

## The Allergen Bank: A Source of Extra Contact Allergens for the Dermatologist in Practice

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The Allergen Bank was established to give dermatologists easy access to special test materials in order to make early diagnoses of special cases of allergic contact dermatitis. The Allergen Bank comprises a computer system to register several hundred contact allergens in appropriate patch test concentrations available at the allergy laboratory and the patch test results. At the request of dermatologists in practice the Allergen Bank may supply special contact allergens for aimed patch testing of contact dermatitis patients.

The organization of the Allergen Bank and the procedure of its use are described. During its first 23 months 28 dermatologists asked for 2,209 allergen samples for testing of 386 patients, an average of 6 allergens per patient and 14 patients per dermatologist. A total number of 164 positive reactions have been registered, and 440 of the 540 allergens have been in use. One third of the positive reactions were caused by the 16 most frequently ordered allergens, which amounted to 340 allergen samples. The allergens included plant chemicals, acrylates, animal feed additives, fragrance chemicals and preservatives.

Selected allergens were investigated for stability during handling and shipping under varying conditions relevant to the function of the Allergen Bank. The possible inhomogeneity of petrolatum based allergen preparations is discussed in relation to diagnostic patch testing. *Key words: contact dermatitis; patch tests; patch test materials; stability; contact allergy; occupational dermatitis; chemical analysis.*

(Accepted September 21, 1995.)

Acta Derm Venereol (Stockh) 1996; 76: 136–140.

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Contact allergy plays an important role in about 20% of the patients with hand eczema (1). The choice of patch test allergens is crucial for the evaluation of a contact dermatitis patient. The correct diagnosis of allergic contact dermatitis may be missed if the allergen in question is not tested. The failure of diagnosing a contact allergy may affect advice given to the patient, and consequently treatment and prognosis of the eczema. The fact that allergic contact dermatitis is often a complicating factor aggravating other eczematous diseases stresses the importance of choosing the right allergen for testing. The use of a standard series comprising the most common allergens is widely accepted. However, supplementary tests with properly diluted working materials and extra allergens selected on the basis of patient history and known exposure are often required to assure the correct diagnosis (2). A recent short communication showed that 5–23% of the patients in different clinics only had contact allergies to compounds outside the standard series (3). If contact allergies are diagnosed early and early intervention is carried out, the prognosis may be better.

According to de Groot (4) there are about 2,800 known contact allergens. Every year new ones are added to the list. Only the 24 most frequently occurring contact allergens are included in the standard series. Selected dermatology departments with special interest in contact dermatitis have a big stock of contact allergens available for routine use. The allergens may be bought from the suppliers or made up in petrolatum in the proper test concentrations at the hospital pharmacy. Against this background the allergen bank concept was developed. This paper describes the idea behind the Allergen Bank, which supplies extra contact allergens to dermatologists in practice, and the preliminary experience of its use. Further, the problems of stability of contact allergens during handling and shipping have been investigated. The possible inhomogeneity of the allergen preparations is discussed.

### MATERIALS AND METHODS

#### *The Allergen Bank*

The Allergen Bank was established through a grant from the county administration, with the purpose of improving the collaboration between the dermatologists in practice and the department of dermatology. The basic idea was to make available to the dermatologists in practice extra allergens, which can be ordered from the bank for testing specific patients guided by actual exposure histories. The bank serves the dermatologists working in counties referring patients to the hospital.

The Allergen Bank comprises three separate parts:

(1) A "bank" with about 540 numbered contact allergens obtained from different sources. The majority are bought from the commercial patch test suppliers, but many are prepared at the hospital pharmacy from chemicals or products obtained from manufacturers, working places etc. The contact allergens are kept in syringes in the absence of light at 4°C. A few allergens are stored in dark bottles in appropriate vehicles.

(2) A list of the contact allergens available from the Allergen Bank, which is sent to dermatologists in practice. The list is regularly updated and contains information on the contact allergens which pertains to either inclusion in a screening series or chemical groups. In addition, the allergens are listed in alphabetical order with generic names and synonyms included. The patch test concentrations and vehicles are given. The dermatologist lists the allergens required for the particular testing. The nurses working in the allergy laboratory register the orders and ship the allergens requested. Each allergen sample is filled in a small flat plastic container with a plastic lid, a spatula (Van der Bend, the Netherlands) and a cardboard fixed to it for registration of the content. This transport system is suitable for patch test material made up in petrolatum, and the container holds about 25–30 mg, sufficient for a Finn chamber. Liquid material is filled in small tight polyethylene vials. The allergen samples are then shipped to the dermatologist by mail. The dermatologist is asked to provide information on the individual patient to be tested with allergens from the bank. Information is required about age, sex, present and relevant previous occupation, previous patch test results and the localization of the eczema. After testing has been performed, the dermatologist



returns the form with the patch test results to the Allergen Bank for registration in the database.

(3) A software program developed by the computer department of the hospital based on a relational database (DSI System, Birkerød, Denmark). The program is commercially available and contains information about contact allergens available, supplier, concentrations, vehicles and day of purchase or manufacturing. Further, it contains a patient database developed for registration of the referring dermatologist, age, sex and clinical data of the patient as well as the patch test procedures and results. Information about the relevance of the patch test results is also listed when given. The program also contains a statistical package that makes it possible to extract patch test data in various ways according to our needs, such as the frequency of positive tests to an allergen, doubtful reactions, irritant reactions to individual allergens, and lists of patients with reactivity to an allergen. The data from the first 23 months of use are reported.

#### Stability of contact allergens

The stability and homogeneity of contact allergens prepared in various vehicles have previously been studied to a limited extent only (5). Many compounds are quite stable for years when kept in a refrigerator at 4°C, prepared in a suitable vehicle like petrolatum and stored in syringes. However, the stability and homogeneity may vary from chemical to chemical and from batch to batch (6, 7). A prerequisite for the value of an Allergen Bank is a high quality of the allergen samples shipped to the dermatologist for testing each patient. Therefore, we selected six allergens from the bank for chemical analysis using headspace gas chromatography (HSGC), as an appropriate method for analysing chemicals prepared in petrolatum. The allergen samples were investigated after varying conditions of storage. The selected chemicals exhibit varying vapor pressures as well as boiling points. At each sampling time 2 samples of the allergen preparations were placed in transport chambers, one was kept in the refrigerator at 4°C and one at room temperature, until shipping for analysis. Samples were examined following 8 days, 2 days and 1 day of storage. *Chemicals.* Cinnamic aldehyde, Hermal, BN 1301267922, exp. date 6.95; hydroxycitronellal, Hermal, BN 1304267922, mfg 06.93; eugenol, Hermal, BN 1302267922, exp. date 9.95; hydroxyethyl methacrylate, Chemotechnique, BN 88502A; benzoyl peroxide, Hermal, BN 13825, mfg 9.91 and carvone, hospital pharmacy FAS, BN 4119481, exp. date 3.96.

*Analysis.* HSGC of 20 mg allergen samples was performed as described previously (8, 9), except that the samples were preheated for a minimum of 6 h at 110°C before the chromatographic analysis. The prolonged heating time was chosen to ascertain equilibrium of allergen concentration between the gas phase and the sample matrix. The allergens, except benzoyl peroxide, will not degrade at 110°C. Production of benzene, the degradation product of benzoyl peroxide (10), was monitored for the stability test of benzoyl peroxide by HSGC. The area counts of major GC peaks of the allergens, stored for various time intervals, were compared for the elucidation of stability of these substances.

The single series of samples were analyzed in reversed order, i.e. the samples stored for 8 days were analyzed first, followed by the samples stored for 2 and 1 days, respectively, in order to eliminate possible effects due to varying preheating times before the chromatographic analyses. The carvone concentration in the day 1 sample was not analysed, as the sample was lost during handling.

## RESULTS

### Preliminary experience of the Allergen Bank

The Allergen Bank has been in operation since February 1st, 1992. During the first 23 months 28 dermatologists have asked for a total of 2,209 allergen samples for testing of 386 individual patients, corresponding to an average of six allergens per patient and 14 patients per dermatologist (range 1–162).

A total of 164 positive reactions have been registered (7%), and 440 of the 540 allergens (81%) have been applied. One third of the positive reactions (51/164) were caused by the 16 most frequently ordered allergens, which amounted to 340 allergen samples and a positivity of 15% (Table I). The allergens included plant chemicals, acrylates, animal feed additives, fragrance chemicals and preservatives. The remaining two thirds (113/164) of the positive reactions were caused by rarely ordered allergens.

The relevance of the reactions in relation to the dermatitis of the patients is not detailed in all cases. Further information from the patients and the dermatologists is necessary.

### Analytical results

The results of the chemical analysis are visualized in Fig. 1. Day 1 concentration (area count of the major GC peak) of an allergen is arbitrarily considered as 100%, and the variation in concentration (stability index, SI) during storage is depicted relative to day 1 concentration of the allergen. Considering the relative standard deviation of HSGC analysis to be  $\pm 10\%$  (8, 11), eugenol was found to be stable at room temperature during the storage period (8 days), whereas cinnamic aldehyde apparently was unstable at room temperature. The concentration of cinnamic aldehyde in the allergen sample decreased with the increasing storage period at room temperature. The concentrations of other target allergens during storage of the respective samples both at room temperature and 4°C were relatively higher than those at day 1. On the other hand SI's of these allergens were  $<150\%$  in all the cases, except for hydroxycitronellal stored for 8 days (SI 191%). Furthermore, 163% SI of benzoyl peroxide stored at room temperature for 8 days is due to higher benzene production (10). As the day 1 sample of carvone was not analysed the day 2 carvone concentration (room temperature) was used as 100% SI.

Table I. The 16 most frequently ordered contact allergens from the Allergen Bank

The number of orders of each allergen are shown, together with the frequency of positive reactions.

Allergen	Frequency of positive reactions/numbers ordered
Sesquiterpene lactone mix 0.1% pet	5/35
Methyl methacrylate 2% pet	3/28
Eugenol 1% pet	3/27
Tylan 5% pet	7/26
Carvone 5% pet	1/26
Spiramycin 10% pet	7/24
Lichen acid mix 0.3% pet	1/24
Cinnamic aldehyde 1% pet	2/19
Compositae extract mix (Hausen) 6% pet	8/18
Triethyleneglycol dimethacrylate 2% pet	2/18
Spearmint 5% pet	1/17
Ammonium persulphate 2.5% pet	2/17
Avoparcin lauryl sulphate 5% pet	5/17
Ethyleneglycol dimethacrylate 2% pet	2/16
Usnic acid 0.1% pet	1/14
Bronopol 0.5% pet	1/14
Overall	51/340



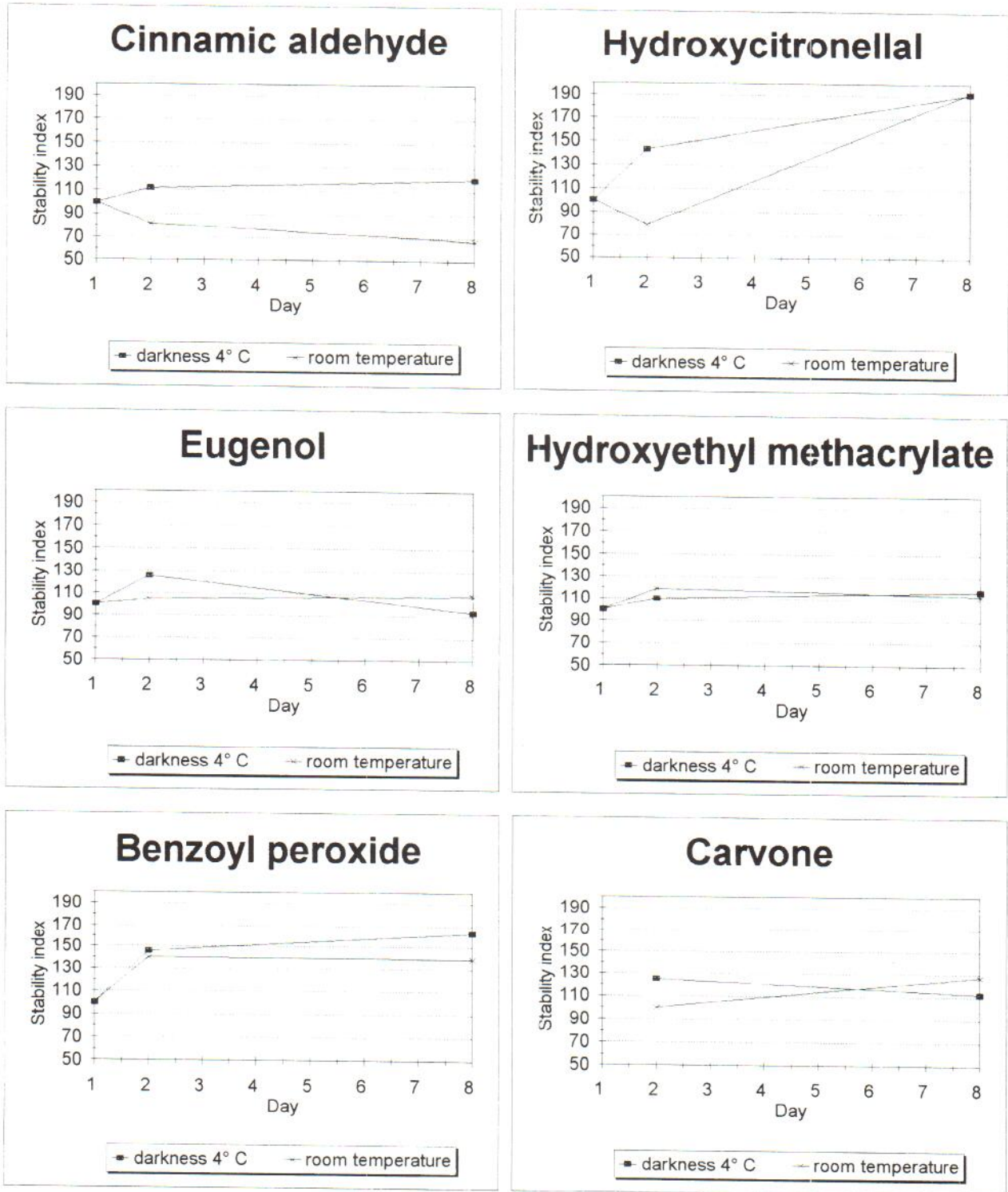


Fig. 1. Stability of allergen samples during varying storage conditions. Day 1 concentration is depicted as stability index 100, and the results of analyses of other samples are shown in relation to the concentration for day 1. Day 1 concentration for carvone was not tested.

DISCUSSION

The Allergen Bank service has been well received by dermatologists. Many of them practise in towns situated in rural areas with agricultural industry. The results (Table I) suggest that the animal feed additives in particular caused a number of

allergic contact dermatitis reactions in agricultural workers, and that the allergens are easily identified by exposure history combined with product information and subsequent testing (12).

The value of the Allergen Bank service for patients and dermatologists in practice remains to be evaluated. The fre-



quency of the draw from the Allergen Bank varies considerably from practice to practice depending on many factors. Among these are the number of contact dermatitis patients seen, the interest and training of the dermatologist, and the number of allergens, besides those of the standard series, in possession. Many dermatologists in Denmark have bought a limited number of extra allergens, which they find useful in their area. They order rare allergens only occasionally.

The Allergen Bank service may have certain advantages. A contact dermatitis patient may be tested with the relevant allergens soon after referral to the practice. This may improve the treatment and advice given by the dermatologist, as certain allergies may be discovered faster, leading to discontinuation of exposure. This may in certain cases lead to healing of the eczema (13). The availability of extra allergens may encourage the dermatologist to perform tests with standardized allergens instead of crude working materials based on the exposure history of the patient. Information about product ingredients is not always easy to obtain and it may take a relatively long time. In this respect the Allergen Bank may be an additional help. A further advantage is that patients far from the hospital may save time, reduce absence from work as well as travel expenses, when the allergens are made available to the dermatologist in his practice, since the alternative is referral of the patient to the dermatology department for further evaluation.

Most contact allergens are bought from the commercial patch test suppliers, and as such they are up to their standards. As a routine new allergens prepared in the hospital pharmacy are checked in the department on 25 consecutive controls to assure that they are not irritant. The allergens are renewed on a regular basis and preparations older than 5 years are discarded. The staff of the allergy laboratory gains experience with the preparations of the Allergen Bank and can to a certain extent advise the dermatologists regarding product information and the choice of contact allergens for a particular patient. An epidemic of a contact allergy in a certain geographical area due to the appearance of new techniques or products in a working place may be discovered sooner than before, since the patch test results are collected in the patient database and the statistics are evaluated regularly. The data collected in the Allergen Bank constitute a source of information for research and development in the field of contact dermatitis.

The allergens selected for the stability test represent both aliphatic and aromatic organic compounds. HSGC is the most appropriate technique for the analysis of allergen preparations prepared in petrolatum. It can only be used to analyse materials with a vapour pressure. The results of the analysis revealed that eugenol was stable at room temperature during the storage period of 8 days, but cinnamic aldehyde was not. For other target allergens, variations in the allergen concentration during storage, both at room temperature and at 4°C, were found only in the positive direction. This can possibly be explained by the inhomogeneity of the allergen preparations (5). A reliable and "true" test of stability of allergen preparations cannot be performed in such preparations before even distribution of the chemical in petrolatum is ascertained. Provided that these allergen preparations were stable, a variation up to 50% in the allergen concentration was revealed in most cases. This variability may appear high, but during daily routine patch testing a variation in the dose of allergen applied to the skin around 100% will not invalidate the procedure in the majority of cases, since most significant contact allergies in a

patient will show up in a patch test even when testing within this large variability.

The inhomogeneity of allergen samples is obviously a problem shared by all dermatologists using petrolatum-based preparations and not only related to the function of the Allergen Bank. However, many false positive, false negative, and non-reproducible patch tests may be explained by this variation in allergen content from sample to sample. The development of better patch test materials is necessary to avoid this problem, although great efforts and resources will be required to validate allergen preparations according to pharmaceutical standards. In many cases releasability of the allergen from the vehicle and homogeneity of the preparation should be ascertained, as has been done with the TRUE test® (14). However, for the more rarely used contact allergens it is utopian to request complex pharmaceutical testing prior to clinical use. You still need the experienced contact dermatologist who can examine the patient using all his/her senses, and according to availability carefully use crude patch test materials chosen from the depths of his/her experience. The contact allergic patients are not exposed only to pure, stable chemicals but to environmental compounds as they appear with all their mixtures and impurities. In the present study a preparation of methyl methacrylate was included. However, large variations in the SI (>600%) could be observed, which was ascribed to the inhomogeneity of the actual preparation. Similar variations have been reported before with nickel, partly due to variations in dosing of the allergen (15). The subject was not pursued further in the present context but it calls unambiguously for additional studies in order to develop techniques suitable for the preparation of homogeneous petrolatum-based allergens.

There are also some problems related to the use of the Allergen Bank. Patch test reading and interpretation may vary from practice to practice. This may be minimized through information and further training. The problem of economy is not yet solved. So far the Allergen Bank is financed by a research grant. It has to prove its value in a future study performed in close collaboration with selected dermatology practices, where patient records and follow-up details in relation to the outcome of patch testing can be evaluated. The conclusion of the stability studies performed here is that the dermatologist should order the allergen samples from the bank shortly before testing of the patient in order to minimize the time necessary for the allergen to be stored in the transport container, and that the samples should be kept in the refrigerator until the time of the test.

#### ACKNOWLEDGEMENT

Mrs. Gitte H. Jensen provided skillful technical assistance in connection with the HSGC analyses.

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