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The Guinea Pig Maximization Test—with a Multiple Dose Design

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The guinea pig maximization test (GPMT) is usually performed with one moderately irritant induction dose of the allergen and gives a qualitative assessment – hazard identification – of the allergenicity of the chemical.

We refined the GPMT by applying a multiple dose design and used 30 guinea pigs in a test divided into a control group and 5 test groups of 5 animals. Each group was treated with different induction concentrations of the allergens: formaldehyde, cinnamic aldehyde, propyl paraben, lidocaine, mercaptobenzothiazole or chlormethylisothiazolinone/methylisothiazolinone. The test results were analysed using a logistic multidose response model. The precision of the results depends only on the total number of animals, the dose design and the response pattern.

The maximal sensitization rate for a chemical was determined, and the intracutaneous induction concentration that sensitized 50% of the animals (EC_{50}) (or another percentage) was estimated. Further studies are needed to prove the validity of this idea. However, improvements in protocols for the GPMT are needed to reduce interlaboratory variability in results and to reduce the number of animals used for allergenicity tests. ***Key words: contact allergy; toxicology; animal experiments; dose response; formaldehyde; cinnamic aldehyde; propylparaben; lidocaine; mercaptobenzothiazole; chlormethylisothiazolinone/ methylisothiazolinone; Kathon CG.***

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The albino guinea pig is so far the laboratory animal of choice for evaluating the allergenic potential of contact allergens. The test results may vary considerably from laboratory to laboratory due to the use of different methods, animal strains and differences in the choice of test concentrations and vehicles (1, 2).

The new OECD guideline (Organisation for Economic Cooperation and Development) (# 406) for sensitization tests in laboratory animals (3) limits the number of recommended tests to two: the guinea pig maximization test (GPMT), an adjuvant method, and the Buehler test, which is a non-adjuvant assay. A minimum of 10 animals should be used in the treatment group and at least 5 animals in the control group. When fewer than 20 test and 10 control guinea pigs have been used, and it is not possible to conclude that the test substance is a sensitizer, testing in additional animals to give a total of at least 20 test and 10 control animals is strongly recommended (3). The new guideline also emphasizes the importance of the use of control animals, blind reading, repeated challenges and the choice of vehicle. These recommendations try to reduce interlaboratory variability of test results.

In the standard GPMT one single concentration is used for intracutaneous and topical induction. According to the OECD guideline the concentration used for each induction exposure should be well tolerated systemically and should cause mild to moderate skin irritation.

However, what is a mild or moderate irritant reaction in the skin? This is not further defined, and the literature shows considerable variation in the choice of induction concentrations for a chemical tested with a GPMT (4). The choice of a single induction concentration for a chemical may be fortuitous in relation to the concentration giving the maximal sensitization rate in a GPMT. This may be a significant cause of variation in results between laboratories and experimenters. For some chemicals, e.g. formaldehyde, the dose response relationship in a GPMT is non-monotonous or "bell shaped" (5, 6). This variation in methodology and lack of comparability may also lead to repeated tests to verify earlier results, and thus an unnecessary use of guinea pigs.

The ethics of animal experimentation is a high priority issue in the public debate today, and legislation concerning laboratory animals is being tightened up in many countries (7). Research is encouraged to develop alternative allergenicity test methods that can replace the animal methods used in this field of toxicology. With regard to predictive contact allergy tests there is no reliable *in vitro* assay available. The immunological mechanism behind contact allergy is very complex and the contact allergy requires a complete individual (animal or man) to develop. Within a foreseeable time *in vitro* tests will not be able to replace the use of animals for predictive allergy tests. Guinea pigs may in the future be replaced to some extent by mice. The murine local lymph node assay (LLNA) is a new promising method (8). However, in its present design substances with exclusively irritating properties could falsely be classified as allergens by the LLNA or, alternatively, the allergenicity of chemicals with both allergenic and irritant properties could be overestimated (9).

In the development of a new strategy for contact allergy testing we will still need to use guinea pigs to some extent. One important reason is that there is about 50 years' experience of this animal model, and new models must be compared to and validated against current routine methods, such as the GPMT. However, the GPMT method may be further improved in order to increase the reproducibility of the test and thus the value of the test.

In the Principles of Human Experimental Technique Russell & Burch (10) describe how methods applied constantly should be subject to improvements by **R**efinement of the method, **R**eduction of the number of individuals (animal or man) used, and **R**eplacement of "higher" animals with "lower" animals or *in vitro* techniques – the 3 Rs. The *in vitro* methods and the LLNA constitute an attempt to *replace* the use of guinea pigs. The purpose of the present study was to *refine* the GPMT by introducing modern statistical methods in the evaluation of the test results, and by that also to *reduce* the total number of guinea pigs used for contact allergy tests (11). We used a multidose response design for the GPMT to evaluate 6 contact allergens with varying allergenic potencies. Such designs do not necessarily lead to an increase in the total number of

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animals required for a test, because a decrease in the group size can counterbalance the increase in the number of groups (12).

MATERIALS AND METHODS

Chemicals

Freund's complete adjuvant (FCA) was purchased from Difco, Chicago, Illinois, USA. Propylene glycol was of analytical grade and purchased from Merck. Petrolatum or water was used for the patch tests.

The allergens were: formaldehyde pro analysi 37–38%, Merck, Darmstadt, Germany; mercaptobenzothiazole (MBT) from Sigma Chemical Co., St. Louis, USA; propylparaben from Sigma Chemical Co., St. Louis, USA, cinnamic aldehyde from Merck–Schuchardt, Hohenbrunn, Germany, and lidocaine from Nomeco, Denmark. Chlormethylisothiazolinone/methylisothiazolinone (Kathon CG) was a gift from Rohm & Haas.

Animals

Outbred albino female guinea pigs (Dunkin– Hartley, Møllegaard, Ll. Skensved, Denmark) weighing between 350 to 450 g at receipt were housed in groups of 2 or 3 in plastic cages. The animals were kept on a 12-h photoperiod at a room temperature of 21°±1 C, a relative humidity of 55%±5% with food and water available *ad libitum* (standard guinea pig pellets, Altromin® 3123, Chr. Petersen A/S, 4100 Ringsted, Denmark). Beech wood chips were used as bedding (Glamsbjerg Træindustri A/S, 5620 Glamsbjerg, Denmark). The animals were randomly assigned to test and control groups, ear marked and allowed to adapt for 1 week before use. Hair was removed by clipping and shaving. All animals were weighed once a week.

Guinea pig maximization test

The procedure described by Magnusson & Kligman (14) was followed, comprising intracutaneous induction with FCA on day 0; topical induction on day 7 and a subsequent challenge on day 21 by closed patch tests to the flank of the animal. However, pretreatment of the topical induction area with sodium lauryl sulphate was not carried out. Thirty animals were used in each test because it is the number of guinea pigs recommended for the performance of a GPMT with a chemical with low sensitizing potential (3).

Patch test technique: For topical induction, filter paper mounted on Leukoflex® (Beiersdorf AG) saturated with the chemical was used; Finn Chambers®(Epitest Ltd., Helsinki, Finland) on Scanpor® (Norgesplaster A/S, Oslo, Norway) were used for challenge. Two patches were mounted side by side, one with the challenge preparation and one with vehicle control. When liquid preparations were tested 2 pieces of filter paper were placed in the Finn Chambers® and filled to saturation. Liquid preparations were dosed using an Eppendorf Varipette 4710®, and petrolatum preparations were dosed manually using a 5-ml syringe.

Vehicles: Water was the vehicle of choice for all injections, as it mimics most human exposure situations. Propylene glycol was the alternative for chemicals not soluble in water: mercaptobenzothiazole, cinnamic aldehyde, and propylparaben. Water was used for the challenge with formaldehyde and Kathon CG, whereas petrolatum was used for the challenge with mercaptobenzothiazole, propylparaben, lidocaine and cinnamic aldehyde.

Concentration: The range of concentrations for induction and challenge to be used in a multiple dose GPMT was decided from a pilot study utilizing FCA-treated naive guinea pigs for each chemical (Table I). Reading was performed 3 and 24 h after removal of the patch test in the pilot experiments.

Dose response design: The 30 animals in each test were randomly divided into 5 test groups of 5 animals each receiving different concentrations of the allergens and one control group of 5 animals treated simultaneously during the induction phase with vehicle and FCA alone. As every animal in each of the 5 test groups received both an intracutaneous and a topical induction treatment, there is a possibility of using 5 different intracutaneous and 5 topical induction concentrations. Table II shows a complete 5 × 5 factorial design, which for practical reasons cannot be carried out with only 30 animals. The dose combinations shown in brackets are suggested as a fractional design, which still can detect whether the response depends on both dose factors, one of them or none. This scheme allows for the treatment of different groups with all 5 intracutaneous doses and the use of a high or low topical dose.

Table I. *Concentration range for induction and challenge*
Intracutaneous concentration (ic), epicutaneous concentration (ec)

	Induction	Challenge
Formaldehyde	0.03%–3% ic	
	0.1%, 10% ec	1% aq
Mercaptobenzothiazole	0.03%–3% ic	
	0.3%, 30% ec	10% pet
Cinnamic aldehyde	0.03%–3% ic	
	0.3%, 30% ec	3% pet
Propylparaben	0.1%–10% ic	
	0.3%, 30% ec	10% pet
Lidocaine	0.1%– 3% ic	
	0.3%, 30% ec	10% pet
Chlormethylisothiazolinone/ methylisothiazolinone (Kathon CG)	3–100 ppm ic	
	30, 3000 ppm ec	300 ppm aq

Reading: The challenge reactions were read blindly after 48 h and 72 h. The following grading scale was used: 0 = no visible change, 1 = discrete or patchy erythema, 2 = moderate and confluent erythema and 3 = intense erythema and swelling (14). A grade 1 reaction was not regarded as a positive challenge because discrete or patchy erythema frequently occurs in both test and control animals in this model, due to non specific irritation from clipping, shaving and bandaging. The number of sensitized animals (grade 2 and 3) in each group was used in the statistical analyses.

Statistical methods

The relation between the response probability p(x) and the dose x is according to the standard logistic dose response model given by: $\log(p(x)/(1-p(x))) = a + b\log(x)$, where a is the intercept and b is the slope of the linear logistic response versus log dose relation. The dose corresponding to a response probability of 0.5, i.e. EC_{50}, is given as exp(-a/b). The model is extended to describe the joint effect of two dose variables x and z by adding the term clog(z) to the right hand side of the above equation, where c represents a slope parameter analogous to b. By letting $\log(z) = (\log(x))^2$ the model is modified to describe a non-monotone dose response relation. A further extension is to allow for a non specific background response rate P(0) at dose 0 and a maximal response rate P(inf), which is the limiting rate as

Table II. *Multiple dose design*
The concentrations shown here are an example to illustrate the distribution of doses. The concentrations shown in brackets were used.

		Epicutaneous dose				
		0.1%	0.3%	1%	3%	10%
Intracutaneous dose	0.01%	[X]	X	X	X	X
	0.03%	X	X	X	X	[X]
	0.1%	[X]	X	X	X	X
	0.3%	X	X	X	X	[X]
	1%	[X]	X	X	X	X

the dose increases towards infinity. This model is defined by: $P(x) = P(0 + (P(inf) - P(0))p(x)$. The observed response rates at different dose levels are analysed by maximum likelihood estimation, and asymptotic likelihood ratio tests are applied to assess the significance of the parameters in order to determine the simplest model that gives the best fit. The general strategy for the dose response analysis is as follows: If there are no positive responses in the control group the model without P(0) and P(inf) is used with both intracutaneous and the topical dose variables. If the data allows for estimation of this model the results are compared with those of the simpler models with only one dose variable, and these are subsequently compared with the model that assumes constant response rate in all groups, i.e. no dose response relation. With the design shown in Table II it is also possible to estimate and test a non-monotone dose response model with respect to the intracutaneous dose. If the control group shows positive response it is necessary to use the extended model with background response. A PC program designed to analyse these multidose response models, version 1.0, Copyright STSC Inc. and the Danish Environmental Protection Agency, was used for analysis of the GPMT data (12). A generalisation of Fisher's exact test of the same response rate in all groups was carried out by means of the program StatExact®, copyright Cytel Soft Ware Corporation, Cambridge, MA, USA.

RESULTS

Formaldehyde

Table III shows the 48- and 72-h data for formaldehyde. The result suggests a non-monotone dose response curve, as seen previously (5). The data from both readings gives the best fit with a non-monotone logistic model. With the 48-h responses there is, however, no statistically significant dose response relation ($\chi^2(2) = 0.57$, $p > 0.05$). Fig. 1a shows the fitted dose response curve for the 72-h data. The topical dose was not significant for the sensitization rate, in accordance with previous studies (5).

Cinnamic aldehyde

Cinnamic aldehyde readings at 48 and 72 h are shown in Table IV. One control animal showed at 48 h a moderate erythematous response, considered non specific. The logistic model with both dose variables could not be estimated because 3 of the 6 groups showed maximal response at 48 h. Dose response analysis with background response and the intracutaneous dose only gave a good fit to the data, and the logistic model was highly significant ($\chi^2(2) = 17.8$, $p < 0.001$). Fig. 1b shows the fitted dose response curve for the 48-h data. Analysis with the topical dose only showed a significant lack of fit. The cinnamic aldehyde readings at 72 h gave only one group with an intermediate response between the minimal and the maximal response rates. Therefore, a dose response curve could not be estimated. Fisher's exact test showed a significant ($p = 0.0003$) difference in response between the control group and the pooled groups with cinnamic aldehyde.

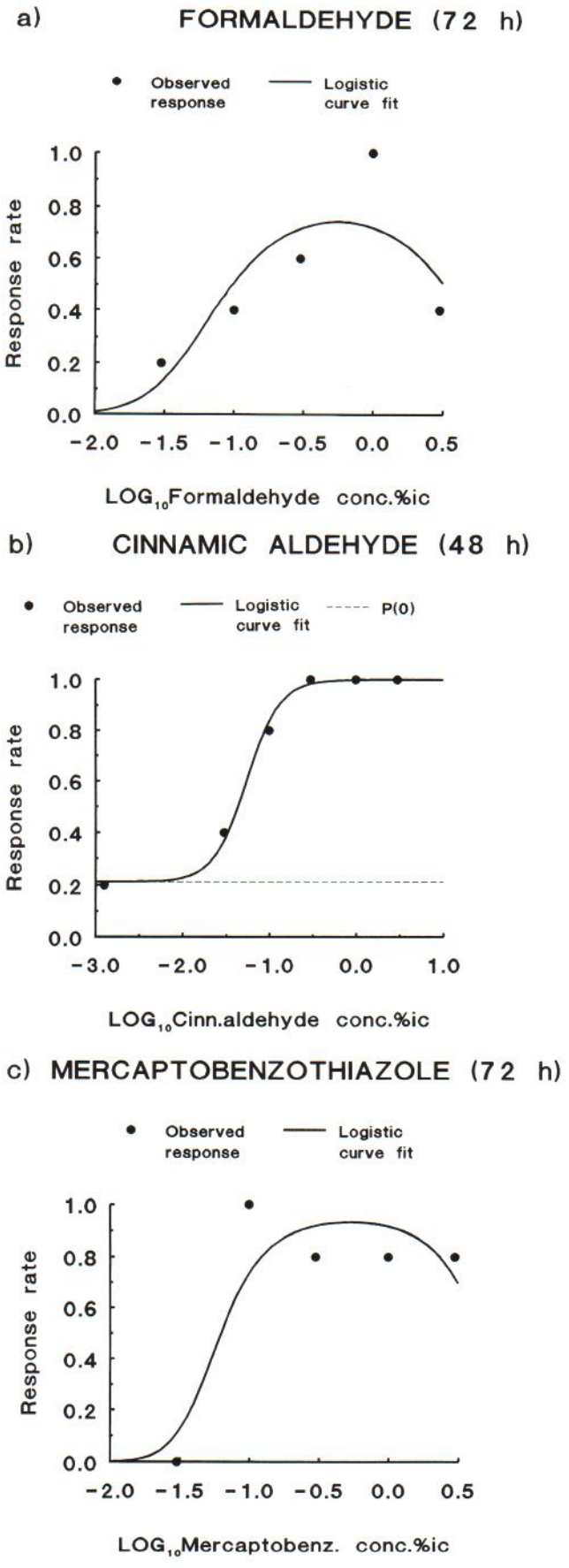

Fig. 1. GPMT with formaldehyde, cinnamic aldehyde and mercaptobenzothiazole. The dose response curves are shown for formaldehyde (*a*)(72-h readings), cinnamic aldehyde (*b*)(48-h readings) and mercaptobenzothiazole (*c*)(72-h readings). The response rate in relation to log intracutaneous dose is given. The dose response curves are based on the parameter estimates shown in Tables III, IV and V.

Table III. *Results of GPMT with formaldehyde*

Concentration (%)		Positive/total	
Intracutaneous	Topical	48 h	72 h
0	0	0/5	0/5
0.03	0.1	2/5	1/5
0.1	10	2/5	2/5
0.3	0.1	3/5	3/5
1	10	3/5	5/5
3	0.1	2/5	2/5
Parameter estimates:			
intracutaneous dose			
constant a (SE)		0.16(0.54)	0.96(0.60)
linear b (SE)		−0.23(0.50)	−0.40(0.52)
quadratic c (SE)		−0.13(0.18)	−0.35(0.22)
χ^2 dose response relation (df)		0.57(2)	5.10(2)

Fisher's exact test 72-h response $P=0.0033$

Table IV. *Results of GPMT with cinnamic aldehyde*

Concentration (%)		Positive/total	
Intracutaneous	Topical	48 h	72 h
0	0	1/5	0/5
0.03	0.3	2/5	4/5
0.1	30	4/5	5/5
0.3	0.3	5/5	5/5
1	30	5/5	5/5
3	0.3	5/5	5/5
Parameter estimates:			
intracutaneous dose			
P(0) (SE)		0.21(0.19)	dose response
constant a (SE)		6.60(3.97)	analysis not
linear b (SE)		2.28(1.60)	possible
χ^2 dose response relation (df)		17.8***(2)	

*** $P<0.001$; Fisher's exact test 72-h response $P=0.0003$

Propylparaben and lidocaine

These two chemicals showed no significant sensitization potential in this model. Only one animal in the lidocaine group reacted at 48 h. It is not possible to calculate a dose response relationship from these data. For lidocaine the maximal dose tolerated by the animals was 3% intracutaneously. Higher doses were lethal within minutes to 2 out of 6 animals during pilot experiments, probably due to the cardiac effect of lidocaine.

Mercaptobenzothiazole

MBT readings at 48 h are shown in Table V. The dose response curve with the intracutaneous dose is steep with a good fit, and the logistic model was highly significant ($\chi^2(1)=16.5$, $p<0.001$). The topical dose alone gives a significant lack of fit, and both doses cannot be fitted simultaneously. Inverse estimation of the dose sensitizing 50% of the animals (EC_{50}) gave a concentration of 0.09% with 95% confidence limits from 0.02% to 0.2%. The MBT readings at 72 h are also shown in Table V. The results suggest a non-monotone dose response relation with intracutaneous dose, and the statistical analysis confirms it. The linear logistic model shows a significant lack of fit ($\chi^2(3)=11.4$, $p<0.01$), while the non-monotone logistic analysis gives an acceptable fit ($\chi^2(2)=5.78$, $p>0.05$), and a significant dose dependence ($\chi^2(2)=10.6$, $p<0.01$) (Fig. 1c).

Table V. *Results of GPMT with mercaptobenzothiazole*

Concentration (%)		Positive/total	
Intracutaneous	Topical	48 h	72 h
0	0	0/5	0/5
0.03	0.3	0/5	0/5
0.1	30	4/5	5/5
0.3	0.3	4/5	4/5
1	30	5/5	4/5
3	0.3	5/5	4/5
Parameter estimates:			
intracutaneous dose			
constant a (SE)		5.03(2.20)	2.41(0.84)
linear b (SE)		2.05(0.89)	−0.71(0.63)
quadratic c (SE)			−0.57(0.27)
χ^2 dose response relation(df)		16.5***(1)	10.6**(2)

*** $P<0.001$; ** $P<0.01$

Table VI. *Results of GPMT with Kathon CG*

Concentration (%)		Positive/total	
Intracutaneous	Topical	48 h	72 h
0	0	1/5	0/5
3	30	5/5	5/5
10	3000	4/4	4/5
30	30	5/5	5/5
100	3000	5/5	5/5
100	30	4/5	5/5
Parameter estimates:			
intracutaneous dose			
P(0) (SE)		0.20(0.18)	
constant a (SE)		4.63(5.61)	1.78(2.12)
linear b (SE)		−1.67(3.90)	0.51(0.79)
quadratic c (SE)		0.25(0.62)	
χ^2 dose response relation (df)		11.3**(3)	0.45(1)

** $P<0.01$; Fisher's exact test 72-h response $P=0.0003$

Kathon CG

Kathon was, as expected, an extreme sensitizer in the GPMT. The readings at 48 and 72 h are shown in Table VI. The highest intracutaneous induction concentration was 100 ppm, based on the pilot experiments. One control animal showed at 48 h a moderate erythematous response considered non specific. Even the lowest concentrations (3 ppm ic and 30 ppm topically) sensitized all animals in the group. The dose response analysis of the 48 h readings required a non-monotone model in intracutaneous dose with a background response ($\chi^2(3)=11.3$, $p<0.01$). It corresponds to fitting an almost constant

significantly elevated response rate around 90% at doses above 0 and the background rate of 20% at dose 0. With the 72-h readings it is possible to fit a non significant monotone dose response curve that increases from about 80 to 90% over the dose range from 3 to 100 ppm. Fisher's test showed a significant ($p=0.0003$) difference in response between the control and the pooled Kathon CG-treated groups. The Kathon CG animals were rechallenged on day 35 with a concentration of 100 ppm to confirm the results from the first challenge on day 21. Chiefly, the same outcome was seen – all test animals were sensitized.

Table VII gives for all chemicals an overview of the test results based on the 48- and 72-h data, the fitted dose response curves and the inverse estimation of probabilities for sensitization.

DISCUSSION

The chemicals were chosen because they are allergens with a varying sensitization potential from extreme to very low, and for most of them results from standard guinea pig allergy tests have been published.

The total number of animals included in the test was equal to the requirements following the old OECD guideline 406 – i.e. 30 guinea pigs. Instead of getting a response frequency based on one single induction concentration it was possible to get a sensitization profile covering several induction doses.

The advantage of the logistic regression model is that data from all animals are included in a simultaneous analysis. The precision of the results depends only on the total number of animals, the dose design and the response pattern, and it is in this sense independent of sizes of groups given the same doses. This has been shown by Finney (13), who calculated the precision of bioassays based on binary responses and the logistic dose response model. It was for example shown that the same precision was achieved when doses of one drug were given to 2 groups (doses) of 12 animals, to 3 of 8 or to 4 groups of 6 animals and in each case compared to results obtained with another drug given to similar groups of animals. The larger number of smaller groups does, however, allow for testing of a wider range of doses and will give a better characterization of the shape of the dose response curve. This makes it preferable to the traditional analysis consisting of group wise comparisons. The method may be used for analysis of any dose response experiment giving data on a nominal or classificatory scale (15). We decided to accept grade 2 or 3 challenge reactions as evidence of sensitization because discrete or patchy erythema (grade 1) is often caused by irritation from clipping, shaving, bandaging and/or marginally irritating challenge preparations. Of course, if all controls are completely negative and the test animals show doubtful reactions, then the doubtful reactions may be relevant and an evidence of contact allergy. This phenomenon did not occur in this series of experiments. Repeated challenge with more than one concentration can solve this issue.

The dose response design was chosen to cover a dose range encompassing 2–3 decades. The choice of doses applied in the present study was decided from the pilot experiments, and a factor 3 increase in concentration was used from one dose level to the next. This choice of a factor 3 increase is based on the experience of several other allergens (16). A concentration range for induction which covers a factor 100– 300 is often used in sensitization experiments. There is a tradition in immunologic experiments to include concentrations which cover several decades from very low to the highest tolerable. However, for some chemicals a factor 10 increase from dose level to dose level might be applied, covering a total dose range of a factor 10,000. For other chemicals a factor 2 increase might be appropriate, at least within a certain dose range.

Previous GPMTs have shown that the intracutaneous induction treatment for many chemicals contributes most to the development of the sensitization. The statistical analysis showed for both nickel sulphate and formaldehyde in the GPMT that the topical induction gave no significant contribution to the sensitization rate (5, 17). The omission of pretreatment of the topical induction area with sodium lauryl sulphate may be an alternative explanation why the topical inductions seemed to be of minor importance. However, for chlorocresol the topical dose was important (18). The reasons for these differences between allergens are not clear and should be studied further. If the topical induction treatment could be omitted from the GPMT, the dose response design would be simplified and the protocol would correspond closely to the single injection adjuvant technique (SIAT) (19).

The shape of the dose response curve may be monotonous (linear) or non-monotonous ("bell shaped"). The dose response test with formaldehyde using 5 concentrations and 30 animals gave essentially the same "bell shaped" dose response curve as the larger experiment including 120 animals and 6 concentrations (5). When comparing the non-monotone and the monotone logistic models for formaldehyde (Table III) for the 72-h data, the difference between the χ^2 for goodness of fit was $7.3 - 4.32 = 2.98$ with 1 df. This is not statistically significant at the 5% level, and therefore one cannot draw the conclusion from the present data that the non-monotone logistic model is superior to the standard model. From a toxicological point of view this may not be very important,

Table VII.

Allergen	*Maximal Sensitization Rate*	EC_{50}[a]	'Threshold Conc.'[b]
Formaldehyde	0.8	0.96%	<0.03%
Cinnamic aldehyde	1	0.04%	<0.03%
Propylparaben	0	>10%	>10%
Mercaptobenzothiazole	0.9	0.07%	<0.1%
Lidocaine	0	>3%	>3%
Kathon CG	1	<3 ppm	<3 ppm

[a] EC_{50} is the intracutaneous concentration sensitizing 50% of the animals.
[b] 'Threshold concentration' is the lowest concentration which can sensitize the guinea pigs in a GPMT.

because the maximal sensitization rate and the EC_{50} may be decisive for labelling. However, the "bell shaped" dose response curve is of scientific interest and may cause a "false" low sensitization frequency in a standard test that has used a too high induction dose of the allergen. The concentration threshold for sensitization with formaldehyde in the GPMT was below 0.03%. The calculated maximal sensitization rate was about 0.8 equivalent to the result from former study. The EC_{50} was estimated to 1% and 0.2% after 48 and 72 h based on Table III. However, these estimates are not very precise since they are calculated from the fitted curves, which do not represent a statistically significant dose response dependence.

The strong sensitization potential of cinnamic aldehyde was confirmed. The dose response analysis (Table IV) showed that the intracutaneous dose is decisive for the response. The curve is very steep and the χ^2 for goodness of fit is 0.25 showing a good fit. The EC_{50} is 0.04% and the maximal sensitization rate is 100%. When the cinnamic aldehyde results are compared with the formaldehyde data it is seen that cinnamic aldehyde gives a steeper curve with a higher maximum sensitization rate, suggesting that it is a stronger allergen. The threshold concentration for sensitization is below 0.03% and data with concentrations below that are desirable.

In this study propylparaben did not sensitize one single animal in spite of a wide dose range used for induction and a high challenge concentration. Results of a rechallenge on the opposite flank 1 or 2 weeks later would have been interesting. However, rechallenge was not performed in this test. Likewise, lidocaine did not sensitize the guinea pigs. No animal data on lidocaine have previously been published.

Published GPMT data with MBT have shown a sensitization frequency of about 40% to 60%. The choice of induction concentration has varied from 0.4% to 1% intracutaneously and 10% to 25% topically. The challenge concentration was 10% and 15% (4). The present study confirmed that MBT is a strong allergen. The curve is steep with a good fit. The topical dose had little effect on the outcome as seen for formaldehyde and cinnamic aldehyde. The maximal sensitization rate was 0.9 to 1 depending on which reading (48 or 72 h) was used. The EC_{50} was 0.07% to 0.08%. For the 72-h readings the non monotonous logistic model gave the best fit.

The results with Kathon CG are shown in Table VI and confirmed the strong sensitizing potential of this preservative (20, 21). The concentrations used in the test are given in ppm active ingredient. Even the lowest concentrations 3 ppm intracutaneously and 30 ppm topically sensitized all animals. It would have been desirable to include further data with lower concentrations. The challenge concentration was 300 ppm, which is high compared to human experience. There was one positive control animal. However, the challenge concentration was decided from the pilot experiments using FCA-treated animals. Rechallenge with Kathon CG 100 ppm on day 35 confirmed the strong reactivity.

In conclusion, the multiple dose response design and the multiple dose response analysis program showed advantages in relation to the standard GPMT:

(1) Multiple doses are tested and a dose response relation is estimated when the intracutaneous and topical induction treatments are analysed separately or in combination.
(2) The maximal sensitization rate can be determined.
(3) The intracutaneous induction concentration that sensitizes 50% of the animals (EC_{50}) (or another percentage) can be estimated if the curve fit is good and the allergen is a strong sensitizer.
(4) The effect of intracutaneous and topical dose on the sensitization frequency can be evaluated.
(5) The statistical method can handle positive control animals.
(6) The laboratory animals are used in a more efficient way, because the test result allows for more detailed evaluation of the chemical.

Further studies with more chemicals are desirable to confirm the usefulness of the multidose response analysis, and more data are needed to determine if such dose response results can be used directly for regulatory purposes to classify allergens. The results from a GPMT dose response test cannot stand alone and must – as the standard GPMT – be validated by comparison to other sources of information, such as experience of the compound from limited use tests, complaint data from industry and consumers, epidemiologic data and diagnostic tests in dermatologic clinics (2).

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