluble oils, textiles, paper, detergents, glues, topical drugs
Preventol GD (Bayer)	2,2'-methylenebis-(4-chlorophenol)	Soluble oils, textiles, cosmetics and toiletries
Proxel XL (ICI, Manchester, England)	1,2-benzisothiazolin-3-one, 20% in aqueous propylene glyol	Soluble oils
Proxel HL (ICI)	1,2-benzisothiazolin-3-one, 30% in a mixture of morpholine di- and triethanolamine	Soluble oils, paints, resins
Cytox 3522 (American Cyanamid, Wayne, N.J., U.S.A.)	Methylene-bis-thiocyanate 10% in solvent	Soluble oils, paper, cooling water
Preventol D2 (Bayer)	Hydroxymethylene and polyhydroxymethylene monobenzylether	Soluble oils, textiles, paper, glues, detergents, paints
Forcide 78 (Progiven, Aubervielliers, France).	1,2,5-tris(hydroxyethyl) hexahydrotriazine and 1,3,5-tris(ethyl)hexa-hydrotriazine, 1:1	Soluble oils
Grotan BK (Schülke & Mayr, Norderstedt, FRG)	1,3,5-tris(hydroxyethyl) hexahydrotriazine	Soluble oils, cosmetics
KM 200 (Riedel de Haen, Seelze, FRG)	1,3,5-tris(hydroxyethyl) hexahydrotriazine	Soluble oils
Grotan OX (Schülke & Mayr)	N,N-methylene-bis-5-methyloxazolidine	Soluble oils
Germall 115 (Chemag, Frankfurt, FRG)	Imidazolidinylurea	Drugs and cosmetics

suppliers. We tested the products in the purity in which they were marketed. Preventol CMK (chlorocresol) contained less than 0.1% 3-methylphenol as measured by high-performance liquid chromatography and gas chromatography-mass spectrometry (The Danish State Chemical Supervision Service).

The consumption of metal-working fluids in Denmark is not elucidated in detail. At least 54 companies are registered as manufacturers or distributors of metal-working fluids; they are estimated to represent more than 90% of the Danish market. About 52% of the registered products contain at least 15 different biocides. By December 1983 232 products were registered and the innovation is expected to be 10% per year (Arbejdstilsynet, Danish Labour Inspection Service, 1984).

When the yearly consumption of biocides is calculated based on the registered information about new products, the following figures appear: o-phenylphenol 0.5 tons, chlorocresol 1.6 tons, 1,2-benzisothiazolin-3-one 0.1 tons, 1,3,5-hexahydrotriazine 0.3 tons (personal communication, Produktregistret, Arbejdsmiljøinstitutet (Danish National Institute of Occupational Health, Register of Substances and Materials, Copenhagen, 1985)). These are minimum figures and they give no information about the number of individuals exposed.

The estimated consumption of phenolic compounds in metalworking fluids in Denmark is about 12 tons per year (*Larsen and Hardt,* 1982).

The consumption of biocides in agriculture, horticulture and in industrial production is unknown, because no registration is made in this area, except for products marketed as pesticides and herbicides.

Based on the total sales of cosmetics in Denmark the yearly consumption of biocides in cosmetics has been estimated at about 35 tons (*Valeur,* personal communication, 1984).

About 50 tons of formaldehyde is used per year in cleaning products, cosmetics and toiletries (calculated as 37% formalin). Formaldehyde exposure is difficult to estimate because the chemical, besides being manufactured, imported and used as such, is incorporated into a large variety of products and reactants in many chemical processes, i.e. formaldehyde releasers, polymerized plastics, foams and resins, textile finishes, paper industry, metal-working fluids, medicaments, cosmetics and detergents (Miljøprojekter 61, 1984). Formaldehyde was the 25th chemical on the top 50 list of chemicals (production volume) produced in the United States in 1980 (*Adams,* 1983).

The survey of biocide consumption is complicated by the multitude of synonymous names, e.g. formaldehyde releasing compounds are marketed under about 80 trade names and synonyms (*Fiedler,* 1983) and chlorocresol is marketed under 14 different names in Denmark (Arbejdstilsynet, Danish Labour Inspection Service, 1984).

AIM OF STUDY

The present studies examine the sensitizing potential of selected biocides in guinea pig sensitization assays and relate the results to the clinical experience following exposure to the compounds. The initial studies (*Andersen and Hamann,* 1983, 1984a; *Andersen et al,* 1984a) (III, I, IV) posed several questions, subsequently investigated in methodological studies.

(a) Discrepancy between guinea pig test results and clinical experience: The sensitizing potential of chlorocresol found experimentally in the guinea pig maximization test (GPMT) was high in contrast to our clinical experience. Supplementary tests studied the relationship between the induction dose and sensitization rate. Further, consecutive dermatitis patients were patch tested with chlorocresol to obtain more detailed information about the clinical findings in relation to the experimental results (*Andersen and Hamann,* 1984b) (II).

(b) Time-related variations in reactivity: The chlorocresol sensitization rate in guinea pigs decreased from the 1st challenge (day 21) to the 2nd challenge (day 35). This was unexpected and formed the basis for a study aimed at explaining this decrease in reactivity (*Andersen,* 1985a) (VI).

(c) Variations of vehicles: Chlorocresol is only slightly water soluble and therefore propylene glycol was used as vehicle for the intradermal chlorocresol induction, and for emulsification with Freund's complete adjuvant. This suggested a study of the effect of 3 types of Freund's complete adjuvant emulsions on chlorocresol sensitivity in guinea pigs (*Andersen,* 1985b) (VIII).

(d) Variations in reading: Quantification of contact allergy in guinea pigs is difficult and variations in reading may explain some of the discrepancies in test results between laboratories and investigators. We measured the skin blood flow and the skin fold thickness and related the results to the visual scoring method (*Andersen and Staberg,* 1985) (VII).

(e) Variations between laboratories: Guinea pig maximization tests with formaldehyde in Copenhagen and Stockholm yielded significantly different sensitization rates, probably due to differences in animal strains and choice of induction concentrations (*Andersen et al,* 1984a)(IV). A dose-response study of formaldehyde sensitivity was designed to clarify this point (*Andersen et al,* 1985) (V).

(f) Variations in allergen bioavailability: The high sensitizing potential of chlorocresol, which contrasted with the clinical experience, was the basis for a study of

the influence of different vehicles on the induction of chlorocresol sensitivity in guinea pigs. The bioavailability of chlorocresol was measured in similarly treated animals using »disappearance measurements« (*Wahlberg,* 1965) from bandages and the skin. Gas chromatography-mass spectrometry combined with an isotopic dilution technique was used (*Andersen et al,* 1985) (IX).

GUINEA PIG SENSITIZATION ASSAYS USED IN AUTHOR'S STUDIES

Three methods were utilized:

Guinea pig maximization test

The guinea pig maximization test (GPMT), selected as the standard procedure, was developed and named by *Magnusson and Kligman* (1969) and is considered among the most sensitive procedures for detecting the sensitizing potential of a compound. The procedure is developed through a systematic series of experiments, in which the investigators »maximized« each single component of the technique to achieve the maximum level of sensitization.

The guinea pig maximization test combines the use of intradermal injections, Freund's complete adjuvant and topical applications under occlusion. The test was designed to be as simple as possible to reduce the need for man-power; therefore, only 2 induction treatments are employed (Fig. 2).

The test concentrations used for induction should produce mild to moderate skin irritation. However, what is moderately irritating is a matter of judgment and may differ from investigator to investigator and with animal strains. If the test material is not locally irritating, the skin over the induction area is treated with sodium lauryl sulphate 10% in petrolatum 24 hours before the topical induction on day 7. Challenge is performed after a 2-weeks rest period (day 21) as closed patch tests with the test substance in a maximum non-irritating concentration and dilutions thereof.

The test gives the potential sensitizing capacity of a chemical. In many instances, substances which are potent sensitizers in the assay can be used without giving rise to frequent contact sensitivities, because the conditions for use of the substances (concentration, mode and route of exposure) are much less hazardous than the conditions of the test situation (*Magnusson and Kligman,* 1969; *Klecak,* 1983; *Andersen and Hamann,* 1983, 1984b) (III, II). Furthermore, the risk of causing contact allergy is dependent upon several other factors, such as individual susceptibility, presence of skin disease, occlusive effects, and the ability of the chemical to penetrate the skin.

Finished products composed of several ingredients should not be tested with this method. Usually one or two ingredients are potential allergens, while the rest

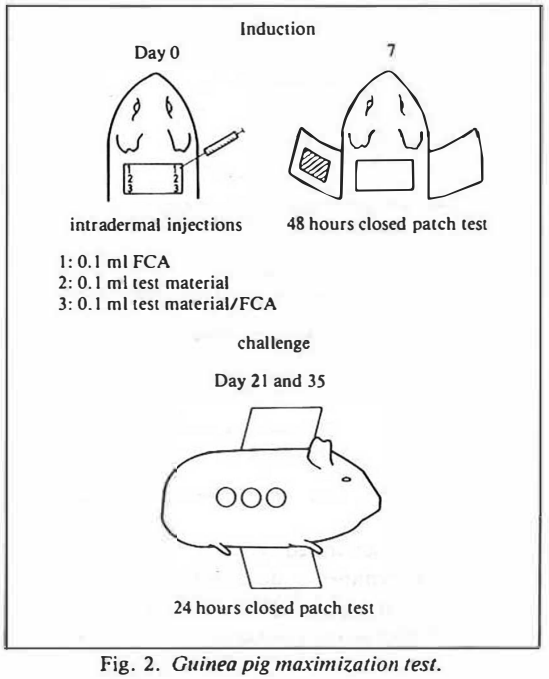


Fig. 2. *Guinea pig maximization test.*

are water and non-allergenic substances. These non-allergenic components, however, may influence the choice of induction concentration. A locally irritating non-allergenic ingredient, i.e. a solvent, will decrease the test concentration of an allergenic ingredient, which is mildly irritating, if they are tested together. The decreased test concentration may hamper the detection of an allergen.

Open epicutaneous test

The second test method used in the dose-response study of chlorocresol allergy (*Andersen and Hamann,* 1984b) (II) was the open epicutaneous test (OET) developed by *Klecak et al* (1977). The induction technique simulates the consumer situation with repeated, uncovered topical applications to the flank daily for 3 weeks. Several induction concentrations are used in separate animal groups (Fig. 3). Freund's complete adjuvant is not used, which makes the test less sensitive than those employing this immune enhancer. Open challenge is done on days 21 and 35, with application of 3-4 concentrations of the test substance, and the reactions are subsequently scored using an all or none criterion.

The sensitivity of the test was comparable to that of the guinea pig maximization test of 32 fragrance materials (*Klecak,* 1983), but in other experiments it was less sensitive (*Fahr et al,* 1976; *Andersen and Hamann,* 1984b)(II).

One advantage of the open epicutaneous test is its suitability for testing finished products and the prescribed use of progressive dilutions of the test

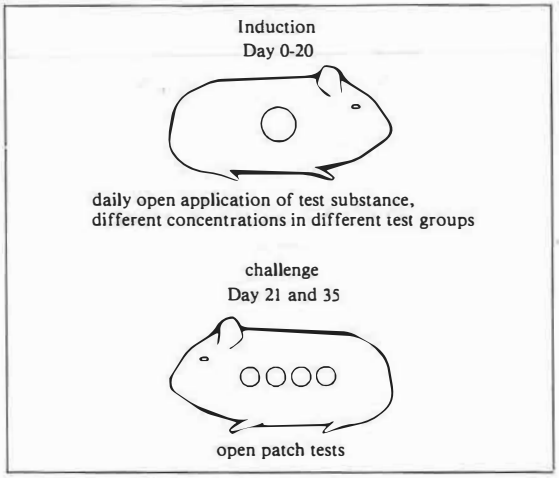


Fig. 3. *Open epicutaneous test.*

compound in separated groups of animals. The procedure determines a dose-response relationship, both for the induction phase and, by the use of different challenge concentrations, for the elicitation phase.

The open epicutaneous test was chosen for the chlorocresol study as a less sensitive counterpart to the guinea pig maximization test, which found chlorocresol to be a strong experimental sensitizer in contrast to the clinical experience.

Cumulative contact enhancement test

A third assay was chosen for the study of chlorocresol contact sensitivity in different vehicles and the relation between the sensitization rate and the chlorocresol bioavailability through the skin. The method should prescribe topical application for induction on unmodified skin and be sensitive enough to detect the allergenicity of chlorocresol. This implied that Freund's complete adjuvant had to be included as an immune enhancer. The recently described cumulative contact enhancement test (CCET) (*Tsuchiya et al,* 1982) fulfilled the criteria. The technique combines the use of intradermal injections with Freund's complete adjuvant and covered epidermal applications (Fig. 4). The highest response rate is expected when the adjuvant is injected before the third of the four induction patch tests and not at the start of the test. The method appears to be as sensitive as other methods employing Freund's complete adjuvant.

CLINICAL BIOCIDE ALLERGY AMONG WORKERS AND CONSUMERS RELATED TO THE RESULTS OF GUINEA PIG SENSITIZATION ASSAYS

Metal-working fluid dermatitis

Machinists constitute a potential risk group for development of contact allergy to biocides due to the frequent and prolonged exposure to the compounds in metal-working fluids.

Several reports indicate increasing usage of water-based metal-working fluids in the industrialized world between 1950 and 1970, along with an increase of dermatitis localized at the hands and arms of those occupationally exposed to metal-working fluids (*Arndt,* 1969; *Braun and Sitgraves,* 1958; *Squire et al,* 1950). The frequency of allergic versus irritant contact dermatitis in machinists with dermatitis varies: the ratio 20% allergic to 80% irritant reactions is frequently stated (*Robertson and Storrs,* 1982), but others expect 50% of metal-working fluid eczemas to be allergic in nature (*Fisher,* 1979). In contrast, *Menter et al* (1975) did not find a single positive reaction in 206 individuals patch tested with a limited panel of coolant fractions, and *Rycroft* (1981) found no biocide sensitive among 22 patients with soluble oil dermatitis.

One of the problems of evaluating metal-working fluid dermatitis is to ensure that the relevant allergens are used for patch testing. Testing with the metal-working fluid actually used is recommended, but the results may be difficult to interpret. False negatives as well as false positive results from patch tests may occur. Metal-working fluids based on oil in water emulsions or aqueous solutions are locally irritating, probably due to their detergent content and alkalinity in combination with wetness (*Rycroft,* 1982). For patch testing they must be appropriately diluted to decrease the risk of irritant reactions. Following dilution, the allergen content may be reduced below the threshold required for elicitation of an allergic patch test reaction in a sensitive subject. One way to circumvent this problem is to patch test with the metalworking fluid ingredients separately; another, is to test with the fluid undiluted and subsequently perform retests with the chemical constituents if the first test was positive. However, the composition of the metal-working fluids may change during use, and some of the compounds formed under heat and mechanical action may be allergens, although they are difficult to identify (*Jansson,* 1984).

The allergens frequently responsible for allergic contact dermatitis among machinists include metals, tall oils (contains a mixture of carboxylic and resin acids), and biocides (*Rycroft,* 1982). Among the prominent allergenic biocides are phenolic compounds and formaldehyde releasers.

Results with phenolic compounds

In spite of extensive use for decades, o-phenylphenol (preventol 0 extra) and dichlorphene (Preventol GD) have sensitized few workers and consumers, which is in accordance with the weak sensitization potential found in the guinea pig maximization test (*Andersen and Hamann,* 1984a) (1).

Chlorocresol (Preventol CMK) sensitivity occurs sporadically and case reports continue to appear. Among 1462 consecutive dermatitis patients patch tested with chlorocresol 2% in petrolatum from 1979 to 1982, 11 had a positive patch test (0.8%). When we reevaluated 10 of these patients with an initial positive chlorocresol patch test, none showed reproducible chlorocresol sensitivity during retests and provocative use-tests with repeated open applications to one elbow flexure daily for 5 days (*Andersen and Hamann,* 1984b) (II). Interestingly, the use of chlorocresol in topical corticosteroids is extensive; thus about 16% of the amount sold in Denmark in 1980 was chlorocresol preserved. If chlorocresol were a strong human contact sensitizer we should expect hypersensitive patients to appear more frequently due to repeated applications of chlorocresol containing topical medicaments to diseased skin.

Guinea pig maximization tests with chlorocresol showed a strong sensitization potential, with positive reactions ranging from 15% to 84% of the animals tested, depending on the concentration used for induction (*Andersen and Hamann,* 1984b) (II). The lowest concentrations used for induction were 0.2% intradermally and 0.4% topically. These concentrations are only 2 to 4 times higher than the use concentration in many products. Thus, there was a considerable discrepancy between the results of guinea pig maximization tests with chlorocresol and our clinical experience. In the less sensitive open epicutaneous test, chlorocresol did not show sensitization. There are other examples of the guinea pig maximization test showing a strong sensitizing potential of chemicals which during actual use have shown a low frequency of clinical contact allergy, i.e. gold salts, malathion, (*Kligman,* 1966b; *Magnusson and Kligman,* 1970).

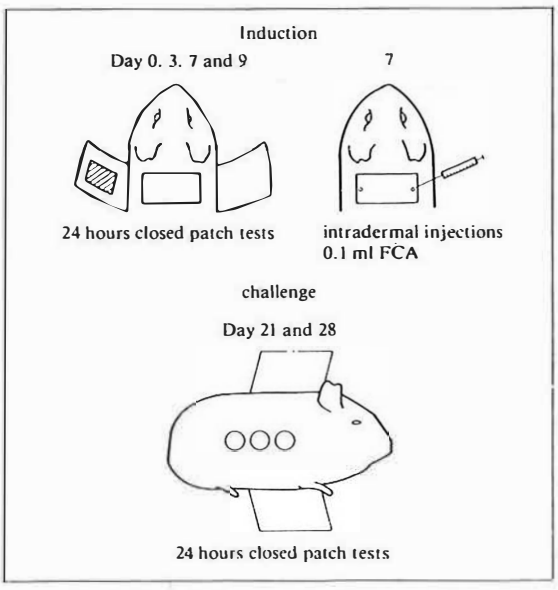


Fig. 4. *Cumulative contact enhancement test.*

Results with Cytox 3522

Cytox 3522, containing 10% methylene-bis-thiocyanate, sensitized all animals in the guinea pig maximization test and the majority of the animals in the open epicutaneous test (*Andersen and Hamann,* 1983) (III). In spite of this, human sensitization has not been reported. Cytox 3522 and equivalent products are used as wide spectrum biocides in industrial water systems, solutions and emulsions. Limited exposure, low use concentration, and lack of diagnostic patch test materials may partly explain the discrepancy between the animal data and the clinical experience. The current use of Cytox 3522 in Denmark is unknown, but it is probably low. Patch tests with Cytox 3522 0.2% in petrolatum in 1606 consecutive patients in Danish dermatology clinics revealed 5 positive reactions, which could not be explained (*Andersen and Veien,* 1985). Evaluation of the allergenicity of this chemical is delayed because we have been unable to obtain further information or samples of trade product ingredients from the manufacturer (American Cyanamid Company). In a Finnish paper-manufacturing plant the annual consumption of methylene-bis-thiocyanate containing chemicals is about 25 tons. In spite of this, contact dermatitis is apparently rare. Thirty-four exposed workers were patch tested with a standard battery of 25 substances plus Cytox 3522 0.2% in petrolatum. The patch tests were applied for 24 hours and read on days 3 and 5. The Cytox material gave no definitely positive allergic skin reaction, although three persons showed a mild reaction graded as (+) one plus according to the ICDRG (International Contact Dermatitis Research Group) recommendations (*Wilkinson et al,* 1970). The reactions were interpreted as being of irritant nature (*Jäppinen,* personal communication, 1984).

Results with formaldehyde and formaldehyde releasers

Formaldehyde sensitivity has been reported since the twenties (*Horsfall,* 1934; *Bonnevie,* 1939). *Bonnevie* (1939) reported 12 patients with contact allergy sensitized by formaldehyde in disinfectants, antiperspirants, glues, and phenoplasts (polycondensation resins). Formaldehyde appears among the 10 most frequent allergens and is incorporated in the patch test standard series recommended by the International Contact Dermatitis Research Group (ICDRG). However, many positive reactions not explained by the history and clinical features, may result from irritant responses (false positives) and are often related to the presence of status eczematicus (*Björnberg,* 1968) and/or the excited skin syndrome (ESS) (*Epstein and*

Maibach, 1966; *Mitchell and Maibach,* 1982). This hyper-irritability phenomenon may be seen in guinea pigs too (*Andersen and Maibach,* 1980) Formaldehyde 2% in water is a marginally irritant patch test preparation and the concentration has recently been reduced to 1% (ICDRG, 1984).

Formaldehyde condensate products constitute about 60% of the total biocide sales for use in metal-working fluids (*Rossmoore,* 1981). Exposure often occurs in a hidden fashion since at least 80 trade names and synonyms are used in the marketing of formaldehyde-releasing compounds (*Fiedler,* 1983).

INTERLABORATORY VARIATIONS WITH FORMALDEHYDE AND FORMALDEHYDE RELEASERS

We evaluated the sensitizing potential of formaldehyde and 6 formaldehyde releasers (Forcide 78, Germall 115, Grotan BK, Grotan OX, KM 200 and Preventol D2) (Table 2) using the guinea pig maximization test in two laboratories, one in Copenhagen and one in Stockholm (*Andersen et al,* 1984a) (IV). Discrepancies between the results from the two laboratories were observed.

Formaldehyde and Grotan BK were tested in both laboratories. The sensitizing potential was in general stronger in Stockholm than in Copenhagen: formaldehyde sensitized 95% of the guinea pigs in Stockholm and 50% in Copenhagen. For Grotan BK, the corresponding rates were 74% and 20%, respectively. Interestingly, higher intradermal induction concentrations were used in Copenhagen. According to the guinea pig maximization test procedure, the induction concentration should provoke a moderately irritant reaction which, however, is determined by a subjective evaluation and may vary with the strain og guinea pigs used (*Stampf et al,* 1982). The variations were possibly due to the use of different animal strains, test concentrations and vehicles.

Our data demonstrated that guinea pig sensitization assays, in spite of being standardized, may yield significantly different test results, complicating the comparison of findings in different laboratories.

FORMALDEHYDE DOSE-RESPONSE RELATIONSHIP

Our varying results with formaldehyde in the guinea pig maximization test prompted us to evaluate the relationship between induction dose and sensitization rate, in two different animal strains. The same batch of formaldehyde was used in both laboratories. The sensitization rates were analysed using a generalized logistic dose-response model and the best fitting logistic curves were calculated. Three important observations were made (Fig. 5) (*Andersen et al,* 1985a) (V). Firstly, in the guinea pig maximization test with formaldehyde, the intradermal induction concentration was crucial for the sensitization rate, while the topical induction showed no significant effect. The topical induction dose, however, may be important for other allergens, i.e. for chlorocresol (*Andersen and Hamann,* 1984b) (II).

Secondly, the dose-response relationship was non-linear (non-monotonous), suggesting that there was an optimal induction dose above and below which a decreased response rate was seen (Fig. 5). The maximum sensitization rates calculated from the fitted curves were 80% in Copenhagen and 84% in Stockholm following intradermal induction with 0.65% and 0.34% formaldehyde, respectively. The EC_{50} for formaldehyde (concentration sensitizing 50% of the population) was 0.061% in Copenhagen and 0.024% in Stockholm (72 hours).

The non-linear dose-response relationship found for formaldehyde is concordant with previously reported data for picryl chloride and dinitrochlorobenzene (*Macher and Chase,* 1969), sultones (*Ritz et al,* 1975),

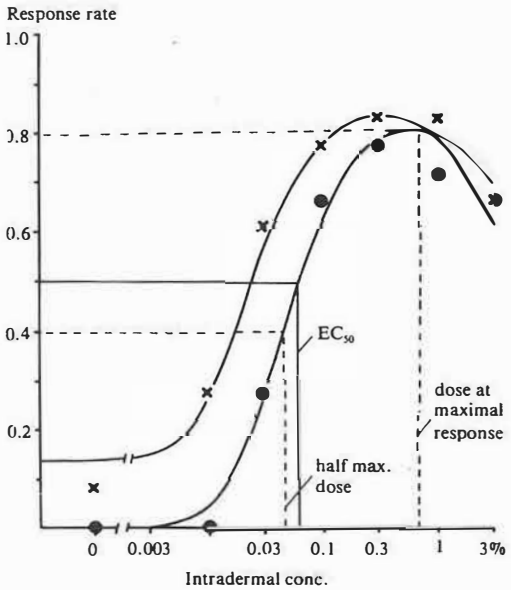


Fig. 5. *Observed response rates and best fitting logistic curves[a)] for formaldehyde sensitivity (72 hr reading) in the guinea pig maximization test in two laboratories (o – – – o, Copenhagen, x – – – x Stockholm). The maximal response rate and the corresponding formaldehyde concentration, the concentration at the half maximal response, and the EC_{50}[b)] for the Copenhagen results are shown (Andersen et al, 1985).*

[a)] The fitted curves are given by the equations:

$$P_{(x)} = 1/(1 + \exp(-1.42 + 0.225 \times \log x + 0.262(\log x)^2))$$

for the Copenhagen data and for the Stockholm data:

$$P_{(x)} = 0.14 + 0.86/(1 + \exp(-1.42 + 0.225(0.657 + \log x) + 0.262(0.657 + \log x)^2))$$

[b)] the EC_{50} is the formaldehyde concentration, which can sensitize 50% of the test animals.

p-nitrobenzyl compounds (*Roberts et al,* 1983), and to some extent for chlorocresol (*Andersen and Hamann,* 1984b) (II). The level of contact sensitivity in guinea pigs is determined by a balance between activated effector and suppressor cells, and the balance is influenced by the dose given (*Macher and Chase,* 1969).

The value of a sensitization test improves when a dose-response relationship is included in the procedure, as in the open epicutaneous test (*Klecak,* 1983). This is possible without increasing the number of animals. Usually, 40 animals are required for the guinea pig maximization test, – 20 test animals and 20 sham-treated control animals. The 40 animals may be divided into 1 control group of 5 animals and 7 test groups of 5 animals; each group is treated with different induction concentrations. The reduced number of animals in each group is balanced by the increased number of groups, and valid statistical calculations are still possible.

By means of dose-response studies, with a range of wellknown allergenic chemicals, it is possible to determine the susceptibility of an animal strain to sensitization. It is not enough to use one chemical as marker of the susceptibility of the strain as this may change from chemical to chemical (*Parker et al,* 1975; *Polak et al,* 1968).

Thirdly, the best fitting dose-response curves from the 2 laboratories were parallel, suggesting that the guinea pig strain used in Stockholm was significantly more sensitive to formaldehyde than the animals used in Copenhagen. A difference in reading between the two laboratories cannot be excluded. However, owing to frequent contact between the investigators, the interlaboratory reading variation is presumably small. Further tests involving exchange of animals are desirable.

One advantage of the statistical model designed for the present study was the possibility of analyzing positive challenge reactions among the control animals. Such reactions may occur now and then because of biological and technical variations, which can be minimized but not prevented, by standardization.

SUCCESSIVE CHALLENGES WITH CHLOROCRESOL

Positive tests in a guinea pig sensitization assay should include the rigorous follow-up now employed in human testing, in order to minimize the risk of non-specific positive reactions caused by the excited skin syndrome. Usually, the test result in a guinea pig maximization test is based on the day 21 challenge response. In other test procedures, e.g. Freund's complete adjuvant test, the Buehler test and the open epicutaneous test, successive challenges are prescribed (*Klecak,* 1983). Generally, persistence of contact allergy in guinea pigs is expected (*Frey et al,* 1965) and repeated challenges may have a booster effect which increases the degree of sensitivity (*Maurer,* 1983). However, transient sensitivity in guinea pigs has been reported for sodium zirconium lactate (*Turk and Parker,* 1977) and di(meth)acrylates (*van der Walle et al,* 1983). Our guinea pig maximization tests with chlorocresol showed a decline in reactivity after challenge from day 21 to day 35 (*Andersen and Hamann,* 1984b) (II). The decline was noted in 3 tests using intradermal induction concentrations ranging from 0.2% to 5% in propylene glycol and the same topical induction concentration of 10% chlorocresol in petrolatum. The dose and time dependent reactivity warrants further studies.

In supplementary tests cyclophosphamide was used as an immune response modulator. The effect of cyclophosphamide on contact sensitivity depends on the time of administration in relation to hapten exposure. If the drug is given 3 days before induction it eliminates suppressor cells, and the subsequent challenge shows increased sensitivity (*Polak,* 1980). However, each challenge stimulates both effector and suppressor cells. If cyclophosphamide is administered 3 days before the first challenge, the intensity of the skin reaction to a later challenge, performed 1-2 weeks later, is markedly increased (*Polak,* 1980).

When cyclophosphamide (250 mg/kg) was injected intraperitoneally in chlorocresol-treated guinea pigs 3 days before the first challenge, the decrease in reactivity from day 21 to day 35 was reversed (*Andersen,* 1985a) (VI). This suggested that the reduction in chlorocresol sensitivity previously seen (*Andersen and Hamann,* 1984b) (II) was caused by an immunologic phenomenon – probably the formation of suppressor cells. This »down-regulation« of the sensitivity may operate in chlorocresol sensitive patients and may provide an alternative explanation of the non-reproducible positive patch tests. Other possible explanations are false positives due to a too high patch test concentration and the excited skin syndrome. Further studies are required to evaluate this hypothesis.

The »down-regulation« of the chlorocresol sensitivity in the guinea pig maximization test may partly explain the discrepancy between the strong sensitization potential found on day 21 and the clinical experience with this widely used biocide (*Andersen and Hamann,* 1984b) (II). These findings should encourage the use of successive challenges in standard guinea pig sensitization assays.

READING OF TEST REACTIONS

Reading of the skin reactions is usually accomplished by scoring according to non-linear descriptive reading scales, i.e. *Magnusson* and *Kligman's* 4 point scale

(Table 3) (*Magnusson and Kligman,* 1970). This adds a subjective element to the ratings. Simultaneously sham-treated control animals should always be included in guinea pig allergy tests to allow blind reading of the challenge response. Interindividual differences between raters must be expected (*Weil and Scala,* 1971; *Steinberg et al,* 1975). The use of sham-treated control animals and blind reading reduces the risk of misinterpreting the test reactions, e.g. false positives due to an excited skin reaction (*Andersen and Maibach,* 1980; *Bruynzeel et al,* 1983; *Mitchell and Maibach,* 1982).

Non-specific effects are greater immediately after removal of the patch. As in human patch testing, much information is gained by delayed readings.

The problems of visual scoring must be added to the variations in technique and animals. They can be alleviated by the training and experience of the staff. Quantitation of guinea pig contact allergy by measuring changes in skin blood flow and skin fold thickness may be a useful supplement to the visual scoring as a measure of interobserver and interlaboratory differences (*Andersen and Staberg,* 1985) (VII). Blood flow was measured by laser Doppler flowmetry (Periflux microvascular flowmeter, Perimed, Stockholm, Sweden), which produces an output related to perfusion characteristics, i.e. volume and flow rate, but do not quantitate skin blood perfusion as volume/weight/-unit time. Skin fold thickness was measured by a Mitutoyo skin fold caliper (Mitutoyo MFG, Co. Ltd., Tokyo, Japan). The laser Doppler flowmetry statistically separated sensitized (grade 2 or 3) from non-sensitized animals (grade 0 or 1), and the skin fold thickness measurement separated grade 3 (edematous) from grade 2 (non-edematous) reactions (Table 4). High frequency pulsed ultrasound is another alternative method for skin thickness measurements, which was found suitable for the assessment of human patch test reactions (*Serup et al,* 1984). The visual judgment of a trained observer may still be the best and fastest method of reading, because it includes all the facets of an allergic patch test reaction, i.e. a combination of redness, thickness, scaling, edema, etc. It may be inappropriate to transfer this complex biological phenomenon into one or a few physico-chemical parameters.

However, to assess interobserver and interlaboratory variations, the methods mentioned above are useful. Different animal sources and varying test sites provide additional risks for variation in results between observers and laboratories.

Table 3. *Magnusson and Kligman's grading scale for the evaluation of challenge patch test reactions.*

0	= no visible change
1	= discrete or patchy erythema
2	= moderate and confluent erythema
3	= intense erythema and swelling

Table 4. *Evaluation of contact sensitivity reactions in guinea pigs. The maximum agreement between visual grading and two quantitative measurements (laser-Doppler flowmetry (LDF) for erythema, and skin fold thickness (SFT) for edema) is shown. Control animals and chlorocresol sensitized animals were used (Andersen and Staberg, 1985).*

	Increase in LDF	
Score	<15 (negative)	>15 (positive)
0 and 1 (negative).....	21*)	3
2 and 3 (positive).....	2	22
	Increase in SFT	
	<0.6 mm	>0.6 mm
0, 1 and 2.....	34	2
3.....	2	10

*) Number of animals.

OTHER SOURCES OF RESULT VARIATION

The following factors should also be considered: the patch test technique, type of Freund's complete adjuvant emulsion, and the choice of vehicle.

Patch test technique

The importance of proper occlusion is well known from diagnostic and predictive patch tests (*Hjorth,* 1983; *Magnusson and Kligman,* 1970). *Ritz and Buehler* (1980) restrained their guinea pigs with a rubber dam for 6 hours to assure proper occlusion. They claimed that an elastic bandage circulating the trunk is ineffective and that inefficient occlusion may explain why some investigators found the Buehler guinea pig assay less efficient than other assays in detecting allergens (*Buehler,* 1982).

For the challenge procedure, the quality of occlusion can be verified if the Finn Chamber (Epitest Ltd., Helsinki, Finland) or a similar aluminium cup is used as patch test unit. Skin indentations from the aluminium flange immediately after removal of the patches assures proper occlusion.

The size of the challenge patch test may have an effect. *Frosch and Kligman* (1979) found increased challenge reactivity in humans with increasing size of the patch tests, even with identical concentrations.

The location of the challenge patch test on the guinea pigs was of less importance in *Magnusson and Kligman's* (1970) study using strong experimental allergens in alcohol (2,4-dinitrochlorobenzene, p-nitroso-dimethylaniline, and p-phenylenediamine), except for reactions over scapula which were stronger than over sacrum. However, I found a decreased challenge response in chlorocresol sensitized guinea pigs when the challenge patch tests were 2 cm nearer the abdomen compared to the usual site on the mid-flank

(*Andersen,* 1985b) (VIII). *Björkner and Niklasson* (1983) found a similar regional variation in challenge reactivity when testing acrylates in petrolatum, but the difference was not apparent when acetone was used as vehicle. Presumably, the regional difference is more important with weaker allergens.

Adjuvant emulsion

Freund's complete adjuvant (FCA) has been utilized in guinea pig sensitization assays since *Landsteiner and Chase* (1940) described the promoting effect on contact sensitivity. *Maguire* (1974) found that the complete adjuvant should be injected into or nearby the allergen application site, but that emulsification of allergen and adjuvant was not necessary. However, several guinea pig sensitization assays prescribe the use of mixtures of allergen and adjuvant.

The preparation of the adjuvant-allergen emulsion is dependent on the solubility of the allergen. *Magnusson and Kligman* (1970) prescribed blending of the adjuvant with an equal volume of water. Water-soluble allergens should be dissolved in the water phase; oil-soluble or water-insoluble chemicals should be dissolved first in a small quantity of ethanol or propylene glycol, and then mixed with saline. The proportion of ethanol or propylene glycol should not exceed 30% w/w of the final aqueous solution (*Maurer,* 1983). Chlorocresol is only slightly water soluble. Therefore, I investigated the effect of 3 types of adjuvant-chlorocresol emulsions on the sensitivity using the guinea pig maximization test (*Andersen,* 1985b) (VIII). The adjuvant was mixed with propylene glycol, saline with 30% (v/v) ethanol or saline, respectively. The relative viscosity of the emulsions was determined as a physico-chemical parameter. The sensitization rates reached the same level with the 3 types of emulsion, however, higher degrees of sensitivity were obtained at the 48 hours challenge reading with the oil/propylene glycol and oil/ saline + ethanol emulsions compared to the saline/oil emulsion. It is suggested that the type of adjuvant emulsion should be specified in reports on contact allergy tests to aid comparison between laboratories. Only chlorocresol was used as allergen, but other allergens may show a different relationship between sensitization and type of emulsion.

Effect of vehicle

Effects of the vehicle on diagnostic and predictive patch tests are well documented, e.g. formaldehyde is tested in water, most other standard allergens in petrolatum (*Marzulli and Maibach,* 1980; *Andersen et al,* 1984a; *Christensen et al,* 1984) (IV). No vehicle is optimal for all substances. Most investigators prefer to dissolve the test substance because dispersions are prone to form a sediment which makes exact dosing difficult. *Magnusson and Kligman* (1970) found 70% ethanol superior to petrolatum, propylene glycol and acetone in topical sensitization to DNCB. For intradermal sensitization, ethanol was unsatisfactory and propylene glycol or a vegetable oil was preferable. Conflicting results on the sensitizing potential of mono(meth)acrylates may be explained partly by differences in the choice of vehicle (*Chung and Giles,* 1977; *van der Walle et al,* 1982; *Björkner and Niklasson,* 1984).

The discrepancy between our initial animal data on chlorocresol and the clinical experience with the allergen prompted us to study further the sensitization rate in relation to different vehicles. To induce contact allergy, a topically applied compound must diffuse out of the vehicle and bandage material and penetrate into the skin and form conjugates with skin proteins (*Polak,* 1980). Therefore, we compared the chlorocresol sensitization and release from 5 preparations (*Andersen et al,* 1985) (IX): (1) A 5% aqueous suspension stabilized with 0.15% of a gelling agent carbomer 941 (Carbopol 941, Goodrich Chemical Company, Cleveland, Ohio, USA); (2) a saturated aqueous solution, containing about 0.4%; (3) 5% in olive oil and acetone (4 + 1); (4) 5% in propylene glycol, and (5) 5% in propylene glycol containing 0.15% Carbomer 941.

The cumulative contact enhancement test was chosen as the guinea pig assay, because it prescribes topical occluded application of the allergen on unmodified, shaved skin and Freund's complete adjuvant injections into a nearby skin site. Chlorocresol bioavailability was determined by analyzing the fraction of the applied amount that remained in the bandage and application skin site after 24 hours. An isotopic dilution technique was used.

There was a significant difference in the sensitization rates produced by the 5 chlorocresol preparations (Table 5). The 5% chlorocresol suspension in

Table 5. *Cumulative contact enhancement tests with chlorocresol in 5 preparations. Closed challenge day 21 with chlorocresol 1% in petrolatum, 48 hour readings. The frequency of sensitization is related to the bioavailability of the preparation (Andersen et al, 1985b).*

Chlorocresol preparation	Challenge result	Bioavailability*)
5% in water + carbomer 941 0.15%	12/19**)	75%
Saturated aqueous	6/20	54%
5% in olive oil/acetone	11/20	34%
5% in propylene glycol	4/20	35%
5% in propyleneglycol + carbomer 941 0.15%	4/20	not tested

*) Bioavailability means the fraction of the amount applied to the skin that penetrates the skin. The value is determined by measurements of the amount of chlorocresol that disappears from the bandage used for induction of chlorocresol allergy, and from the skin of the guinea pig.

**) Grades 2 and 3 denote sensitization.

water was the strongest sensitizer, while the 5% chlorocresol solution in propylene glycol showed the least sensitizing capacity. The saturated aqueous solution sensitized 6 animals compared to 4 animals sensitized from the propylene glycol preparations, which contained 13 times more chlorocresol. When the concentration reaches the saturation point, the maximum thermodynamic activity of the drug in the vehicle is achieved. This provides optimal conditions for skin absorption (*Barry,* 1983).

There was an ambigous relationship between the sensitization rates and the chlorocresol bioavailability. The greatest absorption appeared from the suspension which gave the highest sensitization rate, while the olive oil/acetone preparation showed a proportionally low chlorocresol bioavailability, in spite of the fact that it produced an equivalent sensitization rate. Our absorption study determined the amount absorbed, but not the penetration rate, which might affect the sensitization. From physico-chemical considerations the absorption rates from the aqueous preparations are considerably larger than from the propylene glycol preparations, which accord with the low chlorocresol solubility in water and high solubility in propylene glycol. This gives a higher skin/vehicle distribution coefficient for the aqueous preparations.

No simple relationship appears between the induction of contact allergy and the bioavailability of an allergen. This is in agreement with our present knowledge of the sensitization process (*Polak,* 1980). The critical amount of allergen necessary in presentator cells in the skin (Langerhans cells, macrophages) (*Stingl et al,* 1981) is small and only the amount that is bound to presentator cells is of relevance (*Blomberg-van der Flier et al,* 1984). For toxicological routine tests it seems appropriate to use a test vehicle which resembles that of the compound intended for use among consumers.

KEY FACTORS IN INTERPRETATION

To facilitate interpretation of test results, the future application of the test substance and test data should be taken into consideration when choosing the experimental design. Sensitization assays using Freund's complete adjuvant are usually more sensitive than those without adjuvant. The guinea pig maximization test establishes to what extent a particular substance has the potential for contact sensitization (*Magnusson and Kligman,* 1970). A positive test does not necessarily exclude the substance from application, since the stimulus in the test procedure may be stronger than in usage conditions. On the other hand, two negative test results from experienced laboratories using the guinea pig maximization test suggest that the substance is unlikely to sensitize in the concentration employed. It must be emphasized that this result does not imply that the substance will never sensitize anyone. For example, lanolin allergy is a clinical problem for leg ulcer patients, even though the lanolin batch used by *Magnusson and Kligman* (1970) did not sensitize guinea pigs under experimental conditions. Recently, alkane-diols were identified as the major allergenic constituents of hydrogenated lanolin. These compounds sensitized guinea pigs (*Takano et al,* 1983). Hypersensitivity produced in guinea pigs using one of the less sensitive non-adjuvant techniques (e.g. the open epicutaneous test) is more alarming. So, a reasonable approach would be to use an adjuvant test as a screening procedure and to add a non-adjuvant technique in case of sensitization. Such a step-wise approach is a more satisfactory and cost-effective solution.

Positive tests in any of the assays should lead to as rigorous follow-up as is now employed in human testing, in order to minimize the likelihood of non-specific positives from the excited skin syndrome (ESS). This entails, at a minimum, verification and often inclusion of additional controls. Repeating the assay and using open challenge (to avoid non-specific effects of tape) are of great scientific and practical value.

Guinea pig allergy test results cannot stand alone. The results should be corroborated by comparison with other sources of information, such as:

1) Use tests with a final formulation on a limited scale prior to marketing.
2) Industry, worker and consumer complaint data.
3) Retrospective and prospective epidemiological data.
4) Monitoring programs, such as those of the Department of Health, Education, and Welfare (HEW) – sponsored investigation of consumer's perceptions of adverse reactions to cosmetic products.
5) Diagnostic test results in dermatologic clinics (*Marzulli and Maibach,* 1983).

A risk-benefit analysis is appropriate when results from guinea pig sensitization assays are interpreted. For cosmetic chemicals intended to be used extensively on normal and sometimes diseased skin, no substantial risk will be accepted. However, because of their inherent beneficial effects, many topical drugs are fully acceptable in spite of having a sensitizing potential.

The investigator should know – if possible – the basic chemical purity of the test substance, and its relationship to known allergens (e.g. cross-reactions).

Statistical considerations are involved when extrapolating from a small test population to large numbers of consumers. *Henderson and Riley* (1945) discussed this issue by drawing attention to the confidence limits. Many guinea pig allergy tests, however, amplify the

exposure to such an extent that negative results ensure a considerable safety margin.

WHAT TO TEST AND NOT TO TEST?

A priority list can be made. Some soaps, dish washing liquids, detergents and emulsifiers are examples of products that have been used for a long time and by many people, without causing allergic contact dermatitis, although they may cause irritant dermatitis in susceptible persons. Unfortunately, some common chemicals, long thought to be »safe« have turned out to be allergens after all, so a high index of suspicion is indicated (*Ritz et al,* 1975; *Andersen et al,* 1984b).

New substances intended for skin contact, rubber chemicals, preservatives, drugs and reactive additives in plastics industry are examples of compounds, which should be tested for sensitizing capacity (*Magnusson et al,* 1982).

Toxicologists may be tempted to test indiscriminately and perform many tests. But such an unstructured procedure is not only problematic from a scientific point of view, but also wasteful with regard to economy and laboratory animals, and leads to a mass of redundant information. This approach is little more than a statutory ritual to create the impression that something is being done in the interest of the public (*Kligman,* 1966a).

Consumer groups and regulatory agencies will continue to demand evaluation of toxicity to assure safety of environmental substances. The demand for toxicological data ought not to be a bottle neck that delays the development of new products to the benefit of the people. Guinea pig sensitization assays are an important tool for safe development of new products when the tests are executed and interpreted by trained people. So far, *in vitro* tests that can replace the guinea pig assays have not been developed.

CONCLUSIONS

The introduction of guinea pig sensitization tests with everyday contact chemicals and industrial products has been extremely valuable for estimating the risk of sensitization to environmental chemicals. It is obvious, however, that they only serve to assess the likelihood of sensitization under certain conditions of use.

The sensitizing potential of chemicals is best disclosed by guinea pig assays using Freund's complete adjuvant. However, a positive test in an adjuvant assay may rate the chemical as a stronger sensitizer than it appears to be during actual use. On the other hand, a negative test result ensures a considerable safety margin concerning the risk following human use, provided appropriate test conditions are chosen (animals, technical aspects, concentrations and vehicles).

In a series of studies of the sensitizing properties of chlorocresol, formaldehyde and 13 other biocides, using the guinea pig maximization test and two alternative guinea pig methods, further limitations in the predictive value appeared. The present essay describes the discrepancies between the test results and the registered human sensitization and discusses some of the causes of these differences.

Chlorocresol and Cytox 3522, containing 10% methylene-bisthiocyanate, were potent allergens in the guinea pig maximization test in contrast to the low frequency of human sensitizations reported.

Results from guinea pig sensitization tests cannot stand alone, but must be corroborated with other sources of information, such as limited usage tests with the products in question, complaints from exposed groups of people, retrospective and prospective epidemiologic data, and diagnostic patch tests from dermatologic clinics. Supplementary guinea pig tests using alternative methods increase the predictive value, whereas product labeling for allergenicity hazard based on a single test is unsatisfactory.

Varying test results from different laboratories can chiefly be attributed to the use of different animal strains and differences in the choice of concentrations and vehicles.

The reliability of the test results can be improved by including as a routine procedure dose-response determinations for both induction and challenge, because the choice of one single concentration is fortuitous in relation to the concentration giving maximal sensitization. Successive challenges are useful to confirm initial challenge results and adds to the reliability of the sensitization assay.

Further standardization of the test methods is desirable. More uniform readings of the challenge reactions can be achieved through training of the investigator and comparison with the so-called objective measuring techniques, such as laser-Doppler flowmetry and skin thickness measurements. The patch test technique, the quality of occlusion and the choice of vehicle have profound effects on the results. Test reports should include arguments for the choice of vehicle.

To increase the predictive value of the sensitization assays, further studies are needed. These should include dose-response determinations on a wide range of chemicals and examine the effect of induction route, find ways to characterize the susceptibility of the animal strain and assure optimal technical performance.

The currently available guinea pig assays allow testing of all types of chemicals and products. A higher priority should accordingly be given to standardization of the methods rather than the development of new guinea pig assays. In vitro tests and tests using other animal species might profitably be considered.

SUMMARY IN DANISH

Kontaktallergitests på marsvin har til formål at forudsige allergifremkaldende egenskaber ved kemikalier og produkter. Flere testmetoder er tilgængelige, men de varierer m.h.t. anvendelsesområde og er af varierende følsomhed.

Nogle metoder er designede til testning af kemikalier og andre er velegnede til testning af færdigvarer. De metoder der anvender Freund's komplette adjuvans er generelt mere følsomme end de metoder, der ikke anvender denne immunmodulator. Der er mangel på undersøgelser, som sammenligner flere testmetoders effektivitet til at fastslå en lang række stoffers allergifremkaldende egenskaber og samtidig vurderer resultaternes betydning for human eksposition.

Guinea pig maximization test, som har været anvendt i ca. 15 år, er en følsom adjuvansmetode til rutinebrug for testning af kemikalier. Den har sammen med 2 andre metoder (open epicutaneous test og cumulative contact enhancement test) været anvendt til vurdering af de potentielle allergifremkaldende egenskaber ved klorkresol, formaldehyd og 13 andre markedsførte konserveringsmidler, som anvendes industrielt og i husholdningsprodukter.

Guinea pig maximization tests med produkterne viste sensibiliseringsfrekvenser, som ikke altid stemte overens med den kliniske erfaring med de samme produkter (artiklerne I, II, III og IV). Testresultaterne gav ikke grundlag for en rimelig rangstilling af produkternes allergifremkaldende egenskaber i praksis.

De indledende arbejder rejste en række spørgsmål vedrørende årsager til variation mellem testresultater. Flere af dem er søgt belyst i supplerende undersøgelser.

Tests med formaldehyd og formaldehydfrigørere i 2 laboratorier (København og Stockholm) viste en betydelig variation mellem resultaterne (artikel IV). Dette dannede baggrunden for en sammenlignende dosis-respons undersøgelse med formaldehyd i København og Stockholm. De marsvinestammer der blev anvendt i de 2 laboratorier var forskellige: Ssc:AL fra Seruminstitutet i København, og i Stockholm, Dunkin-Hartley fra Sahlin, Malmö. Resultaterne blev analyseret statistisk ved hjælp af en logistisk dosis-respons model. De viste, at Dunkin-Hartley marsvin var mere følsomme for lavere doser af formaldehyd end Ssc:AL marsvin (artikel V). Den intradermale formaldehyddosis givet i induktionsfasen var afgørende for sensibiliseringsfrekvensen. Endvidere påvistes en ikke-lineær afhængighed mellem induktionsdosis og allergihyppighed. Dette fremhæver betydningen af at anvende flere forskellige induktionsdoser, når et stofs allergifremkaldende egenskaber skal vurderes. Om den »klokkeformede« dosis-respons kurve er et mere generelt fænomen for eksperimentel kontaktallergi kan ikke afgøres på det nuværende grundlag.

Klorkresol var i modstrid med de kliniske erfaringer stærkt sensibiliserende i guinea pig maximization test bedømt efter lappeprøveprovokation dag 21, men vi så et uventet fald i reaktivitet fra dag 21 til dag 35 (artikel II). Normalt forventes sensibiliserede dyr at bevare deres specifikke reaktivitet i en længere periode. Supplerende undersøgelser med klorkresol ved hjælp af cyclofosfamid som immunmodulator bekræftede det initiale fund og påviste, at den stedfundne »nedregulering« af kontaktallergien sandsynligvis kunne forklares ved fremkomsten af specifikt aktiverede suppressor-celler (artikel VI). Yderligere undersøgelser må vurdere betydningen af dette fænomen for eksperimentel og klinisk kontaktallergi.

Den visuelle aflæsning af lappeprøvereaktioner på marsvinehud er subjektiv og udgør en anden kilde til varierende testresultater. Kvantitative målinger af erythem og af ødem, de to karakteristika som indgår i den visuelle aflæsning, blev sammenholdt med aflæsning efter en 4-punkts rangskala. Der var god korrelation mellem den visuelle aflæsning af erythemet og hudens blodgennemstrømning målt med laser-Doppler flowmetri. Sammenligning mellem visuel-palpatorisk vurdering af ødem og måling af hudfoldtykkelse gav også god korrelation. De objektive målemetoder er et godt supplement til den visuelle aflæsning og kan med fordel anvendes til vurdering af variationer mellem aflæsere (artikel VII).

Freund's adjuvans anvendes ved guinea pig maximization test blandet med allergenet. Da klorkresol i ringe grad er vandopløselig, blev propylenglykol brugt som vehikel for klorkresol i de indledende forsøg. En emulsion lavet af propylenglykol og Freund's komplette adjuvans var ustabil, og derfor undersøgtes forskellige typer adjuvansemulsions effekt på klorkresolsensibilisering (artikel VIII). Variationer i typen af Freund's komplette adjuvansemulsion påvirkede ikke i væsentlig grad sensibiliseringshyppigheden. I denne undersøgelse vurderedes også betydningen af placeringen af provokationslappeprøverne på marsvinenes flanke. De prøver der blev placeret 2 cm lavere på siden af dyrene gav signifikant svagere reaktioner sammenlignet med den rutinemæssige placering.

På grund af forskellen mellem klorkresols allergifremkaldende egenskaber i guinea pig maximization test og i klinikken undersøgtes betydningen af forskellige vehikler på klorkresolsensibilisering i en anden marsvinemodel, cumulative contact enhancement test. Denne metode foreskriver lappeprøveinduktion med klorkresol i kombination med injektion af Freund's komplette adjuvans. Samtidig blev klorkresols biotilgængelighed i de forskellige vehikler målt. Biotilgængeligheden betyder her den mængde af allergenet, som når frem til huden. Den mængde klorkresol, der blev tilbage i lappeprøvebandager og i hud, blev bestemt ved hjælp af en isotopfortyndingsteknik med deuteri-

um-mærket klorkresol. Der var ingen enkel sammenhæng mellem biotilgængelighed og sensibiliseringsevne (artikel IX).

Resultaterne peger i retning af, at en yderligere standardisering af de anbefalede allergitestmetoder på marsvin er nødvendig for at øge metodernes reproducerbarhed og resultaternes anvendelighed.

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