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A Tape-Stripping Method for Measuring Dermal Exposure to Multifunctional Acrylates

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Current methods for measuring dermal exposure to skin irritants and allergens, such as acrylates, have significant drawbacks for exposure assessment. A noninvasive sampling method has been developed and tested for measuring dermal exposure to a multifunctional acrylate employing a tape stripping of the nonviable epidermis (stratum corneum). Samples were subsequently extracted and a gas chromatographic method was employed for quantitative analysis of tripropylene glycol diacrylate (TPGDA). This method was tested in 10 human volunteers exposed to an a priori determined amount of TPGDA or a UV-radiation curable acrylate coating containing TPGDA (UV-resin) at different sites on hands and arms. On the average, the first tape stripping removed 94% (coefficient of variation 16%) of the theoretical quantity of deposited TPGDA and 89% (coefficient of variation 15%) of the theoretical quantity of deposited TPGDA in UV-resin 30 min after exposure. Quantities of TPGDA recovered from two consecutive tape strippings accounted for all of the test agent, demonstrating both the efficiency of the method to measure dermal exposure and the potential to determine the rate of absorption with successive samples over time. In general, the amount removed by the first stripping was greater for TPGDA than for UV-resin while the second stripping removed approximately 6 and 21% of TPGDA and UV-resin, respectively. However, when the amounts removed with the first tape stripping for TPGDA or UV-resin from the five different individual sites were compared, no significant differences were observed (P = 0.111 and 0.893, respectively). No significant difference was observed in recovery between TPGDA and UV-resin for the first tape stripping when calculated as a percentage of the theoretical amount (P = 0.262). The results indicate that this tape-stripping technique can be used to quantify dermal exposure to multifunctional acrylates. © 2000 British Occupational Hygiene Society. Published by Elsevier Science Ltd. All rights reserved

Keywords: dermal exposure; multifunctional acrylate; tape stripping

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INTRODUCTION

Multifunctional acrylates (MuFAs) are widely used in ultraviolet radiation curable acrylate coatings (UV-curable coatings) and their use has increased steadily by an average rate of 15% per annum during the last 15 years (Wolff *et al*., 1982; Jongeneelen *et al*., 1988; van Rooij *et al*., 1992, 1993). One of the active components in UV-curable acrylate coatings, tripropylene glycol diacrylate (TPGDA), is an irritant and allergen to the skin (Björkner, 1984). Skin irritation and allergic contact dermatitis due to TPGDA exposure in various occupations has been reported (Jolanki *et al*., 1994; Koppula *et al*., 1995; Kanerva *et al*., 1996). To our knowledge, only two investigations of dermal effects in wood surface coating industry workers have been published (Voog and Jansson, 1992; Fischer *et al*., 1994). Fischer *et al*. (1994) reported that 28% of the workers exposed to UV-curable coatings had eczema or a history of eczema at the time of the study. Recently, TPGDA and two other methacrylate compounds (ethylene glycol dimethacrylate and 2-hexyl propylene methacrylate) have been classified as skin sensitizing agents in Finland (The Finnish Scientific Committee on Health Effects of Chemicals, 1996).

The use of MuFA-containing resins in the wood surface coating industry involves a significant poten-

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tial risk for dermal exposure (Fischer *et al*., 1994; Nylander-French *et al*., 1994a). A method for measuring exposure to airborne MuFAs in the wood surface coating industry has been published (Nylander-French *et al*., 1994b) but no method for assessing dermal exposure to MuFAs has been developed.

Although assessment of dermal exposure to potentially hazardous chemicals in the workplace is important, relatively little research has focused on methods to measure dermal exposures. Primarily, investigations have focused on exposures to pesticides (Anonymous, 1986), polycyclic aromatic hydrocarbons (Wolff *et al*., 1982; Jongeneelen *et al*., 1988; van Rooij *et al*., 1992, 1993), and toxic metals (Ness, 1994) using various sampling methods.

A novel approach to investigate physical, chemical, or structural properties of the stratum corneum as well as the penetration of the stratum corneum (absorption) by chemical compounds employed physical removal of the surface cell layers by tape stripping (Porter and Schuster, 1967; Jenkins and Tresise, 1969; Rougier *et al*., 1983, 1985, 1986; Dupuis *et al*., 1984, 1986; Serup *et al*., 1989; Serup and Jemec, 1995). The experimental procedure required a single application of a chemical followed by immediate washing of the application site with a suitable solvent after 30 min. The upper layers of the stratum corneum (nonliving keratinized layers) were then removed by six successive strippings using adhesive tape. The concentration of a substance in the cells attached to the adhesive strip from each layer of epidermis was then correlated to the total amount of substance absorbed. These studies offer a starting point for development of techniques and methods useful for measuring dermal exposure in occupational field studies. However, these studies did not allow for investigation of dermal exposure *per se* since the contaminant was washed from the skin prior to the application of the tape. Thus, the total amount of the contaminant that was in contact with the skin at that time was not determined. Since the purpose of the current investigation was to measure dermal exposure rather than (or in addition to) absorption, we omitted skin washing from our sampling protocol.

Although promising, tape stripping of the stratum corneum for estimation of dermal exposure has been used only with a few compounds of limited interest for occupational health. Cullander *et al*. (1997) described a tape-stripping method for the analysis of metals in the skin. However, to our knowledge, the method was not used for characterization of dermal exposure to hazardous metals in occupational or environmental settings.

The goal of this study was to apply tape stripping of the nonviable epidermis for measuring dermal exposure to a multifunctional acrylate in human volunteers. Preliminary data were obtained from 10 volunteers exposed to TPGDA and a reference UV-curable acrylate coating (UV-resin) in a laboratory setting to determine the utility of the method for measuring dermal exposure under field conditions.

MATERIALS AND METHODS

Chemicals

Tripropylene glycol diacrylate (TPGDA, CAS No. 42978-66-5, technical grade) was obtained from Akzo Nobel Industrial Coatings AB, Malmö, Sweden. A commercially available UV-resin was obtained from Becker Acroma Klintens AB, Märsta, Sweden. The purity of the TPGDA was 84.3% and the amount of TPGDA in the UV-resin was 29.5% [determined by a gas chromatographic analysis using a 90.3% pure TPGDA (Aldrich, Milwaukee, WI) as a reference]. The determined purity for the TPGDA is consistent with previously published studies where TPGDA was used in dermal patch testing and determined to be 81–91% pure (Henricks-Eckerman, 1997; Kanerva, 1997).

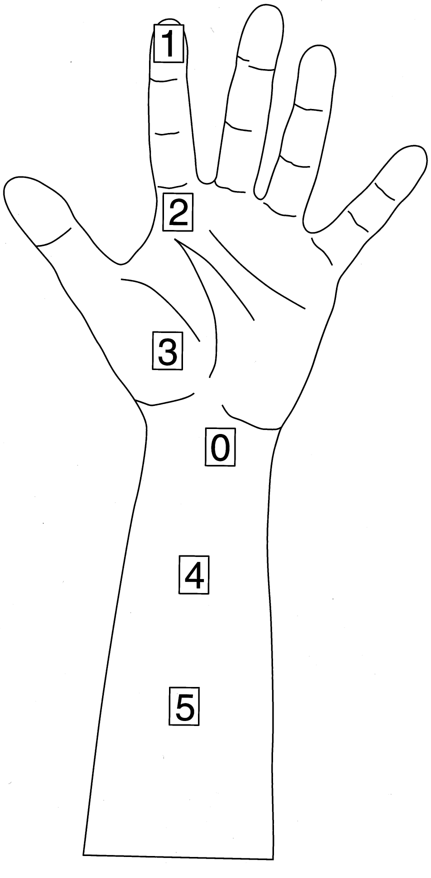

Fig. 1. Tape-stripping sites on the volunteer's palms and the lower volar region of the arm. Numbers 1–5 correspond to the sites where 2.5 µl of either TPGDA or UV-resin was deposited prior to tape stripping; #1 fingertip, #2 upper palm, #3 lower palm, #4 lower arm, #5 upper arm. Tape strip from the site #0 served as an unexposed control site (field blank).

Tape stripping

Fixomull® tape (Beiersdorf AG, Germany), precut to size 2.5 cm×4.0 cm, was tested on 10 human volunteers for its capacity to remove the deposited acrylate material from the skin. Predefined areas on subject's fingertip (one site), palm (two sites), and volar region of the lower arm (two sites) were exposed to either TPGDA (left hand and arm) or UV-resin (right hand and arm) (Fig. 1). Also, an unexposed control sample (field blank) was obtained from each arm (Fig. 1). TPGDA or UV-resin (2.5 μl on each site) was applied neat to the skin using a sterile micropipette tip. Since both TPGDA and UV-resin are viscous liquids, residue outside the pipette tip was carefully removed by a lint free lens tissue before application in order to deliver a constant amount of the material. In order to provide an even exposure, the applied dose was spread with the side of the pipette tip to cover an area of about 1 cm². The exposed site was left uncovered for 30 min before the Fixomull® tape (10 cm² area) was applied over the 1 cm² exposed area. Prior to application, Fixomull® tape was inspected for any defects and the spreading pattern of the applied material was inspected to see whether it was larger than the area of the adhesive. After the first tape was applied to the skin, the site was marked using a dermal marker. After 2 min, the tape was removed carefully by pulling at an angle of 45 deg (Prall, 1966) using clean forceps (rinsed in acetone between each stripping). A second tape was carefully applied to the same site immediately after the first tape was removed and retained on the skin for 2 min. After sampling, the tapes were placed into labeled 20 ml scintillation vials (Wheaton, Millville, NJ) and the vials were filled with 10 ml of acetone (Merck, Darmstadt, Germany) containing 300 ppm n-nonane (Merck, Darmstadt, Germany) as an internal standard and closed with aluminum-lined screw caps. Since a residue of UV-resin was observed on the skin after the first tape stripping of the first three volunteers, the tape-stripping procedure was modified to include two consecutive strippings from each site from the seven remaining volunteers. Thus, the two consecutive strippings represent the total amount of the analyte recovered and the difference between the two strippings may reflect absorption or penetration of the test compound at that point in time.

This study was approved by the Institutional Review Board on Research Involving Human Subjects, School of Public Health, The University of North Carolina at Chapel Hill.

Chemical analysis

Samples were placed onto a rotation shaker, shaken at 250 rev min^{-1} for 30 min and allowed to rest for 30 min before aliquoting into autosampler vials for gas-chromatographic analysis. Blank samples (one for every 20 samples) were prepared using the same procedure. After extraction, the samples were stored at −18°C until analysis by gas chromatography.

A modification of a previously published gas chromatographic (GC) method for analysis of airborne MuFAs (Nylander-French *et al.*, 1994b) was developed for the quantitative analysis of TPGDA from the samples. A HP 5890A Series II (Hewlett-Packard, Waldbronn, Germany) equipped with a HP 7673 GC/SFC injector and a DB-5 column (30 m, 0.32 mm inside diameter, 1.0 μm phase thickness of 95% dimethyl-, 5% diphenylpolysiloxane; J&W Scientific, Inc., CA) was used. The injector and the flame-ionization detector temperatures were 250 and 300°C, respectively. The initial oven temperature was held at 50°C for 1 min and increased at 20°C min^{-1} to 230°C where it was held for 6 min. After analysis, the oven temperature was increased to 280°C for 6 min to remove late-eluting compounds. Injections were made in the split/splitless mode using helium as a carrier gas. The retention time for TPGDA peaks was 11 min.

Storage stability. Eight sets of tape samples were prepared (in triplicate) by spiking the Fixomull® tape (2.5 cm×4.0 cm) with 2.5 μl of TPGDA. The samples were placed into 20 ml scintillation vials, sealed with aluminum-lined screw caps, and stored in the dark at room temperature for 2, 6, 18, 30, 42, 66, 90, or 114 h. At the end of the storage period, the samples were extracted with 10 ml of acetone and extracts were stored at −18°C until analysis by GC as described previously. No trend for loss of TPGDA from the Fixomull® tape nor significant differences between the storage periods were observed (one-way ANOVA, $P = 0.07$).

Analytical limit of detection. The analytical limit of detection (ALOD), determined as a signal-to-noise ratio of 3:1, for TPGDA was 1.65 μg per sample, which corresponds to a surface concentration of 165 ng cm^{-2} for the sampling area of 10 cm². For the UV-resin used in this study the respective ALOD corresponds to an amount of 0.005 μl distributed evenly over a skin area of 10 cm².

Statistical analysis

Recovery was calculated based on the theoretical maximum amount applied of TPGDA in 2.5 μl of media (TPGDA or UV-resin) as μmol amount recovered from each tape. The calculated theoretical amount of TPGDA in 2.5 μl of applied TPGDA mixture was 7.3 μmol or 2.2 mg and in 2.5 μl of applied UV-resin it was 2.7 μmol or 0.8 mg.

Average recoveries and coefficients of variation were calculated for each site and for all sites pooled. A one-way analysis of variance was used to investigate differences in recovery between the individual sites and between hand, palm, and arm. A two-way analysis of variance was used to investigate differences between the two test compounds. All statistical analyses employed the SAS System Software (SAS Institute, Cary, NC) and all differences were evaluated at a significance level of 0.05.

RESULTS

The average recoveries calculated as a percentage of the theoretical μmol amount applied (7.3 μmol for TPGDA and 2.7 μmol for UV-resin) for the first tape stripping and for the total of the first and second tape stripping are shown in Table 1. On the average, the first tape stripping removed 94% of the theoretical amount of deposited TPGDA and 89% of the corresponding TPGDA in UV-resin. Two consecutive tape strippings removed 102 and 113%, respectively. The coefficient of variation ranged between 5 and 24%. These positive biases in removal efficiencies are most likely due to minor variations in the delivery of the test agents.

Since recoveries were observed to be greater than the theoretical maximum for the total with two tape strippings, the amount (μmol) of TPGDA recovered in each stripping was investigated and compared with the total amount removed by the two consecutive strippings (Table 2). This analysis showed that the average amounts removed with the second tape strippings were approximately 6 and 21% for TPGDA and UV-resin, respectively. In general, recoveries for the first tape stripping were 10–15% higher and for the second tape stripping 10–15% lower for TPGDA than for the UV-resin.

When the recoveries in the first tape stripping for TPGDA or UV-resin from the five different individual sites were compared, no significant differences were observed (*P*-values for the global tests were 0.111 and 0.893 for TPGDA and UV-resin, respectively).

In order to investigate whether there were any differences in recoveries between arm and hand, data from these sites were pooled and comparisons were made between the arm (sites 4 and 5) and hand (sites 1–3) or palm (sites 2 and 3). When comparing the arm and hand, the recoveries for TPGDA or UV-resin were not significantly different for the first tape strippings (*P*-values for the global tests were 0.188 and 0.961, respectively). However, the recovery for TPGDA was significantly higher from the hand than from the arm when it was calculated as a total of the first and second tape strippings (*P*-value for the global test was 0.021). When the arm and palm were compared, recoveries were significantly higher from the palm than from the arm for the first tape stripping ($P = 0.032$) and for the total amount in the first and second tape stripping ($P = 0.006$). No significant differences were observed between these sites exposed to UV-resin. Furthermore, no significant difference was observed in recovery between TPGDA and UV-resin for the first tape stripping when calculated as a

Table 1. Efficiency of adhesive tape stripping of the nonviable epidermis to remove TPGDA from different sampling sites exposed to purified TPGDA (84.3% monomer) or UV-resin (29.5% TPGDA monomer). The average recoveries (percentage calculated from the theoretical μmol amount of TPGDA applied) for the first tape stripping (10 subjects) and for the total of the first and the second tape stripping (six or seven subjects) are included

	TPGDA		UV-resin	
	1st strip	Total	1st strip	Total
Fingertip (#1)	*N*=10	*N*=6	*N*=10	*N*=7
Average removal efficiency (%)	88	101	89	106
Coefficient of variation (%)	19	14	18	16
Upper palm (#2)	*N*=10	*N*=6	*N*=10	*N*=7
Average removal efficiency (%)	103	111	91	119
Coefficient of variation (%)	8	5	12	6
Lower palm (#3)	*N*=10	*N*=6	*N*=10	*N*=7
Average removal efficiency (%)	96	105	88	108
Coefficient of variation (%)	14	10	22	24
Lower arm (#4)	*N*=9	*N*=5	*N*=10	*N*=7
Average removal efficiency (%)	92	96	92	119
Coefficient of variation (%)	14	11	10	6
Upper arm (#5)	*N*=10	*N*=6	*N*=10	*N*=7
Average removal efficiency (%)	88	96	86	113
Coefficient of variation (%)	21	11	14	11
All areas	*N*=49	*N*=29	*N*=50	*N*=35
Average removal efficiency (%)	94	102	89	113
Coefficient of variation (%)	16	11	15	14

Table 2. Quantification of TPGDA removed from two consecutive tape-stripping samples of skin from subjects exposed to purified TPGDA (84.3% monomer) solution or UV-resin (29.5% TPGDA monomer). The average amount (µmol) removed by the first and the second tape strippings and the cumulative amount removed are shown

	TPGDA			UV-resin		
	1st strip	2nd strip	Total	1st strip	2nd strip	Total
Fingertip (#1)	*N*=6	*N*=6	*N*=6	*N*=7	*N*=7	*N*=7
µmol removed	6.808	0.555	7.363	2.169	0.653	2.882
S.D.[a]	1.09	0.14	1.01	0.38	0.12	0.46
Upper palm (#2)	*N*=6	*N*=6	*N*=6	*N*=7	*N*=7	*N*=7
µmol removed	7.735	0.306	8.041	2.501	0.642	3.143
S.D.	0.52	0.26	0.37	0.18	0.14	0.20
Lower palm (#3)	*N*=6	*N*=6	*N*=6	*N*=7	*N*=7	*N*=7
µmol removed	7.217	0.394	7.611	2.322	0.539	2.861
S.D.	0.83	0.25	0.75	0.63	0.21	0.67
Lower arm (#4)	*N*=5	*N*=5	*N*=5	*N*=7	*N*=7	*N*=7
µmol removed	6.513	0.489	7.002	2.501	0.653	3.155
S.D.	0.99	0.29	0.76	0.18	0.05	0.20
Upper arm (#5)	*N*=6	*N*=6	*N*=6	*N*=7	*N*=7	*N*=7
µmol removed	6.354	0.590	6.944	2.398	0.596	2.994
S.D.	0.78	0.14	0.73	0.34	0.10	0.34
All areas	*N*=29	*N*=29	*N*=29	*N*=35	*N*=35	*N*=35
µmol removed	6.940	0.466	7.406	2.378	0.617	2.995
S.D.	0.95	0.23	0.81	0.38	0.13	0.41

[a] S.D.=standard deviation.

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percentage of the theoretical amount (*P*-value for the global test was 0.262).

DISCUSSION

Dermal exposure to irritating, allergenic, or carcinogenic agents, or substances causing systemic toxicity that may interact directly or indirectly (via electrophilic metabolites) with the skin is poorly understood. A major limitation to our understanding and development of this area of research has been the absence of a noninvasive method to determine chemical-specific skin exposure.

We have developed and tested, under laboratory conditions, a noninvasive tape-stripping technique coupled with analytical chemistry (gas chromatography) for measuring dermal exposure to TPGDA from the skin. With this method we were able to remove applied TPGDA from the skin of human volunteers very efficiently with Fixomull® tape (Beiersdorf, Germany) 30 min after application.

No significant differences were observed in the recovery of TPGDA in the first tape strippings of the skin for TPGDA applied neat or in an UV-resin. The average recoveries from the hands and arms for the first tape strippings of TPGDA and UV-resin exposure were 94% (CV 16%) and 89% (CV 15%), respectively. Recoveries for the first tape strippings were 10–15% higher while for the second tape strippings they were 10–15% lower for TPGDA applied neat rather than with the UV-resin. Approximately 6% of the amount of TPGDA and 21% of the amount of UV-resin was removed with the second tape strippings, suggesting absorption of TPGDA into the upper levels of the epidermis. This indicates that when using this method for measuring dermal exposure in the field at least two or more consecutive tape strippings should be performed at the same site. In addition to acquiring more accurate estimations of the steady-state level of cumulative dermal exposure, the second stripping may also demonstrate absorption into the skin with little additional investment in resources. UV-resin is a chemical mixture containing compounds that may enhance dermal penetration of TPGDA. Since, the second tape stripping recovered more TPGDA from the sites exposed to UV-resin than to neat TPGDA (average 21 and 6%, respectively) it can be postulated that UV-resin enhances TPGDA penetration through the first layers of the stratum corneum.

The upper palm areas had the highest recoveries for the first tape stripping and the least variation. These differences may be due to (1) the thickness of the skin in this area, which would cause slow penetration and facilitate the removal of the compound with tape stripping, (2) chemical composition of the UV-resin, and/or (3) pipetting errors.

Variation in the amount of TPGDA delivered by micropipette and minute amounts of TPGDA remaining after spreading by the micropipette tip most likely contributed to the positive biases observed (recoveries >100%). The average removal efficiencies of 102 and 113% for two consecutive tape strippings of TPGDA and UV-resin correspond to excess amounts of 0.05 and 0.44 μl, respectively, delivered to the skin. This variation can be explained by the viscous nature of the test compound that contributed to difficulty in delivery of an exact amount by micropipetting.

The tape-stripping technique has the potential to meet the requirements for an efficient and reliable method to assess dermal exposures (van Hemmen and Brouwe, 1995). Stripping with an adhesive has been widely accepted as a dermal sampling technique in dermatology (Pinkus, 1951; Prall, 1966; Porter and Schuster, 1967; Jenkins and Tresise, 1969; Serup *et al*., 1989; Gerritsen *et al*., 1994). The primary potential risk associated with tape stripping is mild erythema, which has been observed after 50–60 repeated tape strippings and which may persist for one to two days (Öhman and Vahlquist, 1994). However, no evidence of erythema was observed in the current study.

In order to obtain a better understanding of dermal exposure to MuFAs, knowledge about the capacity of a tape-stripping method must be integrated with occupational exposure measurements. Thus further studies are warranted to gain knowledge about the levels of dermal exposure to MuFAs in the wood surface coating industry. Also, the tape-stripping technique should be generally applicable for assessing exposure to other chemicals, which have low volatility and do not penetrate quickly through the epidermis.

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