A study of chromium induced allergic contact dermatitis with 54 volunteers: implications for environmental risk assessment

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Abstract

Over the past 60 years, dose-response patch test studies by various methods have been conducted in an attempt to identify the minimum elicitation threshold (MET) concentration of hexavalent chromium (Cr(VI)) that produces an allergic response in Cr(VI) sensitive subjects. These data are not adequate, however, to provide an accurate estimate of the MET because of the variability in the patch testing techniques and the variability in diagnostic criteria used. Furthermore, the data were not reported in terms of mass of allergen per surface area of skin (mg Cr/cm²-skin), which is necessary for conducting occupational or environmental health risk assessments. Thus the purpose of this study was to determine the MET (mg allergen/cm²) for Cr(VI) and trivalent chromium (Cr(III)) by patch testing techniques. A patch test method that delivers a controlled amount of allergen per surface area of skin was used. A group of 54 Cr(VI) sensitised volunteers were patch tested with serial dilutions of Cr(VI) and Cr(III) to determine the cumulative response rate at several concentrations. The results indicate that the 10% MET for Cr(VI) based on the cumulative response was 0.089 µg Cr(VI)/cm²-skin. Only one of the 54 volunteers may have responded to 33 µg Cr(III)/cm²-skin, otherwise Cr(III) was unable to produce allergic contact dermatitis in these highly sensitive volunteers. Two supplemental studies were also conducted to assess whether the surface area of the patch and the concentration of Cr(VI) in the patch (related to patch thickness) were likely to influence the results. The data from these studies were used to assess the risk of developing allergic contact dermatitis due to contact with Cr(VI) and Cr(III) in soil. The findings indicated that soil concentrations at least as high as 450 ppm Cr(VI) and 165 000 ppm Cr(III) should not pose an allergic contact dermatitis hazard for at least 99.99% of the people in the community who might be exposed.

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Hexavalent chromium Cr(VI) is one of the most common dermal sensitisers in the occupational setting and accounts for about

5% of clinically reported cases of allergic contact dermatitis in the United States.¹ Cr(VI) related allergic contact dermatitis has been reported in chromium plating workers,^{2,3} lithographers,⁴ diesel repair shop workers,⁵ and leather workers.⁶ Additionally, Cr(VI) in household products such as bleaches and detergents, cosmetics, and shoe polish has been cited as a potential cause of allergic contact dermatitis.^{1,7} In many areas, there can be appreciable human exposure to chromite ore processing residue (COPR) and mine tailings when either is mixed with soil.⁸

Cr(VI) induced allergic contact dermatitis is known as a type IV, delayed, or cell mediated allergic reaction.^{12,9} The localised biological response of allergic contact dermatitis is similar to a "poison oak" hypersensitive reaction, and elicits the standard symptoms of erythema, oedema, and small vesicles.^{1,9,10} Type IV allergic dermatitis reactions are most often not life threatening and their effect is generally limited to the skin. Sensitisation is a threshold response where single or repeated exposure to low doses of an allergen may not produce an allergic response if the threshold dose is not reached.⁸ Epidermal contact with high concentrations of Cr(VI) can also produce irritant contact dermatitis, which is a heterogeneous symptom wherein a chemical induces a non-immunological dermatitis.¹¹⁻¹³ A cutaneous irritant causes direct damage to the skin without prior sensitisation.^{1,12}

It has been known since 1925 that dermal contact with Cr(VI) can elicit allergic dermatoses.¹⁴ Due to the strong sensitising potential of Cr(VI) and the desire to reduce the incidence of allergies in workers, several studies involving patch testing have been conducted to determine the "minimum elicitation threshold" (MET) of Cr(VI) in sensitised persons.¹⁵⁻²¹ In those studies, people known to be Cr(VI) sensitive were tested with patches containing serial dilutions of Cr(VI) (usually as potassium dichromate (K₂Cr₂O₇)) in petroleum jelly, water, and/or acid-glycine.

Dose-response studies of dermal sensitisers conducted before 1988 were much less complex than those used today. For example, much of the existing patch test data for Cr(VI) were collected before the improved and standardised diagnostic criteria developed by the North American Contact Dermatitis Group (NACDG)²² and the International Contact Dermatitis Research Group (ICDRG).²³ One deficiency in prior studies is believed to be that some irritant reactions were scored as an allergic response. Early

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reports of Cr(VI) patch test data often failed to disclose information regarding the diagnostic criteria to determine allergy, duration of application, and the analytical methods used to validate the chromium concentration and valency state. Also, it is known that the patch preparation methods were inconsistent and that interpatch variability of the amount of Cr(VI) applied could be as high as an order of magnitude.²⁴ In some of these previous studies,¹⁵⁻²¹ a patch test was simply a gauze pad that was immersed into the aqueous Cr(VI) solution before application, an open area of skin covered with tape, or a measured amount of Cr(VI)-petroleum mixture loaded into Finn chambers. At least two recent papers have attempted to identify a MET for Cr(VI)-induced allergic contact dermatitis from these studies. Based on a statistical analysis of patch test data, it has been suggested that 10 ppm Cr(VI) was the MET at the 10% response level.^{25,26} Paustenbach *et al*²⁷ examined the data from the same studies and suggested that the 10% MET should be about 50 ppm Cr(VI) in solution.

Certainly, the most important deficiency in these studies with respect to their usefulness in environmental risk assessment is that the data were not reported in terms of mass of allergen per unit area (for example, mg/cm²). As has been discussed elsewhere, by contrast with using patch tests to identify those chemicals to which a person is sensitised (a qualitative decision), in order to perform health risk assessments patch testing data must be presented in terms of mg of chemical per skin area.^{24,28-31} For example, when determining acceptable surface concentrations for toxicants on walls, process equipment, or in soils, uptake per unit area of skin (for example, mg/cm²-skin) is needed.³² This is important because at least one regulatory agency has suggested that allergic contact dermatitis is a proper health endpoint for regulating levels of Cr(VI) in soils.²⁵ Because the existing data do not seem to provide an accurate estimate of the Cr(VI) MET and the data are not adequate to quantitatively predict the health risks related to Cr(VI) in contaminated media, we conducted a carefully controlled study that used dose per unit area as dosimetric index.

This paper describes the results of a patch test study designed to determine the area based MET ($\mu\text{g Cr/cm}^2$ skin) of solubilised Cr(VI) and Cr(III) that will elicit allergic contact dermatitis in chromium sensitised subjects. Under the direction of several members of the NACDG, a group of 54 volunteers known to be sensitised to Cr(VI) were patch tested with serial dilutions of Cr(VI) and Cr(III). To reduce the variability inherent in

earlier patch preparation methods, "TRUE-Test" patches were specifically manufactured for use in this study.

Materials and methods

In general, as the water solubility of a Cr(VI) salt increases, its ability to penetrate the skin barrier and elicit allergic contact dermatitis increases.³³ Due to its high degree of water solubility, K₂Cr₂O₇ is one of the most penetrating and therefore most potentially reactive Cr(VI) species.^{8,34} Potassium dichromate is currently used by members of the NACDG and ICDRG as the standard Cr(VI) patch test agent for diagnostic purposes.^{23,35,36} Accordingly, for the purposes of this study, K₂Cr₂O₇ was chosen as the test Cr(VI) compound. Trivalent Cr compounds are not routinely used for clinical patch testing because Cr(III) is considered to have little or no allergic potential.³⁷⁻³⁹ This is due, in part, to the low water solubility of most Cr(III) compounds and the long history of uneventful occupational exposure.^{3,33,40} Chromium trichloride (CrCl₃) was used in this study because it is one of the most water soluble Cr(III) species.³⁴ Chromium trichloride has also been used in some earlier patch testing studies.^{41,42}

TRUE-Test (thin layer rapid use epicutaneous test) gel matrix patches were manufactured by Kabi Pharmacia Research Center AS, Inc, Hillerød, Denmark. Table 1 presents the patch concentrations of K₂Cr₂O₇ and CrCl₃ used in this study. Patches containing K₂Cr₂O₇ were prepared by mixing K₂Cr₂O₇ (purity 98.5-101.5%) with a wet hydroxypropyl cellulose gel to the specified concentrations. The patches containing CrCl₃ were prepared by mixing CrCl₃ with a wet polyvidone gel to the appropriate concentrations. These gels have little or no sensitising potential.^{24,36,43} The allergens were mixed to a specified mass of Cr(VI) or Cr(III) per unit area, printed on a sheet of polyester, and dried to a thin film. These coated water impermeable sheets were then cut into square patches of 0.81 cm², mounted on a piece of adhesive, non-allergenic tape, and packaged in an airtight and light impermeable envelope. The K₂Cr₂O₇ test patches were also sealed with desiccant paper to prevent adsorption of moisture.

The TRUE-Test patches are specifically designed to hydrate by perspiration when taped to the skin under occlusion. The dried film is hydrated into a gel thickness of 50 to 70 μm from which the allergen migrates to the skin. The hydrated gel, occluded by adhesive backing and plastic, ensures maximal contact with the skin, thus enabling high allergen bioavailability.³⁵ With this approach, the allergen is evenly distributed over the test area and the quantitative dose of allergen challenge is accurately controlled.^{24,30,44} This provides a significant advantage over other current techniques, such as the Finn chamber, in which the mass of allergen loaded on to the skin may vary by up to an order of magnitude from patch to patch.²⁴

Table 1 Chromium (Cr(VI) and Cr(III)) concentrations used in the patch testing study

Potassium dichromate ($\mu\text{g K}_2\text{Cr}_2\text{O}_7/\text{cm}^2$)	Concentration per unit area ($\mu\text{g Cr(VI)}/\text{cm}^2$)	Chromium trichloride ($\mu\text{g CrCl}_3/\text{cm}^2$)	Concentration per unit area ($\mu\text{g Cr(III)}/\text{cm}^2$)
12.5 (diagnostic)	4.4	100	33.0
2.5	0.88	20	6.6
0.5	0.18	10	3.3
0.25	0.088	2	0.66
0.05	0.018		

ANALYTICAL VALIDATION OF PATCH CONCENTRATIONS

Analytical confirmation of the patch concentrations was conducted before performing the study to ensure that each patch contained the appropriate valency and concentration of Cr. The concentrations of Cr(VI) in the patch were determined with Modified NIOSH Method 7600⁴⁵ as the extraction procedure and inductively coupled-visible absorption spectrometry (IC-VAS)⁴⁶ to measure the solubilised Cr(VI). Modified NIOSH Method 7600 is essentially a modified version of EPA Method 3060,⁴⁷ with slight differences in preparation and buffer solutions. A single patch was immersed in 25 ml of extraction solution (1.7 g of 0.2 M sodium bicarbonate in 1.0 litre distilled water). The polyethylene bottle was capped and ultrasonicated for about three hours, at which time the bottles were removed and placed in a rotating shaker for 48 hours at room temperature. The extractant was then injected into an ion chromatograph where the Cr(VI) was eluted with an ammonium sulphate and ammonium hydroxide eluent mixture. After elution from the column, the Cr(VI) was reacted with 1,5-diphenylcarbohydrazide (DPC) and the peak area absorbance of the coloured DPC-Cr(VI) complex was photometrically measured at 520 nm. The CrCl₃ patches were also analysed by the modified NIOSH Method 7600/IC-VAS to ensure that Cr(VI) was not present in the Cr(III) patches.

The concentrations of Cr(III) in the patches were determined by EPA Method 3050⁴⁸ for extraction and EPA Method 6010⁴⁹ for analysis. A modified EPA Method 3050 was used to extract the Cr(III) patches. Specifically, single patches were added to 1.5 ml of 0.1 M sodium hydroxide and heated to 85°C for one hour, followed by the addition of 300 µl 30% hydrogen peroxide and heat for another hour. Two millilitres of 0.1 M hydrochloric acid were added to the digestant after heating and the solution was cooled to room temperature. Another 2.2 ml 0.1 M hydrochloric acid was then added to the solution and the volume was adjusted to 25 ml with purified water. The patch digestants were then analysed for total Cr (as CrCl₃) by EPA Method 6010, or Inductively Coupled Plasma (ICP). The EPA Method 6010 measures element emitted light by optical spectrometry at 267.7 nm. After the CrCl₃ is digested, the ICP column is calibrated with stock solutions of 2 ml (1:1) HNO₃ and 10 ml (1:1) HCL and diluted to 100 ml with type II water. The digestant is directly injected into the column (about 2 ml) through the torch and a direct reading is taken from the instrument.

Representative patches of each Cr concentration were analysed in duplicate to evaluate interpatch variability. As part of the confirmation analyses, matrix spikes and matrix spike duplicates (MS/MSDs) were analysed by spiking selected patches with 10 µl of K₂Cr₂O₇ at a concentration 10 times that of the patch concentration to assess recovery. The

K₂Cr₂O₇ patches analysed by modified NIOSH 7600/IC-VAS were within 89–101% of the theoretical Cr(VI) concentrations. Also, the MS/MSDs averaged 121% and 111.5% for the 4.4 µg Cr(VI)/cm² and 0.018 µg Cr(VI)/cm² patches with reported relative percentage differences (RPDs) of 2% for both. Therefore, the patch concentrations were within Contract Laboratory Procedure (CLP) criteria for acceptable variability (± 25%). Additionally, the K₂Cr₂O₇ patches analysed for total Cr using EPA Method 6010 were within 88–97% of the theoretical total Cr concentrations. The MS/MSDs measured for the 4.4 µg Cr(VI)/cm² patch were both 106%. We analysed the data by SYSTAT version 5.0 by the paired *t* test with a 95% confidence level and the data sets were not statistically different. Hence, the Cr(VI) concentrations measured were considered to be stable in the hexavalent form and in the appropriate amounts.

The concentrations of total Cr in the Cr(III) patches ranged from 89%–105% of the theoretical concentrations. The MS/MSDs measured for the 33 µg Cr(III)/cm² concentration were 90% and 80% respectively. The 0.33 µg Cr(III)/cm² MS/MSD concentrations were 110% and 113%, respectively, with reported RPDs of 3% for both. The Cr(III) patches were also analysed for Cr(VI) by the modified NIOSH 7600/IC-VAS method and none was found. In summary, these analyses were reproducible and within the CLP acceptable range. Blank patches were also analysed for Cr(VI) and Cr(III). No Cr was detected on either patches using reported limits of 0.00025 mg/cm² for Cr(III) and 0.000015 mg/cm² for Cr(VI). Representative patches were reanalysed about every three months, and, in every instance, the appropriate valency states and concentrations did not change (less than 5%) throughout the duration of the study (about one year).

STUDY DESIGN

Patch concentrations

The Cr(VI) patch concentrations used in this study were chosen based on our best estimates of a range that would provide a maximal (100%) response at the highest concentration and a minimal response (< 10%) at the lowest concentration. This is similar to the design of other patch testing studies.²⁸ Based on the theoretical underpinnings of the dose-response relation⁵⁰ our review of the literature suggested that an approximate 250-fold range of Cr(VI) concentrations from 0.018 to 4.4 µg Cr(VI)/cm² would yield useful results. Similarly, for Cr(III), a 50-fold range of concentrations (0.66–33 µg Cr(III)/cm²) was chosen based on previously published data.⁵¹

Patch testing strategy

The patch testing study was designed to occur in three rounds. In the first round, all subjects were tested with a diagnostic Cr(VI) patch (4.4 µg Cr(VI)/cm²) to confirm that all volunteers in the study were allergic to Cr(VI).

Those who were confirmed as sensitised in round one were then tested in round two with the two lowest Cr(VI) concentrations and all four Cr(III) concentrations. If the subjects responded to both of the Cr(VI) patches or only to the higher concentration ($0.088 \mu\text{g Cr(VI)/cm}^2$), a threshold was considered to have been identified and the subjects did not complete round three. If no response occurred at either of the two low Cr(VI) concentrations, then the subjects were tested in round three with the two higher Cr(VI) concentrations. For each subject, the lowest Cr(VI) concentration at which a positive response occurred was considered to be the MET for that subject.

The rationale for testing with low Cr(VI) concentrations initially, followed by higher concentrations if necessary, was to minimise the incidence of "false positives" and "excited skin syndrome" that can occur when multiple patches are applied to the subject's skin in a single dosing.⁵²⁻⁵⁴ Indeed, many of the earlier reported positive responses to low Cr(VI) concentrations almost certainly were false-positives resulting from multiple testing.^{8 52-55} Patch testing with Cr(III) was performed in one round of testing (round two) as the Cr(III) patches were not expected to elicit a response in most of the subjects. All test patches were supplied to the physicians under code to double blind the study. The purpose of blinding was to achieve unbiased readings of the test results by the study physicians. Only one physician evaluated each patient. The physicians received approval from their respective human use committees as appropriate.

Population size

Before starting the study, an analysis was performed to estimate the total number of subjects required to achieve acceptable statistical power. Our goal was to identify a lower threshold at which no more than 10% of the Cr(VI) sensitive subjects would respond with 90% confidence. A figure of 10% was used since a number of dermatologists indicated that it would be very difficult to accurately identify through testing a 1%-5% value because 10% is probably very close to the threshold dose for all people (including the most sensitised). Estimating the number of subjects needed for the study required an assumption about the number of responders at a particular test concentration. Based on results of previous reports⁵⁶ we concluded that about 50-80 subjects would be required to meet these criteria.

It was anticipated that a number of the subjects may have actually had irritant, atopic, or "excited skin syndrome" reactions,

rather than a true allergic contact dermatitis response during previous testing and that about 80% of the initial subjects would, on round one testing, show a strong positive response to Cr(VI). Of these, it was estimated that at least 75% of sensitised persons would consent to participate in the proposed studies and complete the required testing rounds. The drop out rate for these subjects was not known, but was not expected to exceed 10%-20%. In summary, to have a study population of no less than 50 subjects, it was determined that we needed to find about 100 Cr(VI) sensitised volunteers.

PARTICIPATING PHYSICIANS AND STUDY POPULATION

Participating physicians

Six practising dermatologists conducted the clinical aspects of the study (Dr Robert Adams, Dr Joseph Fowler, Dr James Marks, Dr Charles Morton, Dr James Nethercott, and Dr James Taylor). They also participated in the study design.

Study population

More than 6000 patient files from various dermatologists (who specialised in patch testing) were examined before 100 possible volunteers were identified. Eventually, a group of 113 potential subjects were found by the participating physicians, of which 102 eventually took part in the study (11 subjects dropped out due to personal reasons). All were believed to be Cr(VI) sensitised based primarily on previous clinical patch tests performed by these physicians. As presented in table 2, this initial study population consisted of 78 men (76%) and 24 women (24%). All were over 18 years of age. Persons taking immunosuppressive or steroidal medications and pregnant women were excluded from the study. Subjects with eczema at the scheduled time of testing were not tested until the dermatitis subsided and it was requested that topical steroids not be used for two weeks before testing. All volunteers provided their doctors with written consent to participate in the study.

Patient questionnaire

Before initial testing, each patient filled out a medical and occupational history questionnaire. Each questionnaire was screened by a qualified person to insure proper completion. The medical history discussed in the form included incidence, type, and duration of past present dermatitis and other known allergies including asthma and any other skin problems or sensitivities. The questionnaire also asked for history of jobs held and corresponding duration, as well as any known or potential exposure to Cr in the workplace. Use of over the counter medications and vitamins was also recorded. The purpose of collecting this information was to assess whether previous exposure to Cr compounds was a significant factor in individual METs.

Information on allergic and atopic dermatitis was available for all of the 102 subjects.

Table 2 Description of Cr(VI) sensitised volunteers who participated in the study

Sex	No originally patch Tested	No who qualified and participated	Range of ages (y)	Average age (y (SD))
Men	78	39	24-74	45.6 (12.6)
Women	24	15	25-59	39.6 (10.3)

Table 3 Summary of occupations of the 54 Cr(IV) sensitised volunteers who participated in the study

Occupation	No of people
Accounting	2
Art dealer	1
Brick mason*	3
Carpenter	2
Carpet layer	1
Cement mason/finisher*	4
Truck driver (concrete)*	1
Core driller*	1
Electrician	1
Engineer	2
Gardener	1
Horse trainer	1
Insulation installer	1
Lab technician	2
Mechanic	1
Medical assistant	1
Photoengraver*	3
Plumber (retired)	1
Print estimator	1
Production manager	2
Publisher	1
Real estate associate	1
Retired	5
Saw cutter	1
Secretary	5
Self employed	1
Student	1
Teacher educator	3
Tyre changer	1
Transit operator	1
Warehouse worker	1
Unemployed	1

*Construction related jobs.

Present or past atopic dermatitis was present in 15% of those who completed the study (eight people). This is in keeping with previous data relating to the prevalence of allergic contact dermatitis. Only 22% of the volunteers worked in the construction industry or a related field and a significant number of participants had no known previous occupational exposure to Cr(VI) (table 3).

PATCH TESTING PROCEDURE

In each round of testing, all patches were applied to the upper sides of the back at 7 cm apart and fixed with Scanpor or paper tape for total occlusion with each patient serving as his or her own control. The patches remained in place for 48 hours, at which time they were removed and readings were taken then and at 96 hours. Test sites with positive reactions after the 96 hour exposure duration were photographed. For each patch in each round, the physicians recorded one of the following responses:

- 1 = Weak (no vesicular) reaction: erythema, infiltration, papules (+)
- 2 = Strong (oedematous or vesicular) reaction (++)
- 3 = Extreme (spreading, bullous, ulcerative) reaction (+++)
- 4 = Doubtful reaction, macular erythema only (?)
- 5 = Irritant morphology
- 6 = Negative reaction (–)
- 7 = Not tested

For the purposes of this study, any degree of positive response (1–3), including a very weak response, was considered to be positive. This is consistent with current NACDG criteria for patch test interpretation.^{3,5}

Round one testing took place between June and September 1992. The first round con-

sisted of testing subjects with a TRUE-Test diagnostic patch concentration of 4.4 µg Cr(VI)/cm² and a control patch (containing only hydroxypropyl cellulose) to verify sensitisation to Cr(VI). The patches were removed after 48 hours and an initial reading of the test site was recorded. The final reading, along with photographs of the test sites, was taken 96 hours after patch application. Those who developed an allergic response were considered to be Cr(VI) sensitised and qualified for round two testing of the study. Three weeks after round one was completed, those with definite positive reactions were tested in round two.

Round two testing took place between July and October 1992. The second test round consisted of patch testing volunteers with 0.018 and 0.088 µg Cr(VI)/cm² and all four Cr(III) concentrations (0.66, 3.3, 6.6, and 33 µg Cr(III)/cm²). Control patches consisting of blank hydroxypropyl cellulose and polyvidone were also tested. The patches were removed after 48 hours and an initial reading of the test site was recorded. The final reading, along with photographs of the test sites, was taken 96 hours after patch application.

Round three testing took place between August and December 1992. Those volunteers showing responses to both 0.018 and 0.088 µg Cr(VI)/cm² or to only the 0.088 µg Cr(VI)/cm² patch concentrations were not tested in round three. Those who did not respond to either of the two lowest Cr(VI) concentrations were tested in the third round with the two highest Cr(VI) concentrations (0.18 and 0.88 µg-Cr(VI)/cm²). Any who had anomalous results in round two (positive reaction to 0.018 Cr(VI)/cm² but not to 0.088 µg Cr(VI)/cm²) were retested in round three with all four Cr(VI) concentrations. The round three patches were removed after 48 hours of application and an initial reading of the test site was recorded. The final reading, along with photographs of the test sites, was taken 96 hours after patch application. All volunteers tested in round three were given a three week interval between round two and round three testing.

Patch test results

Round one

Of the 102 people who were initially selected for the study, only 54 responded positively (+, ++, or +++) to the diagnostic Cr(VI) patch (4.4 µg Cr(VI)/cm²). This response rate (47%) was far less than the projected estimate of 80%, but still yielded enough people (54) to provide statistically significant results. The 54 subjects who proceeded to round two of the study consisted of 39 men (72%) and 15 women (28%). The men ranged in age from 25–74 (mean 47.9) years; the women from 25–59 (mean 41.2) years.

Round two

In round two, four of 54 subjects (7%) responded positively to 0.088 µg Cr(VI)/cm² but not to 0.018 µg Cr(VI)/cm². Accordingly, a

Table 4 Cumulative dermal response of 54 Cr(VI) sensitised volunteers to various concentrations of Cr(VI)

Cr(VI) ($\mu\text{g}/\text{cm}^2$)	Minimum elicitation threshold response (%)	Cumulative response (%)
0.018	1/54 (2)	1/54 (2)
0.088	4/54 (7)	5/54 (9)
0.18	5/54 (9)	10/54 (19)
0.88	22/54 (41)	32/54 (59)
4.4	22/54 (41)	54/54 (100)

Table 5 Cumulative response of 54 Cr(VI) sensitised volunteers to various concentrations of Cr(III)

Cr(III) concentration ($\mu\text{g}/\text{cm}^2$)	Cumulative response (%)
0.66	0/54 (0)
3.3	0/54 (0)
6.6	0/54 (0)
33	1/54 (0)*

*This person, on retest, did not respond to this concentration of Cr(III).

MET of $0.088 \mu\text{g Cr(VI)}/\text{cm}^2$ was recorded for these four people and they were not required to complete round three. Only one of 54 (2%) responded positively to both $0.018 \mu\text{g-Cr(VI)}/\text{cm}^2$ and $0.088 \mu\text{g Cr(VI)}/\text{cm}^2$. Accordingly, a MET of $0.018 \mu\text{g Cr(VI)}/\text{cm}^2$ was recorded for this person and he was not required to complete round three. Forty nine of the 54 subjects did not respond to either concentration and, therefore, proceeded to round three. One person had a weak response at the highest Cr(III) concentration tested ($33 \mu\text{g Cr(III)}/\text{cm}^2$). On retesting to confirm the weak response, this person failed to elicit a positive response to $33 \mu\text{g Cr(III)}/\text{cm}^2$. The remaining 53 volunteers also failed to respond positively to any of the Cr(III) concentrations.

Round three

In round three, one of the 49 subjects responded to both 0.88 and $0.18 \mu\text{g Cr(VI)}/\text{cm}^2$. Accordingly, the MET for this person was recorded as $0.18 \mu\text{g Cr(VI)}/\text{cm}^2$. Also, 22 subjects responded to $0.88 \mu\text{g}$

Cr(VI)/ cm^2 , but not $0.18 \mu\text{g Cr(III)}/\text{cm}^2$, and therefore $0.88 \mu\text{g Cr(VI)}/\text{cm}^2$ was recorded as the MET for those subjects. Twenty two of the volunteers tested in round three had no response to either concentration. Because these volunteers did not respond to any patch concentration less than the diagnostic patch, the MET recorded for them was $4.4 \mu\text{g Cr(VI)}/\text{cm}^2$. Tables 4 and 5 present the MET and cumulative frequency of response for Cr(VI) and Cr(III). The figure presents the dose-response curve.

STATISTICAL ANALYSIS OF RESULTS

The data were analysed by a computer modelled data technique.⁵⁸ A truncated log normal distribution was fitted to the data with maximum likelihood methods, which is a technique for choosing parameters of a selected distribution of the observed data such that the probability of response of the observed data is maximised.⁵⁹ The fit to the data was excellent as confirmed by the χ^2 goodness of fit test, which gave a p value of >0.05 . The 10% cumulative response MET for Cr(VI) was $0.089 \mu\text{g}/\text{cm}^2$. The log normal distribution is conventionally used in the analysis of bioassay data and was used here.⁶⁰ Because a reaction at the maximum tested concentration was a criterion for inclusion in the study, the distribution of response concentrations is truncated. Thus the truncated log normal distribution was fitted to the dataset with the highest truncation point being the highest Cr(VI) concentration tested ($4.4 \mu\text{g Cr(VI)}/\text{cm}^2$).

IMPORTANCE OF PATCH CONCENTRATION

Recently, some dermatologists have concluded that the mass of allergen per unit area of skin is the correct measure of dermal dose, not the applied patch concentration of allergen.²⁹ To assess the influence of the concentration in the patch test material *v* the dose applied in mass per unit area on the potential to elicit a response, two different sets of patches were prepared. The first set was prepared with the gel material normally used to manufacture the $0.88 \mu\text{g Cr(VI)}/\text{cm}^2$ patch. A second patch was constructed from the same gel but it was only 1/7 as thick (the thin patch). Hence, the thin patches contained $0.13 \mu\text{g Cr(VI)}/\text{cm}^2$, but were identical to the $0.88 \mu\text{g Cr(VI)}/\text{cm}^2$ patch with respect to mass of Cr(VI) per mass of patch (in this case, 175 ppm).

These patches were then evaluated with nine volunteers previously shown to have a MET of $0.88 \mu\text{g Cr(VI)}/\text{cm}^2$. The results (table 6) indicate that six of the nine subjects showed a response deemed to be morphologically positive at $0.88 \mu\text{g Cr(VI)}/\text{cm}^2$ but none showed such a response at $0.13 \mu\text{g Cr(VI)}/\text{cm}^2$. Thus although both patches were identical on a mass of Cr(VI)/mass of patch basis (175 "ppm" Cr(VI)) the elicited response was different indicating that mass per unit area is a more appropriate measure of dose. Hence, as predicted by Upadhye and Maibach,²⁹ the mass of allergen per unit area of skin, and not the mass of Cr(VI)/mass of patch (concentra-

Cumulative response of 54 Cr(VI) sensitised volunteers to test patches containing various concentrations of Cr(VI).

Table 6 Response of volunteers to patches containing identical PPM concentrations of Cr(VI) (mass of Cr(VI)/mass of patch) but differing mass loading of Cr(VI)/cm²

Subject	Patch concentration	
	0.13 µg Cr(VI)/cm ²	0.88 µg Cr(VI)/cm ²
1	Negative	Negative
2	Doubtful	Positive
3	Negative	Positive
4	Doubtful	Positive
5	Negative	Positive
6	Negative	Positive
7	Negative	Positive
8	Negative	Negative
9	Negative	Negative

tion in ppm) is the critical factor to quantitatively characterise the dermal hazard.

IMPORTANCE OF PATCH SURFACE AREA

In the past, it has been suggested that if the surface area of the standard patch was significantly increased, then a sub-MET dose per unit area might produce a response because the total systemic uptake of the chemical would be greater. To assess whether patches of a larger surface area might elicit a response at concentrations lower than the METs identified in this study, another study was conducted. Four volunteers who had previously been shown to have a MET of 0.88 µg Cr(VI)/cm² were patch tested with five 0.18 µg Cr(VI)/cm² patches placed side by side. Hence, the total amount of allergen required to elicit allergic contact dermatitis in a standard patch was applied, but over a larger surface area. The results showed that sub-MET concentrations of Cr(VI) applied over a larger skin surface area did not elicit the positive responses seen when the MET concentration was applied in the standard patch. These results support the belief that to produce allergic contact dermatitis, the concentration of allergen delivered to the target organ (the Langerhans cell) in a given area of skin is the correct dosimetric index for evaluating the dermal allergic contact dermatitis hazard.

Discussion

The results of this study corroborate earlier reports that Cr(VI) sensitive persons respond to serial dilutions of Cr(VI) in a fairly linear manner at moderate concentrations.^{16-18 20 21} Interestingly, almost half (22/54) of the Cr(VI) sensitised volunteers in this study did not respond to Cr(VI) concentrations less than the diagnostic concentration of 4.4 µg Cr(VI)/cm². The diagnostic patch contains a fairly high Cr(VI) concentration that is unlikely to be found in the environment and most workplaces.⁸

Many of the subjects who failed to respond in round one had been thought by the physicians to be Cr(VI) sensitive due to earlier instances of positive responses to 0.5% K₂Cr₂O₇ in petroleum jelly or to 0.25% K₂Cr₂O₇ in TRUE-Test matrix. As discussed earlier, 0.5% K₂Cr₂O₇ is no longer commonly used clinically in the United States due to the high rate of irritant (non-allergic) responses,^{1 43} although it is still used in Europe. Many dermatologists in North America believe that

use of 0.25% K₂Cr₂O₇ to identify sensitisation, as was done in this study, minimises or eliminates the incidence of irritant reactions.³⁶ Therefore, it is likely that many of those volunteers who initially reacted to 0.5% K₂Cr₂O₇ exhibited an irritant (false positive) reaction in earlier testing and that the 0.25% K₂Cr₂O₇ patch used in this study failed to produce the irritant response. Alternatively, it is possible that some of the subjects who failed to respond in round one truly are Cr(VI) sensitive but have a threshold that is greater than 0.25% K₂Cr₂O₇. If this is true, then the results of our study could be considered "worst case" as only the hypersensitive volunteers were used to represent the sensitised population.

The results of this study suggest that the 10% MET for Cr(VI) induced allergic contact dermatitis is approximately 0.089 µg-Cr(VI)/cm². No significant correlations between age, sex, or occupation were found at any of the tested concentrations.

Several estimates of the prevalence of Cr(VI) related allergic contact dermatitis in the general population have been published in the past three years. Hostynek and Maibach estimated that less than 1% of the general population is Cr(VI) sensitive based on their review of several years of clinical data.⁷ Paustenbach *et al*⁸ estimated that about 0.7% of the general population is Cr(VI) sensitive. Finally, in a review of clinical data collected from several hundred human volunteers, Nethercott⁵⁷ estimated that "a 0.6% prevalence is likely a reasonable reflection of the prevalence of Cr(VI) sensitivity in the general population, although false positive irritant responses to 0.5% K₂Cr₂O₇ in petroleum jelly may explain even some of these responses." In all three analyses, if one excludes those who have had more than 10 years of experience working with wet cement, the prevalence of Cr(VI) sensitisation in the general population is probably no greater than 0.1%. Therefore, if Cr(VI) sensitised subjects comprise less than 0.1% of the general population, an allergic contact dermatitis based soil clean up criterion that is protective of 10% of the Cr(VI) sensitised population will probably be protective of about 99.99% of the general population.

When attempting to use these data to identify safe concentrations of Cr(VI) in environmental media (soil, dust, etc), it is necessary to consider whether children are more or less prone to Cr(VI) sensitisation than adults. In general, most authorities believe that sensitivity to contact allergens increases with age, due primarily to increased exposure.^{62 63} Children under the age of three react less often and less intensely than older children, whereas children older than three react to contact allergens in a manner similar to adults.^{64 65} In studies of patients in paediatric dermatology clinics, Schachner *et al*⁶⁶ diagnosed contact dermatitis in only 1.2% of children tested with K₂Cr₂O₇. Romaguera *et al*⁶⁷ reported positive reactions in 11 of 1023 children tested (1.1%) and Tunnessen⁶⁸ reported a similar prevalence rate of 2.4%. In a study of

$$\text{Soil concentration (mg-allergen/kg-soil)} = \frac{\text{MET} \left(\frac{\text{mg-allergen}}{\text{cm}^2\text{-skin}} \right) \times \text{CF} \left(\frac{10^6 \text{ mg-soil}}{\text{kg-soil}} \right)}{\text{SA} \left(\frac{\text{mg-soil}}{\text{cm}^2\text{-skin}} \right) \times \text{BVA (unitless)}}$$

422 paediatric patients, Angelini and Meneghini⁶⁹ concluded that: (a) sensitisation can occur in children as well as adults, but is extremely rare in the first few years of life; (b) the incidence of allergic contact dermatitis increases with age and escalates significantly from the age of 12 years, primarily because of increased exposure to environmental sensitizers and, perhaps, a fully matured immune system; and (c) the patch testing technique employed in adults, with the same substances and concentrations, is also well suited for diagnostic purposes in children. Thus the METs identified in our study can be considered applicable to children as well as adults.

It is noteworthy that there is some uncertainty associated with reading a patch test response as an allergic *v* an irritant reaction. Nethercott⁵⁷ has stated that the incidence of "misread" test sites can be as high as 20%. Because the patch test reactions in this study were interpreted by members of the NACDG this rate should be much less as the interpretation of patch tests is a routine part of their practice. Because it is more common to misread irritant reactions as allergic than discount an allergic reaction as an irritant one,⁴³ the MET estimated from this study can be considered conservative (no less than predicted here).

CALCULATING ACCEPTABLE SOIL CONCENTRATIONS FOR Cr(VI)

As described by Horowitz and Finley,³¹ an acceptable concentration of allergen in soil can be derived as above: Where MET = the minimum elicitation threshold determined from patch test data; CF = conversion factor; SA = soil adherence factor; BVA = bioavailability.

If the leachability (bioavailability) of the allergen from soil to skin is less than 100%, then the soil concentration must be adjusted upwards accordingly.

The 10% MET for Cr(VI) in sensitised people, as identified in the patch testing study, was 0.089 $\mu\text{g Cr(VI)/cm}^2$ skin. As discussed previously, a threshold derived from a 48 hour, occluded patch test study is highly conservative (health protective) with respect to realistic environmental exposures. Accordingly, to remain consistent with USEPA's "reasonable maximal exposure" philosophy of risk assessment (wherein average and upper bound values are combined to give a "reasonable" estimate, rather than simply compounding worst case assumptions), it is appropriate to use an average value to represent soil adherence. The USEPA's suggested average value of 0.20 mg/soil $\text{cm}^2\text{-skin}$ was used for this analysis.

With these values for MET and soil

adherence factor, it can be estimated that a concentration of 445 mg Cr(VI)/kg soil (445 ppm) should be acceptable. Hence, even if it were conservatively assumed that all Cr(VI) in chromite ore processing residue adhering to skin was able to leach into skin moisture and cross the skin barrier, a chromite ore processing residue concentration of 445 ppm Cr(VI) should protect the vast majority of the Cr(VI)-sensitised population from the allergic contact dermatitis hazard. As noted by Horowitz and Finley⁷⁰ however, extraction of chromite ore processing residue containing up to 1240 mg Cr(VI)/kg soil failed to leach more than 0.1% of the Cr(VI). Hence, chromite processing residue concentrations much higher than 445 ppm Cr(VI) should also be health protective for even those people who are very allergic to chromium. As soil cleanup concentrations of about 400 ppm Cr(VI) or greater are considered to be health protective for the more traditional exposure pathways (soil ingestion and particulate inhalation), it seems that elicitation of allergic contact dermatitis on contact with chromite ore processing residue could occur only at Cr(VI) concentrations much higher than the soil concentrations that may pose other hazards.

CALCULATING ACCEPTABLE SOIL CONCENTRATIONS FOR Cr(III)

For the purpose of calculating a Cr(III) concentration in soil, if one conservatively assumes a 10% MET at the maximum tested dose used in this study (33 $\mu\text{g Cr(III)/cm}^2\text{-skin}$), the concentration that should not pose an allergic contact dermatitis hazard is 165 000 mg Cr(III)/kg-soil (assuming a soil adherence factor of 0.20 mg-soil/ $\text{cm}^2\text{-skin}$ and 100% leachability. Adjustment for less than 100% leachability would yield a chromite ore processing residue concentration of high hundreds of thousands of ppm. In short, it is implausible that soil contaminated with Cr(III) could pose a dermal hazard.

Conclusions

This patch testing study was designed to determine a MET for Cr(VI) induced allergic contact dermatitis on a mg Cr(VI)/ cm^2 skin basis so that the results could be used to perform occupational and environmental risk assessment. The results suggest that the 10% MET for Cr(VI) induced allergic contact dermatitis is about 0.089 $\mu\text{g/cm}^2$. It was also shown that the concentration (in ppm) in the patch was not as relevant a dosimetric index as dose per area. With quantitative risk assessment principles, these patch test data were used to identify safe concentrations of Cr(VI) in soil.

The regulatory concern about Cr(VI) in

soil was born of the belief that because Cr(VI) sensitisation can occur in the workplace, environmental exposure to Cr(VI) in a soil matrix might also elicit allergic contact dermatitis. Initial estimates of the likely safe concentrations of Cr(VI) in soil were based on patch testing data from the 1950s and 1960s²¹; however, as stated previously, there is a high degree of uncertainty associated with those data. Furthermore, the results of those studies were not reported in terms of mg Cr(VI)/cm² skin.

The results of this patch test study were used to develop soil clean up levels which should prevent the elicitation of allergic contact dermatitis even in sensitised persons.³¹ The results also indicate that concentrations of Cr(VI) in soil due to chromite ore processing residue should not produce allergic contact dermatitis, even in Cr(VI) sensitised persons, below about 450 ppm. Our results indicate that traditional patch testing, which is used routinely to diagnose illness, can be modified so that the data can then be used to resolve issues in environmental medicine and quantitative risk assessment.

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References should be numbered consecutively in the order in which they are first mentioned in the text by Arabic numerals above the line on each occasion the reference is cited (Manson¹ confirmed other reports²⁻⁵ . . .). In future references to papers submitted to *Occup Environ Med*

should include: the names of all authors if there are seven or less or, if there are more, the first six followed by *et al*; the title of journal articles or book chapters; the titles of journals abbreviated according to the style of *Index Medicus*; and the first and final page numbers of the article or chapter. Titles not in *Index Medicus* should be given in full.

Examples of common forms of references are:

- 1 International Steering Committee of Medical Editors, Uniform requirements for manuscripts submitted to biomedical journals. *Br Med J* 1979;1:532-5.
- 2 Soter NA, Wasserman SI, Austen KF. Cold urticaria: release into the circulation of histamine and eosinophil chemotactic factor of anaphylaxis during cold challenge. *N Engl J Med* 1976;294:687-90.
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