

Dose-response studies of contact allergens using 3 guinea pig models

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A multi-dose-response induction protocol for the guinea pig maximization test (GPMT), including a statistical computer program, has earlier been developed to improve the power of predictive tests for identification of contact allergens. This dose-response protocol, with 2 modifications (i.e., increased number of animals in each group and increased number of challenge concentrations) was evaluated in the GPMT, the cumulative contact enhancement test (CCET) and the Freund's complete adjuvant test (FCAT), using potassium dichromate and hydroxycitronellal as model contact allergens. Application of the dose-response protocol on the CCET and the FCAT resulted in either monotone or non-monotone curves with significant dose-response. However, application of the dose-response protocol on the GPMT gave curves with no significant dose-response. The protocol makes it possible to obtain an EC₅₀ value, thus improving the possibility of ranking contact allergens, which is of substantial use for risk assessments. The dose-response protocol could benefit from a few adjustments: a wider span in the induction doses; change to simultaneous increase in intradermal and topical induction doses to obtain a proper dose-response for the GPMT; the addition of further challenge concentrations. In addition the computer program should allow calculation of threshold concentration for sensitization and EC₅₀ value for a non-monotone curve.

Key words: contact allergens; cumulative contact enhancement test; dose-response; Freund's complete adjuvant test; guinea pig maximization test; guinea pig; hydroxycitronellal; potassium dichromate; predictive testing; statistical analysis. © Munksgaard, 1999.

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The guinea pig has been considered to be the most suitable experiment animal in predictive testing for contact allergy and it has been used for decades. About 20 guinea pig protocols have been described and some have been compared and evaluated (1–5). The results from these tests correlate well with the sensitizing properties of chemicals in man (3–4, 6–9) and are generally accepted by regulatory authorities (10). In the first international guidelines set forth in 1959 the Draize test was included (11) to screen out potent sensitizers. Since then several predictive guinea pig test methods have been developed and in recent years predictive mouse test methods also have become available. The current guidelines, the updated version of OECD guidelines 406 for Testing of Chemicals (1992) (12), recommends 2 predictive guinea pig tests – the guinea pig maximization test (GPMT) (6–7, 13) and the Buehler test (14–16), and 2 pre-

dictive mouse tests – the murine local lymph node assay (LLNA) (17) and the mouse ear swelling test (MEST) (18–19).

The GPMT with the use of Freund's complete adjuvant (FCA) is the most extensively used predictive guinea pig method in Europe, whereas the Buehler test without FCA is the method of choice in the USA (4). GPMT has been shown in comparative studies to be more sensitive (2–4, 20) and to correlate better with results in humans (3–4) than the Buehler test. The most commonly used predictive guinea pig methods in our laboratory are GPMT, cumulative contact enhancement test (CCET) (21) and Freund's complete adjuvant test (FCAT) (8), depending on which route of exposure is deemed most suitable to answer the questions raised. GPMT entails both intradermal and topical applications, CCET only topical applications and FCAT only intradermal applications.

To improve further the power of predictive tests for contact allergens, a multi-dose-response induction protocol for the GPMT has been developed (22–25). It is combined with a statistical computer program, i.e., a logistic regression analysis, which uses all available test results in the analysis of the results (23). The program uses this logistic regression analysis to present fitted graphs for both monotone and non-monotone curves and calculates chi-square (χ^2) for goodness of fit to each curve and dose-response relationship. For some chemicals, a non-monotone dose-response relationship in GPMT has been established (22–26). Other parameters that may be of use for risk assessment can also be calculated.

The *aim* was to evaluate the multi-dose-response induction protocol for predictive tests of contact allergens developed for GPMT according to Andersen et al. (23), and also the application of the protocol in 2 other animal models, the CCET and the FCAT. Potassium dichromate ($K_2Cr_2O_7$) and hydroxycitronellal (HC), 2 chemicals with well-known allergenic potential, were used as model contact allergens for the evaluation. 2 modifications of the protocol were introduced: increased number of guinea pigs in each experiment group and increased number of challenge concentrations.

Materials and Methods

Animals

The experiments were carried out on outbred female Dunkin Hartley guinea pigs (average weight 300–350 g) from AB Sahlins Försöksdjursfarm, Malmö, Sweden. They were housed in groups of 3 in Macrolon® cages on hardwood chips bedding under controlled environmental conditions. Pelleted standard diet (SDS pellets from AB Sahlins Försöksdjursfarm, Malmö, Sweden) and water were available ad libitum. The guinea pigs were allowed to acclimatize for at least 7 days prior to the first exposure.

The studies were approved by the local ethical committee.

Chemicals

The chemicals tested were potassium dichromate ($K_2Cr_2O_7$) (p.a.) purchased from E. Merck, Darmstadt, FRG, and hydroxycitronellal (HC) (95%) from K&K Laboratories, Division of ICN Biomedicals, Inc., Cleveland, Ohio, USA. Arachis oil (AO) and olive oil (OO) were purchased from Apoteksbolaget, Stockholm, Sweden. Physiological saline was purchased from Kabi Pharmacia AB, Stockholm, Sweden; the Freund's complete adjuvant (FCA) was from Difco Laboratories, Detroit,

Michigan, USA. All chemicals were used as delivered.

Application of a multi-dose-response induction protocol

To evaluate the sensitizing potency of the model contact allergens with the multi-dose-response induction protocol (23), 3 predictive test methods were used: GPMT, CCET and FCAT. In each experiment the animals were divided into 6 groups with 5 different induction concentrations and 1 sham-treated control group (Table 1). The number of animals in each group varied from 5 in the initial study to up to 9 in following studies, due to modifications in the protocol. Up to 7 challenge concentrations and 1 vehicle control on every animal were used, instead of a single dose and 1 vehicle control according to the original protocols. The animals were numbered individually and randomly distributed to the cages. The hair was removed with electric clipper and shaver at induction and challenge exposure sites. 8 series (369 animals in 48 groups) were performed.

Irritancy threshold studies. $K_2Cr_2O_7$ and HC, respectively, were applied topically on the flanks for 24 h using closed patch tests on 3 FCA-treated animals. The minimal irritant concentration was 0.3 % for $K_2Cr_2O_7$ and 100% for HC. The non-irritant concentration was 0.1% for $K_2Cr_2O_7$ and 10% for HC. These concentrations were chosen as the highest for topical induction and for challenge, respectively. In an irritancy threshold study, where HC was injected in 3 animals, the highest tolerable concentration for intradermal application was 3%. The highest tolerable concentration for intradermal application of $K_2Cr_2O_7$ was 0.3% – based on earlier experience (unpublished data).

Induction. The model contact allergens were applied at 5 different concentrations in 5 of the 6 induction groups respectively. Vehicle only was applied to the control groups. The concentrations are reviewed in Table 1. The inductions were done according to the various methods as described in detail below.

Challenge and re-challenge. On day 22, patch testing with the allergens was performed on the flanks using aluminium Finn Chambers 8 mm Ø (Epitest Ltd Oy, Tuusula, Finland) on Scanpor® (Norgesplaster AS, Norway) and acryliclastic bandage (Beiersdorf, FRG) for 24 h. The patches were randomly distributed to avoid bias from differences in location. Test reactions were read blindly at 48 and 72 h after application. Readings at 72 h were chosen for the calculation of dose-response relationship. The minimum criterion for a positive reaction was a confluent erythema (++) (27). Re-

Table 1. Concentration ranges for allergens used at induction and challenge.

Series	Animal group	Method	Allergen	Induction conc. (%)		Challenge conc. top. (%)	Re-challenge conc. top. (%)
				i.d.	top.		
I	1–6	GPMT	K ₂ Cr ₂ O ₇	0.003–0.3	0.01; 1	0.01–0.1	–
II	7–12	GPMT	K ₂ Cr ₂ O ₇	0.0003–0.03	0.01; 1	0.01–0.1	0.01–0.1
III	13–18	CCET	K ₂ Cr ₂ O ₇	–	0.01–1	0.0003–0.3	0.003–0.3
IV	19–24	CCET	K ₂ Cr ₂ O ₇	–	0.0003–0.03	0.003–0.3	–
V	25–30	FCAT	K ₂ Cr ₂ O ₇	0.0001–0.01	–	0.0003–0.3	–
VI	31–36	GPMT	HC	0.03–3	1; 100	0.01–10	–
VII	37–42	CCET	HC	–	1–100	0.01–10	–
VIII	43–48	FCAT	HC	0.03–3	–	0.01–10	–

Concentrations in % (w/w) were increased by a factor of 3 giving a dose range of 10, 100 or 1000. Potassium dichromate (K₂Cr₂O₇) in saline at induction and challenge. Hydroxycitronellal (HC) in arachis oil at induction and in olive oil at challenge. Intradermal concentrations (i.d.) and topical concentrations (top.). GPMT: guinea pig maximization test. CCET: cumulative contact enhancement test. FCAT: Freund's complete adjuvant test.

challenge was done in 2 series. The concentrations used at challenge and re-challenge are summarized in Table 1.

Guinea pig maximization test (GPMT)

The sensitizing potency of the allergens was studied by using the GPMT method in accordance with the original protocol (6), and with the modifications mentioned. The concentrations used are summarized in Table 1. The first exposure at induction (day 0) was made by 3 pairs of intradermal injections of 0.1 ml in the shoulder region on each animal. The 1st pair was for K₂Cr₂O₇ a mixture of FCA and saline (1:1) (w/w) and for HC a mixture of FCA and water (1:1) (w/w). The 2nd pair was the chemicals dissolved at 5 different concentrations in the vehicle or the vehicle alone and was injected close to the FCA mixture. The 3rd pair was an emulsion of K₂Cr₂O₇ in saline and FCA (1:1) (w/w) or an emulsion of HC in FCA and water (1:1) (w/w) with the same total concentrations as in the 2nd pair, and was injected posterior to the 2 previous injections. 1 week later (day 7) a 2nd exposure of 0.2 ml of K₂Cr₂O₇ or HC solution in the vehicle was applied topically to the same area (each application: 2×4 cm) for 24 h using an occlusive dressing with filter paper (Whatman, UK) on Blenderm® (3M, St. Paul, MN, USA) and acrylic bandage. This topical exposure was applied at 2 concentrations, 1 higher and 1 lower, alternating between the groups in each experiment series, starting with the lower topical concentration in the group with the highest intradermal concentration applied (25). 2 series with different induction concentrations for K₂Cr₂O₇ were done. Patches with dilution series of K₂Cr₂O₇ or HC and the vehicles were applied at challenge. Re-challenge was done in the 2nd series with K₂Cr₂O₇ at the same concentrations 2 weeks after the 1st challenge.

Cumulative contact enhancement test (CCET)

The allergens were studied by following the original description of CCET (21) with the modifications mentioned. The concentrations used are summarized in Table 1. Induction was carried out by 4 topical applications (days 0, 2, 7 and 9) of 0.2 ml test substance in the shoulder region (each application: 2×4 cm) for 24 h using an occlusive dressing as described in the GPMT method. K₂Cr₂O₇ and HC dissolved at 5 different concentrations in the vehicle and the vehicle alone were used. FCA was administered prior to the 3rd topical treatment (day 7) as 2 0.1 ml intradermal injections on each side of the application site. 2 experiments with different induction concentrations for K₂Cr₂O₇ were done. Patches with dilution series of K₂Cr₂O₇ or HC and the vehicles were applied at challenge. Re-challenge was done in the 1st series with K₂Cr₂O₇ at the same concentrations 5 weeks after the 1st challenge.

Freund's complete adjuvant test (FCAT)

The model contact allergens were studied with the FCAT as described originally (8), with the modifications mentioned. The concentrations used are summarized in Table 1. Induction was carried out by giving 3 intradermal injections (days 0, 6, 10) of 0.1 ml of K₂Cr₂O₇ dissolved in saline and emulsified with FCA (1:1) (w/w) or 0.1 ml of HC dissolved in FCA and emulsified with water (1:1) (w/w). Patches with dilution series of K₂Cr₂O₇ or HC and the vehicles were applied at challenge.

Statistical analysis

The results were analyzed using the statistical PC computer program "Program for multi-dose-response analysis", i.e., a logistic regression analysis, included in the multi-dose-response induction pro-

tolocol developed for the GPMT (23). This program calculates and adapts the best fitted monotone or non-monotone curve to the observed response rate in the experiment. It also calculates chi-square (χ^2) for goodness of fit to the curve and dose-response relationship.

The threshold concentration at sensitization was chosen empirically to be at least the lowest possible response rate of 1 out of 8 animals = 0.125, which was used in the analysis. The estimated concentration sensitizing 50% of the animals (EC_{50}) was calculated by the program. The maximal sensitization rate was decided to be the highest number of animals with positive reactions at the chosen induction and challenge series. Since the program failed to calculate the threshold concentration and EC_{50} for the non-monotone dose-response curves, the curves were cut at the point where they started to decrease (at the maximum). This procedure results in monotone curves with fewer induction concentrations, from which it is possible to calculate the mentioned parameters. As the program is not able to calculate the parameters in the analysis of the results from the GPMT where 2 induction

routes, intradermal and topical, are taken into account, the calculations were based only on the intradermal administration.

Results

Sensitizing capacity of the allergens in the guinea pig tests

Both contact allergens resulted in a clear sensitization of the exposed animals in all 3 methods used. There was good agreement between the results from the 48 h and 72 h readings. A typical result with the allergens using the multi-dose-response induction protocol at sensitization and challenge is exemplified in Table 2 with HC at the 72 h reading using the CCET. A variable degree of dose-response was seen both in the challenge concentration gradient and in the induction concentration gradient.

In the initial experiments with $K_2Cr_2O_7$ in the GPMT and the CCET (nos. I and III, Table 1) no dose-response was seen, since the majority of animals were sensitized by all the induction concentrations used (data not shown). Lower induction

Table 2. Results from the cumulative contact enhancement test (CCET) (series VII, Table 1) using the multi-dose-response induction protocol.

Animal group	n	Induction conc. ^{a)} (%)	Challenge conc. ^{b)} (%)							
			10	3	1	0.3	0.1	0.03	0.01	00
Exposed										
37	8	100	8	5	3	1	1	0	2	0
38	8	30	7	7	4	0	1	0	0	0
39	7	10	7	7	4	2	0	0	1	0
40	8	3	7	6	4	1	0	0	0	0
41	8	1	5	3	4	0	1	1	2	0
Control										
42	8	AO	2	2	1	1	2	1	1	1

The number of animals with positive test reactions at 72 h after application is given. Numbers in bold were used for dose-response analysis (Table 4, Fig. 2). Allergen: hydroxycitronellal (HC). ^{a)}Induction: HC in arachis oil (AO) topically (w/w). ^{b)}Challenge: HC in olive oil (OO) topically (w/w).

Table 3. Results from sensitization experiments using the multi-dose-response induction protocol in the GPMT, CCET and FCAT.

Animals	K ₂ Cr ₂ O ₇ challenge conc.						HC challenge conc.					
	0.03% in GPMT		0.1% in CCET		0.1% in FCAT		3% in GPMT		3% in CCET		10% in FCAT	
	group	no. pos/ tested	group	no. pos/ tested	group	no. pos/ tested	group	no. pos/ tested	group	no. pos/ tested	group	no. pos/ tested
Exposed	7	3/4	19	5/9	25	7/8	31	2/8	37	5/8	43	6/8
	8	4/5	20	7/9	26	6/8	32	4/8	38	7/8	44	4/8
	9	3/5	21	3/9	27	5/8	33	3/8	39	7/7	45	3/8
	10	2/5	22	1/8	28	3/8	34	5/8	40	6/8	46	1/8
	11	0/5	23	1/8	29	3/8	35	2/8	41	3/8	47	0/8
Control	12	2/5	24	0/8	30	1/8	36	0/8	42	2/8	48	0/8

The data shown were those fitting best to one of the model curves and they were used for logistic regression analysis (Table 4, Figs. 1, 2). The number of positive animals/number of tested animals at challenge for each induction group are given. Further details are presented in Table 1. Allergens: potassium dichromate ($K_2Cr_2O_7$) and hydroxycitronellal (HC). GPMT: guinea pig maximization test. CCET: cumulative contact enhancement test. FCAT: Freund's complete adjuvant test.

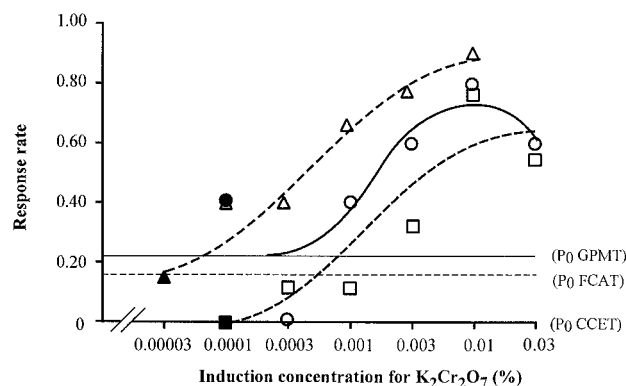


Fig. 1. Dose-response curves at constant challenge concentrations for potassium dichromate ($K_2Cr_2O_7$) using the multi-dose-response induction protocol in the GPMT (○), CCET (□) and FCAT (△). Test results from the 72 h reading were used for the analysis. Data from Tables 1, 3. The $\log_{10}$ for the vehicle control concentration 0% (filled symbols) is not defined, but in the graph assigned a value by the computer program at the onset of each curve by a factor of 3 from the lowest induction concentration used in each experiment. P_0 =baseline for statistical calculation.

concentrations were used in following experiments, resulting in various numbers of positive reactions in each series (Table 3). Re-challenge with $K_2Cr_2O_7$ was done in the GPMT in 1 series (no. II, Table 1) and in the CCET in 1 series (no. III, Table 1), which confirmed the sensitization results from the initial challenge (data not shown).

Dose-response analysis

The results from each sensitization experiment chosen for the dose-response analysis are summarized in Table 3. The best fitted dose-response

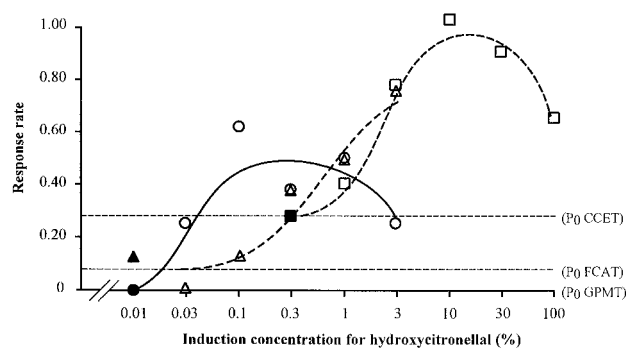


Fig. 2. Dose-response curves at constant challenge concentrations for hydroxycitronellal (HC) using the multi-dose-response induction protocol in the GPMT (○), CCET (□) and FCAT (△). Test results from the 72 h reading were used for the analysis. Data from Tables 1, 3. The $\log_{10}$ for the vehicle control concentration 0% (filled symbols) is not defined, but in the graph assigned a value by the computer program at the onset of each curve by a factor of 3 from the lowest induction concentration used in each experiment. P_0 =baseline for statistical calculation.

curves to the data for each of the methods, GPMT, CCET and FCAT, are shown for $K_2Cr_2O_7$ in Fig. 1 and for HC in Fig. 2. The fitted curves from the results with $K_2Cr_2O_7$ were non-monotone for the GPMT and the CCET and monotone for the FCAT. The fitted curves from the results with HC were non-monotone for all 3 sensitization methods. A significant dose-response relationship was found for the fitted curves from the CCET and the FCAT for both $K_2Cr_2O_7$ and HC. No statistically significant dose-response relationship was seen for the curves fitted from the GPMT with either $K_2Cr_2O_7$ or HC.

The χ^2 values for goodness of fit to the curve and dose-response relationship for the GPMT

Table 4. Results from sensitization experiments in guinea pigs using the multi-dose-response induction protocol.

Allergen	Method ^{a)}	Challenge conc. ^{b)}		χ^2_{FIT} (df) ^{c)}	χ^2_{D-R} (df) ^{d)}	Max. sens. rate ^{e)} (no. pos./no. exp.)	EC ₅₀ ^{f)}		Threshold conc. ^{g)}	
		(%)					(%)	(molal)	(%)	(molal)
$K_2Cr_2O_7$	GPMT ^{h)}	0.03		3.53(2) ns	6.00(3) ns	4/5	2.5×10^{-3}	8.5×10^{-5}	4.4×10^{-6}	1.5×10^{-7}
	CCET	0.1		2.96(2) ns	9.34(2) **	7/9	4.2×10^{-3}	1.4×10^{-4}	6.6×10^{-4}	2.2×10^{-5}
	FCAT	0.1		0.28(3) ns	3.36(2) **	7/8	4.5×10^{-4}	1.5×10^{-5}	1.1×10^{-7}	3.7×10^{-9}
HC	GPMT ^{h)}	3		1.64(2) ns	1.94(2) ns	5/8	6.8×10^{-2}	4.0×10^{-3}	1.6×10^{-2}	9.3×10^{-4}
	CCET	3		1.18(2) ns	15.14(3) **	7/7	1.8	1.1×10^{-1}	0.31	1.8×10^{-2}
	FCAT	10		1.96(2) ns	14.93(3) **	6/8	0.89	5.2×10^{-2}	7.2×10^{-2}	4.2×10^{-3}

Test results from the 72 h reading are used for the analysis; data from Tables 1, 3. Allergens: potassium dichromate ($K_2Cr_2O_7$) and hydroxycitronellal (HC).

^{a)} All methods followed the multi-dose-response induction protocol with a challenge concentration constant.

^{b)} Challenge concentration for the curve chosen.

^{c)} Value for the goodness of fit to the curve. If χ^2 is not significant (ns) then the curve is accepted.

^{d)} Value for the dose-response relationship; ** $p < 0.01$ or not significant (ns).

^{e)} Maximal sensitization rate. The maximal number of animals with a positive reaction/number of animals exposed.

^{f)} Calculated induction concentration sensitizing 50% of the animals, which is given in % (w/w) and molal (mol/kg).

^{g)} Lowest induction concentration that can sensitize, which is given in % (w/w) and molal (mol/kg). Chosen empirically to be at the least the animal response rate $1/8 = 0.125$.

^{h)} Calculated values using results from the GPMT method were based only on the intradermal administration.

GPMT: guinea pig maximization test. CCET: cumulative contact enhancement test. FCAT=Freund's complete adjuvant test.

were statistically non-significant for either $K_2Cr_2O_7$ or HC (data not shown) when both induction routes were used in the analysis. The challenge concentration, the χ^2 values for goodness of fit and dose-response relationship, the maximal sensitization rate, the EC_{50} and the threshold concentration for these 6 curves are summarized in Table 4.

Discussion

In the present study, a multiple dose-response induction protocol was applied to 3 predictive guinea pig methods, the GPMT, the CCET and the FCAT, using $K_2Cr_2O_7$ and HC as model contact allergens. The original descriptions of these methods do all state that predictive testing should be performed using 2 groups of 30 animals altogether, consisting of an exposed group and a control group (6, 8, 21). To get more information from each experiment it has been suggested that the animals should be divided into 6 smaller groups with 5 animals in each and induction brought about according to a multi-dose-response induction protocol (23). With the help of logistic regression analysis of these dose-response data using a PC computer program, additional information of value for risk assessment can be derived (23). Multiple concentrations have been used in many previous studies, at induction (28–29), at challenge (30–31) or at both (8, 32–35) and a dose-response relationship is generally seen. Without the aid of a logistic regression analysis computer program, however, much information is lost. Visualization of the results is usually done graphically but identification of specific concentrations from those figures is sometimes difficult (26, 33). The multi-dose-response induction protocol presented by Andersen et al. has been applied in one other independent study using the GPMT with Tween[®] 80, without receiving a significant dose-response (36).

The original recommendations for *induction concentrations* in the multi-dose-response induction protocol (23) were followed in the initial experiments (Table 1). When testing $K_2Cr_2O_7$ in the FCAT, a monotone dose-response curve (Table 1, Fig. 1) could be fitted to the challenge data as earlier shown with the multi-dose-response induction protocol on GPMT with cinnamic aldehyde (23–24), mercaptobenzothiazole (23) and methyl methacrylate (25). However, when testing $K_2Cr_2O_7$ in the GPMT and the CCET (Table 1), the results indicated that the induction concentrations applied had been too high, as no dose-response relationship at sensitization could be found. Almost maximum sensitization was attained in all exposed groups (data not shown). This was also shown for

Kathon[®] CG, another potent sensitizer, using the same multi-dose-response induction protocol (23–24). In a 2nd experiment with lower induction concentrations a non-monotone dose-response curve could be fitted for the CCET (Table 1, Fig. 1). Exposing guinea pigs to HC, the induction concentrations recommended gave non-monotone dose-response curves with both the CCET and the FCAT (Table 1, Fig. 2) consistent with earlier published results using the GPMT with formaldehyde (22–25), mercaptobenzothiazole (23–24) and nickel sulfate (26). However, exposure to $K_2Cr_2O_7$ (Table 1, Fig. 1) and HC (Table 1, Fig. 2) using the GPMT gave fitted curves which were inconsistent, and the results were equivocal, as no significant dose-response could be seen. This is in contrast to previously published studies, with this multi-dose-response induction protocol using the GPMT, where dose-response curves could be fitted (22–26).

A dose-response relationship could be fitted to all *challenge concentration* gradients used in all 3 methods with both allergens in the induction concentration intervals studied. Dose-response in challenge has been shown earlier (8, 30–35) and points at the importance of choosing an adequate challenge concentration if only 1 is used as recommended in the protocol.

In spite of the efforts to reduce the influence of *irritant reactions*, occasional reactions due to irritation were seen at challenge test sites in the control animals and in the vehicle controls which may have influenced the results and are a matter of concern (37–38). The irritant reactions in the control groups slightly influenced the base level (P_0) in the data assessment (Table 2, Figs. 1–2). The results from the GPMT study with $K_2Cr_2O_7$ (Tables 1, 3) using 5 animals in each group showed a fitted curve with a dose-response relationship, however the dose-response was statistically non-significant due to some irritation observed in the control group. This brought about the increase to at least 8 animals in each experiment group.

The *choice of method* for predictive testing may be influenced by regulatory requirements recommending a specific method, by the amount of test substance available or by an intention to imitate the natural exposure of a chemical to the skin. Several predictive tests to detect the allergenic potential of chemicals have been in use for many years, with humans (39) as well as experiment animals such as guinea pigs and mice (2, 40) as subjects. Great effort is being made to refining and replacing such models, and the development of in vitro methods is currently in progress (41). Quantitative structure-activity relationship (QSAR) studies (42) is a new approach to predict the potential of contact allergens. However, only limited

data are available concerning some groups of chemicals. DEREK (Deductive Estimation of Risk from Existing Knowledge) is one expert system (computer program), which predicts potential skin sensitizers by identification of structural alerts (43). However, the risk of inducing contact allergy is related not only to the inherent allergenic potential (sensitization capacity) of a chemical. Another important factor is penetration into the skin, which depends on the physico-chemical properties of the substance, concentration, occlusion, status of the skin, metabolism of the substance and duration of exposure (13).

As CCET and FCAT have only 1 induction route, administration of the test chemical in an increasing concentration gradient according to the multiple-dose-response induction protocol, a dose-response curve with a good fit to the data and a significant dose-response can be obtained. However, GPMT has 2 induction routes, intradermal and topical, which may be the cause of the inconsistency we noted. According to the protocol (23) the intradermal induction is given in a linearly increasing concentration gradient, which provides a sensitization with a dose-response relationship. However, also according to the protocol (23), the topical induction does not follow a gradually increasing concentration gradient; instead 2 concentrations (1 higher and 1 lower) alternating between the groups in the induction-series are given, which strongly influenced the results with HC. The topical administration thus led to an inconsistent statistically non-significant dose-response in the GPMT for this substance. Our interpretation is that the *influence of intradermal and topical exposure* respectively varies with the chemical under study, as also discussed elsewhere (22).

There was no clear pattern in *sensitivity of the 3 methods* using the multi-dose-response induction protocol with the model contact allergens (Table 4). The FCAT was the most sensitive test for $K_2Cr_2O_7$ and gave the highest response rate and the lowest EC_{50} and threshold concentration. For HC the highest sensitization rate was seen in the CCET. However, the lowest EC_{50} and threshold concentration was seen with the GPMT (Table 4).

The *multi-dose-response induction protocol* with the logistic data analysis adds a powerful tool to predictive tests. However, a small increase in number of animals in each experiment group, compared to what is recommended in the protocol, minimizes the influence of occasional irritant reactions and makes the predictions more reliable (44). The protocol is easily applied to the CCET and the FCAT where the logistic models can be readily fitted to the data. For the GPMT, the topical doses at induction may interact with the logistic model

and the computer program would benefit from a modification where a linear increase in the topical application is included as for the intradermal administration at induction, as previously suggested (26). Analysis of the results from the GPMT was based only on the intradermal administration to make it possible to estimate EC_{50} , the threshold concentration for sensitization and for comparison of the different animal models, because these data could not be calculated by the program for 2 induction application routes. One important advantage of this multi-dose-response induction protocol, compared to standard single dose administration, is that it minimizes the risk of using an induction concentration that is too high. The result could then end up in the down slope portion of the non-monotone dose-response curve, passing the concentration which gives the maximal sensitization rate. The concentration protocol for dose-response studies could preferably be altered with induction doses lowered, when necessary. The use of several patch test concentrations at challenge minimizes the risk of receiving a weak elicitation reaction or no reaction at all in sensitized animals. The available computer program should be modified to allow calculation of threshold concentration for sensitization and EC_{50} for non-monotone curves.

In conclusion, the multi-dose response induction protocol gives better knowledge about the dose-dependency of the sensitizing potential of the test substance. Furthermore, calculating an EC_{50} value, an opportunity not provided by the original methods, improves the possibility for proper ranking of contact allergens and augments the information used in risk assessment.

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