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Subject: Tripropylene glycol diacrylate (TPGDA). CASRN 42978-66-5

Dear Sir:

On July 14, 1994, Henkel Corporation received the enclosed doctoral thesis of Leena Nylander-French, Ph.D. This document was originally sent to Clay Frederick, Ph.D. of Rohm & Haas, who then forwarded it to Specialty Acrylate Manufacturers (SAM) Panel of the Chemical Manufacturers Association (CMA), which, in turn, forwarded it to Henkel. The research reported in this thesis was published by The Royal Institute of Technology, Stockholm, Sweden and the National Institute of Occupational Health, Solna, Sweden.

We believe that this document contains information which meets the reporting criteria under TSCA 8(e), Substantial Risk. Among other findings, skin tumors were reported in a 20-week study on genetically initiated/transgenic TG.AC mice.

For your information, Henkel sells tripropylene glycol diacrylate (TPGDA) to industrial customers who typically formulate products for the radiation curing industry. The current Henkel MSDS and label contain the chemical identity of TPGDA along with proper handling precautions. The MSDS states that the user should avoid contact with eyes, skin and clothing, and avoid breathing mist or vapor. The user is also instructed to wear chemical splash goggles, Neoprene gloves and protective clothing impervious to acrylates when handling TPGDA. Following these procedures and other recommendations is intended to minimize any actual risk.

In March of 1994, Henkel, unaware that the regulation had been stayed, submitted an 8(a) PAIR report in response to the 40 CFR 712 listing of TPGDA in the propylene glycol ethers and esters group. Similarly 8(d) health and safety studies were submitted in response to the corresponding listing in 40 CFR 716.

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Enclosure: Doctoral Thesis

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**IDENTIFICATION OF RISK TO WORKERS IN  
THE ULTRAVIOLET RADIATION CURING  
WOOD SURFACE COATING INDUSTRY**

*An Occupational Hygiene and Experimental Animal Study*

Doctoral Thesis

by

**Leena A. Nylander-French**

**Contains No CBI**

Stockholm  
1994

Department of Environmental  
Technology and Work Science  
The Royal Institute of Technology  
S-10044 Stockholm, Sweden

Division of Industrial Hygiene  
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S-17184 Solna, Sweden

IDENTIFICATION OF RISK TO WORKERS IN THE ULTRAVIOLET RADIATION CURED  
ACRYLATE LACQUER WOOD SURFACE COATING INDUSTRY  
*An Occupational Hygiene and Experimental Animal Study*

Leena A. Nylander-French

## Errata

Abstract	
line 19	Mucociliary to Mucociliary
line 21	form to from
List of Separate Publications	
Paper II	the second author is Pyhä, E.
Abbreviations	-NH to -NH <sub>2</sub>
p. 25, Mechanisms of Action	-NH to -NH <sub>2</sub>
paragraph 1, line 6 and paragraph 2, line 11	
p. 37, UV Radiation Exposure	
paragraph 1, line 3	5 J/cm <sup>2</sup> UV <sub>A</sub> to 1 J/cm <sup>2</sup> UV <sub>A</sub>
p. 47, paragraph 5, line 14	Mucociliary to Mucociliary
p. 50, paragraph 2, line 39	delete or present
p. 52, Figure 9, A and B, x-axis	TPDGA to TPDGA
p. 54, paragraph 3, line 7	control to control
p. 54, paragraph 3, line 8	frequency to frequency
p. 55, Figure 12, text, last line	should read ...for EA (A), TPDGA (B), and Lac A (C).
p. 58, Figure 18, line 3	(5 J/cm <sup>2</sup> ) to (1 J/cm <sup>2</sup> )
p. 71, reference 135, line 2	Technology to Technology
Paper II	
p. 11, Conclusions, second last line	workers exposure to worker exposure
Paper III	
p. 5, paragraph 5, line 7	should read ...odor that smelled stronger.
p. 11, paragraph 2, line 7	control to correlate
Paper IV	
p. 2, Table 1	N values should be shifted left to start under column 1.
p. 2, paragraph 2, line 24 and 25	delete or present
Paper V	
Figure 2, A and B, x-axis	TPDGA to TPDGA
Paper V	
Figure 5, text, line 3 and 4	should read ...for EA (A), TPDGA (B), and Lac A (C).
Paper VI, Table II	P-value 0.650 should be placed under the column
	Mean in %PCE <sup>b</sup>
Paper VII	
Figure 7, line 3	(5 J/cm <sup>2</sup> ) to (1 J/cm <sup>2</sup> )

From the Division of Industrial Hygiene,  
National Institute of Occupational Health, S-17184 Solna  
and  
the Department of Environmental Technology and Work Science,  
The Royal Institute of Technology, S-10044 Stockholm, Sweden

# IDENTIFICATION OF RISK TO WORKERS IN THE ULTRAVIOLET RADIATION CURING WOOD SURFACE COATING INDUSTRY

*An Occupational Hygiene and Experimental Animal Study*

by

Leena A. Nylander-French

Stockholm 1994

# Abstract

## IDENTIFICATION OF RISK TO WORKERS IN THE ULTRAVIOLET RADIATION CURED ACRYLATE LACQUER WOOD SURFACE COATING INDUSTRY

*An occupational hygiene and experimental animal study*

Leena A. Nylander-French

Use of surface coatings containing multifunctional acrylates that are cured with ultraviolet radiation (UVR) is rapidly increasing and the potential health effects to workers have not been adequately investigated. Occupational risks related to the surface coating of wood using UV curable coatings were identified by using an industrial hygiene survey and medical examination of workers. In Sweden, workers are exposed to small amounts of acrylate containing aerosols, vapors, and/or dusts and organic solvents. The potential toxicity of tripropylene glycol diacrylate, the primary acrylate observed in use, was evaluated using rodents and human cells *in vitro*. The most significant findings are that: (1) an estimated 350 out of 8,500 wood surface coating workers are directly exposed to acrylates and UVR, (2) respirable dust particles (28% of the total dust concentration) are present, but overall dust levels are low ( $\sim 0.4 \text{ mg/m}^3$ ), (3) ozone concentration levels did not exceed the background concentration, (4) UVR exposure to unprotected skin at biologically effective dose levels from poorly shielded UV units resulted in erythema and pigmentation, (5) the potential exists for direct exposure to sensitizing agents (acrylates, initiators, and inhibitors) and development of contact dermatitis, and (6) most workers were inconsistent in their work practices and education programs for workers were insufficient. Nasal, pharyngeal, and ocular symptoms were most frequent in UV line workers and finishers of UVR surface coated wood products. Mucociliary clearance and olfaction were impaired in UV line workers indicating changes in the nasal cavity which may be the result of toxicity. Irritant dermatitis was prevalent and low frequency of contact allergy was observed. The principal findings from the toxicology studies indicated that tripropylene glycol diacrylate was moderately toxic and induced skin tumors when applied to the skin of genetically initiated mice and was toxic to normal human skin and lung cells *in vitro*. This technology does involve potential health risks for worker skin and partially cured acrylate coatings must be considered as potentially harmful to airways, eyes, and skin. Control of the UVR curing process in respect to complete curing and shielding of UV units are important measures to reduce potential risk. Education and protective measures are required to reduce worker exposure. Additional research on exposure assessment, risk assessment, and managing risk by process, emission, and exposure control technology is required.

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# List of Separate Publications

This thesis is based on the following papers, which will be referred to by their Roman numeral, I-VII.

- I Nylander-French, L.A.; Fischer, T.; Hultengren, M.; Lewné, M.; Rosén, G.: Assessment of worker exposure in the processing of ultraviolet radiation cured acrylate lacquer coated wood products. *Applied Occupational and Environmental Hygiene* (in press).
- II Nylander-French, L.A.; Berglund, G-B.; Rosén, G.; Pihla, E.: A method for monitoring worker exposure to airborne multifunctional acrylates. *Applied Occupational and Environmental Hygiene* (in press).
- III Holmström, M.; Granstrand, P.; Nylander-French, L.A.; Rosén, G.: Upper airway symptoms in wood surface coating industry workers. *American Journal of Industrial Medicine* (submitted).
- IV Fischer, T.; Nylander-French, L.A.; Rosén, G.: Dermatological risk to workers in ultraviolet curing wood surface coating industry. *American Journal of Contact Dermatitis* (submitted).
- V Nylander-French, L.A.; French, J.E.: Chemical effects in TG•AC (v-Ha-ras) mice. Tripropylene glycol diacrylate, but not ethyl acrylate, induces skin tumors in a twenty week short term carcinogenesis study. *Carcinogenesis* (submitted).
- VI Tice, R.R.; Nylander-French, L.A.; French, J.E.: Absence of systemic *In vivo* genotoxicity after dermal exposure to ethyl acrylate and tripropylene glycol diacrylate in TG•AC (v-Ha-ras) mice. *Environmental and Molecular Mutagenesis* (manuscript for publication).
- VII Nylander-French, L.A.; French, J.E.: Comparative *in vitro* cytotoxicity of ethyl acrylate and tripropylene glycol diacrylate to normal human skin and lung cells. *In Vitro Cellular and Developmental Biology* (submitted).

# Abbreviations

%PCE	percentage of polychromatic erythrocytes
95% CL	95% confidence interval
ACGIH	American Conference of Governmental Industrial Hygienists
AIHA	American Industrial Hygiene Association
BDMK	benzil dimethylketal
BP	benzophenone
bw	body weight
CAS	Chemical Abstract Service register number
D <sub>min</sub>	smallest particle diameter
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DNFB	dinitrofluorobenzene
EDTA	ethylenediamine tetraacetate
ELISA	Enzyme Linked Immuno Sorbent Assay
FCAT	Freund's Complete Adjuvant Test
FEV <sub>1,0</sub>	forced expiratory volume in 1 second
GPMT	Guinea Pig Maximization Test
ip	intrapertoneal
IRPA/INIRC	International Radiation Protection Association/International Non-Ionizing Radiation Committee. Since 1992 ICNIRP (International Commission on Non-Ionizing Radiation Protection).
iv	intravenous
LD <sub>50</sub>	dose lethal to 50% of the animals
LOD	limit of detection
log K	logarithm of rate constant for a chemical reaction
log P	logarithm of <i>n</i> -octanol-water partition coefficient
MN	micronucleus
MN-PCE	miconucleated polychromatic erythrocyte(s)
MoFA	monofunctional acrylate(s) and methacrylate(s)
MSDS	material safety data sheet
MTT	methylthiazol tetrazolium
MuFA	multifunctional acrylate(s) and methacrylate(s)
NHBE	normal human bronchial epithelial cells
NHDF	normal human dermal fibroblasts
NHEK	normal human epidermal keratinocytes
NPSH	nonprotein sulfhydryl(s)
NTP	National Toxicology Program, USA
OR	odds ratio
OSHA	Occupational Safety and Health Administration, USA

OVS	OSHA Versatile Sampler
NPEF	nasal peak expiratory flow
ppm	parts per million
ROS	reactive oxygen species
SCG	Single Cell Gel electrophoresis
SEM	standard error of the mean
TLV	threshold limit value®
TNF <sub>α</sub>	tumor necrosis factor alpha
TPA	12- <i>O</i> -tetradecanoyl-phorbol-13-acetate
TWA	time-weighted average
UV	ultraviolet
UVa	ultraviolet radiation (315-400 nm)
UVb	ultraviolet radiation (280-315 nm)
UVc	ultraviolet radiation (100-280 nm)
UVR	ultraviolet radiation
WEEL	Workplace Environmental Exposure Level
-NH	amino
-OH	hydroxyl
-SH	sulphydryl

Acrylates:

AA	acrylic acid
AMA	allyl methacrylate
BA	<i>n</i> -butyl acrylate
BCA	butyl cyanoacrylate
BDDA	1,4-butanediol diacrylate
BDDMA	1,4-butanediol dimethacrylate
<i>n</i> -BMA	<i>n</i> -butyl methacrylate
<i>i</i> -BMA	<i>i</i> -butyl methacrylate
DEGDA	diethylene glycol diacrylate
DEGDMA	diethylene glycol dimethacrylate
DPOMA	dicyclopentenyloxyethyl methacrylate
EA	ethyl acrylate
EDDA	1,2-ethylenediol diacrylate
EDDMA	1,2-ethylenediol dimethacrylate
EDGA	ethyl diglycol acrylate
EEMA	ethoxy ethyl methacrylate
EGDMA	ethylene glycol dimethacrylate
EHA	2-ethylhexyl acrylate
EMA	ethyl methacrylate
GDMA	glycol dimethacrylate
GPTA	glycerol propoxy triacrylate
HDDA	1,6-hexanediol diacrylate
HDDMA	1,6-hexanediol dimethacrylate

HEA	2-hexylethyl acrylate
HEMA	2-hexylethyl methacrylate
HPA	2-hexylpropyl acrylate
HPMA	2-hexylpropyl methacrylate
IACA	isoamyl-2-cyanoacrylate
IBMA	isobutyl methacrylate
IDMA	isodecyl methacrylate
IOA	isooctyl acrylate
MA	methyl acrylate
MAA	methacrylic acid
MCA	methyl cyanoacrylate
MBMA	methyl methacrylate
NPDDA	neopentanediol diacrylate
NPDDMA	neopentanediol dimethacrylate
NPGDA	neopenthyl glycol diacrylate
NPGDMA	neopentyl glycol dimethacrylate
NPMA	neopentyl methacrylate
OTA-480	oligo triacrylate 480
PDDA	1,5-propylenediol diacrylate
PDDMA	1,5-propylenediol dimethacrylate
PETA	pentaerythritol triacrylate
PMA	propyl methacrylate
TCAA	trichloroacrylic acid
TEGDA	triethylene glycol diacrylate
TEGDMA	triethylene glycol dimethacrylate
TMPTA	trimethylol propyl triacrylate
TMPTMA	trimethylol propyl trimethacrylate
TPGDA	tripropylene glycol diacrylate
TTEGDA	tetraethylene glycol diacrylate
TTEGDMA	tetraethylene glycol dimethacrylate
TTHFMA	tetrahydrofurfuryl methacrylate

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# Introduction

Fundamental changes have occurred recently in the manufacturing of surface coated wood products and in required work practices due to a shift from acid curing to radiation curing systems. This change is due to scientific and political concerns over occupational and environmental exposures to organic solvents and degradation of organic solvent byproducts in the biosphere. Potential occupational risks related to the use of ultraviolet radiation curable (UVR) acrylic coatings<sup>1</sup> in wood surface coating industry have not been sufficiently investigated. Our knowledge of the overall potential health effects of exposure to acrylics, which includes derivatives of both acrylic and methacrylic acid, is inadequate even though they have been produced since the 1930s [123].

## Chemistry, Production, and Use of Acrylates

Acrylates<sup>2</sup> are esters of acrylic or methacrylic acid. Acrylates polymerize readily and are widely used in the chemical industry to make polymeric resins for applications including paints and coatings, printing inks, adhesives, textiles, molded plastic objects, polishes, and leather finish treatments [77].

Acrylates may be classified as either monofunctional or multifunctional depending upon the number of functional acrylate

1 Unless otherwise specified, the term UVR acrylic coating shall include acrylic lacquers, paints, and fillers.

2 Acrylates include both derivatives of acrylic and methacrylic acids. Unless otherwise specified, the term acrylate shall include both groups.

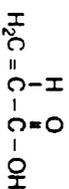
groups present. Monofunctional acrylates

(MuFA) may be presented by the generic formula  $CH_2=CR_1COO-R_2$ , where  $R_1 = H$

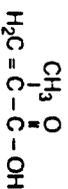
in acrylates or  $R_1 = CH_3$  in methacrylates and  $R_2$  presents the respective alcohol group in the ester (e.g., methyl, ethyl, *n*-butyl, 2-ethylhexyl, hydroxyethyl) [see Fig. 1 for representative generic chemical structures]. Multifunctional acrylates (MuFA)

are defined as those containing more than one acrylate or methacrylate group, respectively (e.g., diethylene glycol diacrylate or dimethacrylate, trimethylol propane triacrylate or trimethacrylate) [see Fig. 1 for representative chemical structures]. One exception to these general features is the absence of the carbon ketone group in the glycol ether family of MuFA (see dashed box area in Fig. 1). Acrylates vary widely in their physicochemical properties depending upon the selection and number of different acrylate or methacrylate functional groups and alcohols in the ester. The carboxyl or carboalkoxy functional group may display electron withdrawing ability through inductive effects of the electron deficient carbonyl carbon atom, and electron releasing effects by resonance involving the electrons of the carbon-oxygen double bond. Therefore, these compounds react readily with electrophilic, free radical, or nucleophilic agents.

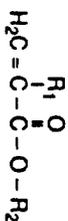
MuFA are generally used as monomers in formulations for cross linking a prepolymer to provide bulk properties such as hardness, abrasion resistance, flexibility, elasticity, and resistance in the cured film [1]. They may also be used as reactive diluents to adjust the desired viscosity of radiation



Acrylic Acid

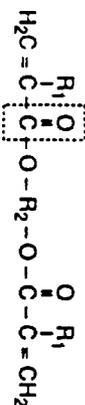


Methacrylic Acid



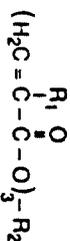
$R_1 = H_2$  Monoacrylate

$R_1 = CH_3$  Monomethacrylate



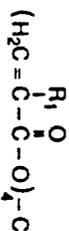
$R_1 = H$  Diacrylate

$R_1 = CH_3$  Dimethacrylate



$R_1 = H$  Triacrylate

$R_1 = CH_3$  Trimethacrylate



$R_1 = H$  Tetraacrylate

$R_1 = CH_3$  Tetramethacrylate

Figure 1. Representative generic chemical structures of acrylates.

curable formulations in much the same way as solvents are used in conventional surface curing systems.

The average annual growth of production of radiation curable acrylate formulations, ~20% in Europe, has been steady for the past 10 years. The total European market for 1990 was estimated at 21,000 metric tons [48] and is expected to grow to 50,000 metric tons by the year 1995 [41]. The total global market for radiation curable resin systems has been estimated at 85,000 metric tons per year (paints 57%, printing inks 10%, and adhesives 3%) [76]. The volume growth in the near future is estimated to continue at 15-20% per annum to the year 2000 with a projected rise in the number of radiation curable applications, in which MuFA are predominantly used. Main users

are in the United States (33%), Western Europe (30%), and Japan (20%).

## Surface Coating of Wood with Ultraviolet Radiation Curable Acrylate Coatings

The manufacturing process for the application of UVR curable wood surface coatings is divided into two parts: (1) surface coating of an object and (2) ultraviolet radiation curing of the surface coating. Wood surface coating includes mainly flat object processing. Other technology, e.g., spraying three dimensional objects using UVR curable polyester coatings, may occur, but was not considered in this study.

## Surface Coating Process

A basic type of UVR surface coating line is described in Figure 2. Primarily, one or two application methods and more than one type of UVR curable coatings are used. A typical manufacturing process begins with feeding the boards onto the conveyor; conveyor speed varying usually between 9-25 m/min. On the conveyor line the wood board passes first through an enclosed sander with local exhaust ventilation and then is cleaned by roller brushes with local exhaust ventilation before resin application.

Surface coating of flat objects is accomplished using roller coaters and/or filling by hand. Application of the coating is usually carried out in a series of coating applications, UVR curing, and sanding of the cured surface. Application of the coating to the product surface is primarily carried out by a roller coater, onto which coating is fed by pumping from a container located beside the roller coater. A  $\approx 0.01$  mm thick ( $5\text{-}25$  g/m<sup>2</sup>) coating is applied by rollers to the substrate during each application. Excess

coating from the roller coater is collected and returned to the process. The first application of the base coat is applied by a roller coater and cured by a UVR unit. In some cases the first base coating may be intentionally only partially cured. This "gelling" produces a tacky surface, which is considered to be a better surface for application of the principal surface coatings (top coat). Application of the base and top coatings is usually carried out 2-3 times each with UVR curing and sanding performed between applications.

In parquet floor manufacturing, fillers (UVR curable acrylate paste) are used to correct for uneven surfaces and are usually applied by hand or by pouring the filler manually onto the roller coater. However, an automatic process to apply fillers is also used, but this may still require manual control and hand filling. If filling is required, it is always performed before coating the product with UVR curable acrylate lacquer or paint.

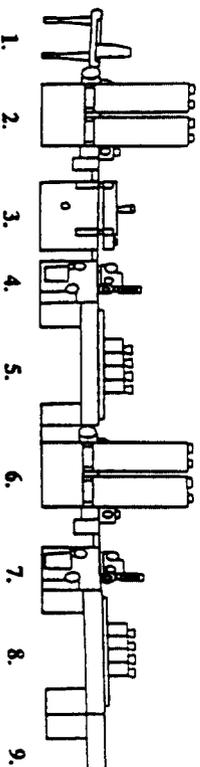


Figure 2. A schematic illustration of a UVR wood surface coating line.

The line consists of: 1. Feeding table, 2. Sanding machine, 3. Cleaner with brushes, 4. Roller coater machine, 5. UVR curing unit with 4 UV lamps, 6. Sanding machine, 7. Roller coater machine, 8. UVR curing unit with 4 UV lamps, and 9. Receiving table.

## Radiation Curing Process

The UVR curing process consists of the application of surface coating, which is polymerized by exposure to UVR. The acrylate coatings used in the UVR surface coating industry are normally composed of three basic components: an acrylated prepolymer (urethane acrylate, polyester acrylate), a MuFA monomer (e.g., tripropylene glycol diacrylate, TPGDA; 1,6-hexanediol diacrylate, HDDA), and a photoinitiator system (e.g., benzophenone, BP; benzil dimethylketal, BDMK) [1]. Depending upon product end use and application, these components may be formulated with stabilizers, pigments, and other additives.

UVR curing equipment consists of UVR source, reflector (semi-elliptical or parabolic aluminum, polished and brightened by either a chemical or electro-brightening process to maximize reflectivity), and ancillary equipment (includes electrical system, shuttering, cooling/extraction, and shielding). The radiation source produces high intensity UVR generated by a mercury arc lamp, pulsed lamp (xenon flash), or electrodeless lamp. The most common source is a mercury vapor lamp, which can be either low, medium, or high pressure. Medium pressure mercury vapor lamps are most widely used for the curing of acrylate coatings [91]. However, gallium lamps, which emit a large portion of visual light (peak 400-450 nm) in addition to the UVR, are used to cure pigmented acrylate paints.

A UVR source emits UVR energy at a wavelength of 200-400 nm, which initiates a photochemical reaction and polymerization of UVR curable acrylate coating. The wavelength of the incident radiation must correspond with the absorption spectrum of the photoinitiator. Also, the absorption of the prepolymer(s) and the photoinitiator(s)

must be adapted to each other to ensure that most of the energy will be transferred to the photoinitiator(s). UVR absorbed by photoinitiator(s) causes homolytic bond cleavage in prepolymer(s) and monomer(s). Generally C-C or C-H bonds are broken (energy requirement, 350-410 kJ/mole), producing active free radicals. These radicals are initiation sites for the polymerization reaction. The active free radicals initiate addition polymerization (chain reaction), ultimately producing a three dimensional cross-linked structure. This principal reaction has two important consequences. First, polymerization is initiated simultaneously at many different points within the coating resulting in a short curing time. Secondly, all constituents of the original uncured coating are included in the polymerization reaction [72, 74, 135, 147].

## Human Health Effects of Acrylates

A rapid and widespread increase in the manufacture and use of acrylic materials have also increased the potential for worker exposure to these materials. Very few studies have been published on worker exposure (acute and/or chronic) to aerosols and/or vapors on MoFA [35, 94, 99, 139, 151], and none on MuFA. General health effects observed in workers exposed to MoFA were not significantly different from those in control groups. However, commonly noted symptoms were nervousness, headache, weakness, dizziness, shakiness, and drowsiness. Neurotoxic effects in dental technicians have been observed after direct skin exposure to methyl methacrylate (MMA) [83, 143, 152].

Skin irritation and allergic contact dermatitis from exposure to acrylates are, potentially, the health effects of the greatest

## Effects of Acrylates on Biological Model Systems

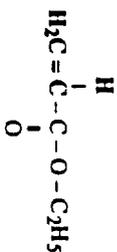
In general, simple MoFA, like ethyl acrylate (EA), are considered to be more toxic, but less potent contact sensitizers, than MuFA, like tripropylene glycol diacrylate (TPGDA; see Table I for chemical structures). The acrylates have been classified for potential biological effects based on a comparison of their structure activity relationships and upon their *n*-octanol-water partition coefficient (log P) values [96]. Acrylates are electrophiles and have an inherent potential for toxicity, including carcinogenicity. As potential direct alkylating agents, their toxicity may be expected to be limited only by their ability to interact with biological systems.

### Absorption, Metabolism, and Distribution

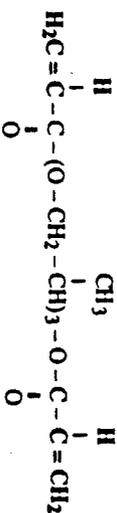
Acrylates vary greatly in their tissue absorption characteristics, based upon their log P values [96]. For example, in guinea pigs methyl acrylate (MA) was absorbed via dermal, oral, or intraperitoneal (ip) routes of administration [42, 153]. After dermal exposure to [<sup>14</sup>C]-MA the majority of radioactivity was observed in skin, kidney, and bladder. Administration by the oral route resulted in the major portion of the radioactivity being observed in the liver and the bladder within 72 h. Intraperitoneal injection of [<sup>14</sup>C]-MA resulted in 35% of the radioactivity being excreted as carbon dioxide (CO<sub>2</sub>) and ~20% excreted in the urine within 72 h. Oral administration of EA to adult rats resulted in ~90% absorption within 4 h and ~75% of the radioactivity was excreted as [<sup>14</sup>C]CO<sub>2</sub> within 24 h [39, 63]. Radioactivity was also observed in the liver, urine, and feces. Orally administered

Table I. The chemical properties and structures of ethyl acrylate (EA) and tripropylene glycol diacrylate (TPGDA). Bolded features of the chemical structure represents electrophilic functional groups.

Compound	Mol. wt. (g/mol)	Density (g/ml)	Vapor pressure (mmHg)	Boiling point (°C)	Solubility
EA CAS: 140-88-5	100.12	0.92	40 (26°C)	99.8	slightly soluble in water, soluble in many organic solvents
TPGDA CAS: 42978-66-5	300.25	1.03	< 0.01 (20°C)	* 315	insoluble in water, soluble in many organic solvents



Ethyl Acrylate (EA)



Tripropylene glycol diacrylate (TPGDA)

[2,3-<sup>14</sup>C]-*n*-butyl acrylate was rapidly absorbed and 75% of the radioactivity excreted in the form of CO<sub>2</sub> within 24 h [148]. Administration of [<sup>14</sup>C]-2-ethylhexyl acrylate by either intravenous (iv) or intraperitoneal (ip) routes to adult rats was rapidly metabolized and 70% was rapidly excreted as [<sup>14</sup>C]O<sub>2</sub> [68]. Radioactivity was also observed in lung, liver, and kidney.

The available data suggests that acrylates most likely act at the site of contact, conjugate available glutathione, via Michael's addition reaction or glutathione-S-transferase [22], and/or are hydrolyzed via carboxylesterases in a tissue specific manner [22, 34, 39, 43, 58, 63, 114, 155]. Conjugation of acrylates by glutathione is expected to be proportional to the number of functional acrylate groups.

After [<sup>14</sup>C]O<sub>2</sub>, depending on the route of exposure, the major metabolites were β-S-(N-acetylcysteinyl)propionic acid and β-S-(N-acetylcysteinyl)isobutyric acid for methyl acrylate (MA) [42] and *N*-acetyl-S-(carboxylethyl)cysteine and *N*-acetyl-S-(carboxylethyl)cysteine ethyl ester for EA [39, 63]. Radioactivity in the urine after [<sup>14</sup>C]-EA oral exposure (intubation) was inversely proportional to the dosage, which suggests that metabolism was glutathione dependent and saturable in the forestomach [39]. The carboxylesterase inhibitor, tri-*o*-cresyl phosphate, administered prior to EA exposure, potentiated the depletion of non-protein sulfhydryls (NPSH) in the liver, but not in the forestomach demonstrating tissue specific differences in metabolism of acrylates [39]. No data on adsorption,

metabolism, and distribution of MuFA were found.

### Systemic Toxicity of Acrylates

Systemic toxicity to acrylates may occur by various routes of exposure. The main potential routes of exposure to workers in an occupational environment are inhalation of aerosols and vapors and/or particles, ingestion of inhaled particles and/or oral contact with contaminated skin or objects leading to oral exposure, and dermal exposure by deposition of aerosols and particles on the skin and/or direct contact with uncured acrylate coatings. Potential systemic toxicity due to acrylates by these routes of exposure are considered in the following sections.

#### Oral toxicity

The acute oral toxicity of MoFA ranges from slightly to very toxic (toxicity categories II to IV) [87]. In general, the oral toxicity of MoFA decreases with an increase in molecular weight. MA appeared to be more toxic than EA, about 5 times more toxic than *n*-butyl acrylate (BA), and less toxic than 2-hexylethyl acrylate (HEA) or 2-hexylpropyl acrylate (HPA) in mice. Methyl methacrylate (MMA), 2-hexylethyl methacrylate (HEMA), and 2-hexylpropyl methacrylate (HPMA) exhibit similar toxicity. The primary symptoms of EA, MMA, ethyl methacrylate (EMA), and *n*-butyl methacrylate (*n*-BMA) acute toxicity in rats were accelerated respiration followed by a slowly developing motor weakness, decreased respiration, dyspnea, loss of reflex activity, and increased defecation and urination [40, 140, 159]. Degenerative changes were observed in the liver, kidney, spleen, and heart in rats and rabbits after exposure to EA and MA [140, 167].

Pozzani et al. [140] and Gordon et al. [67] reported irritation of gastrointestinal tract in rats after gavage dosing of EA and isooctyl acrylate (IOA). Gordon et al. [67] also observed mild central nervous system depression (ataxia and hypoactivity) in rats given 5 g/kg of IOA by gavage. Chanayem et al. [64] reported an increase in cell proliferation, foreign body reactions, and induction of benign and malignant tumors in forestomachs of rats after sustained oral administration of EA by intubation. No data on the oral toxicity of MuFA were found.

#### Inhalation toxicity

Acute toxicity of inhaled MoFA varies with dose and the type of acrylate [21, 40, 131, 140, 155, 159, 162]. Primary effects of acute exposure to MoFA were observed in the respiratory system as irritation, depression preceded by ataxia, and death due to respiratory failure [40, 131, 140, 159].

Subchronic toxicity effects of MMA and EA were observed as irritation in the respiratory system and as degenerative changes in the liver and kidney [62, 140, 159]. Engelhardt and Klimisch [54] reported that inhalation of high concentrations of BA (>4,000 mg/m<sup>3</sup>) did not increase chromosome aberrations or point mutations in either male and female Sprague-Dawley rats (10 wk old) or Chinese hamsters (18 wk old). Gross abnormalities, overt signs of central nervous system toxicity, or respiratory distress were not observed after subchronic exposure to MMA vapors (concentrations 450-4100 mg/m<sup>3</sup>) either in male Sprague-Dawley rats (age not stated), Swiss Webster mice (age not stated), or adult northern grass frogs [162]. Glycol dimethacrylate (GDMA), a MuFA, at 120 ppm was observed to produce slight lethargy, thickened alveolar walls, and periton-

chial lymphatic reaction in female Alderley Park rats (age not stated) exposed 6 h/day for 13 days [62].

Chronic exposure to MA or EA vapors caused irritation to the mucous membranes of the eyes, nose, and mouth, as well as lethargy, dyspnea, and convulsive movements in rabbits, guinea pigs, rats, and a monkey (age, sex, and strain not stated) [167]. Microscopic changes occurred in the form of pulmonary congestion, edema, and toxic degenerative changes in the heart muscle, liver, and kidneys. Later studies confirmed the irritative and degenerative effects of MoFA (MA, EA, BA, and MMA) vapors in the nasal mucosa [28, 90, 115, 145, 162]. The loss of olfactory epithelium and the replacement by ciliated respiratory epithelium were located in the most anterior region. No neoplastic lesions, however, were observed in any of these chronic toxicity studies. Miller et al. [114] reported irritation and degeneration of the olfactory epithelium of the nasal mucosa as well as replacement of the olfactory epithelium to respiratory epithelium in rats after exposure to acrylic acid (AA) vapor (5, 25, or 75 ppm, 6h/day, 5 days/wk, for 13 wk). Gage [62] suggested that AA may be less acutely toxic than MA and EA and that the introduction of a methyl group reduces the irritant action of acrylates. As discussed above, metabolism of MoFA to CO<sub>2</sub> and AA suggests that these toxicities were due to the AA metabolite.

In conclusion, MoFA vapors are irritant to mucous membranes and cause olfactory epithelium degeneration, but are not associated with neoplasia in the nasal cavity. Lung, liver, kidney, and heart are target organs based upon distribution studies, but they are not involved in systemic toxicity. Only one MuFA (GDMA) was tested for

acute inhalation toxicity and that was observed to produce systemic toxicity. No data on potential chronic inhalation toxicity of MuFA was found.

#### Dermal toxicity

Acute dermal toxicity of EA [140, 167], MMA, EMA, and *n*-BMA [40, 159], isomethyl-2-cyanoacrylate (IACA) [9], allyl methacrylate (AMA) [154], dicyclopentylolxethyl methacrylate (DPOMA) [29], and IOA [67] has been extensively studied in rabbits. Pozzani and colleagues [140] patch tested rabbits (24 h exposure) with undiluted EA and reported a LD<sub>50</sub> of 1.8 g/kg bw (range 1.65-1.96 g/kg bw). Treon and colleagues [167] determined the minimum lethal dose of EA to be between 41-50 g/kg bw when exposed up to 5 ml aliquots at intervals up to 20 min. Apparent dose differences may have been due to methods of application and volatility of EA. In these studies MA and EA produced irritation, erythema, edema, thickening, and vascular damage to the skin, but the effects were reversible. Swelling and congestion of the liver, kidneys, and heart, necrosis, and edema and congestion of the lungs were also reported [140, 167]. Deichmann [40] and Spealman and colleagues [159] reported temporary irritation, malaise, and desquamation of the skin after dermal application of MMA, EMA, and *n*-BMA. Spealman and colleagues [159] also observed signs of central nervous system depression with a dose of 37.8 mg/kg MMA. IACA was reported to be moderately irritating to rabbit skin, producing erythema and edema which decreased over time [9]. Acute LD<sub>50</sub> for AMA was reported to be 210 mg/kg bw [154]. DPOMA (acute LD<sub>50</sub> > 5000 mg/kg bw) [29] and

IOA [67] were reported to be nontoxic but slightly irritating to the shaved rabbit skin.

Subchronic dermal toxicity of an undefined acrylic resin [160], IACA [9], AMA [154], DPOMA [29], and IOA [67] was investigated on rabbits. Toxicity ranged from slightly irritating but nonotoxic (DPOMA) to erythema and edema (IACA and IOA) and to hyperkeratosis, erosion, and ulceration (an undefined acrylic resin and AMA); demonstrating the wide range of biological activity of acrylates. In mice, a similar range of dermal toxicity was observed when male C3H/HeJ mice were treated with EA (neat), AA, or BA [45]. AA and BA exhibited limited epidermal hyperplasia and no gross signs of skin irritation whereas EA induced dermatitis, fibrosis, hyperkeratosis, and necrosis.

Sensitization potential of acrylates using guinea pigs and different test methods by dermal exposure has been extensively investigated (Table II). The sensitizing potential may be dependent upon the method utilized. In general, the Freund's Complete Adjuvant Test (FCAT) is more sensitive than the Guinea Pig Maximization Test (GPMT), which is more sensitive than other procedures (Draize, Polak, Split adjuvant, etc.) used to determine sensitizing capacity [88]. Although, the FCAT may be more sensitive, it is not considered to be the most appropriate for determining allegericity under conditions of use [88]. When a set of 18 acrylates tested by both the FCAT and GPMT were compared (Table II), 78% of the acrylates had been judged to be sensitizers in the FCAT and 56% in the GPMT. Percentage do not equal 100 because different investigators reported some

acrylates as both sensitizing and nonsensitizing. This occurrence was the most frequent with the GPMT. Negative results were observed for 28% of the acrylates in the FCAT and 72% in the GPMT. Only one acrylate could not be determined either positive or negative in the FCAT, while the GPMT was not considered capable of determining 5 of the acrylates, in these different studies. MuFA were more effective in inducing sensitization than MoFA. Methacrylates were less effective than acrylates in inducing sensitization (see Table II and Mechanisms of Action for additional discussion). Cross reactivity between acrylates was common within those with structurally similar groups [17, 31, 170]. Inhibitors (e.g., hydroquinone) are added to prevent spontaneous polymerization in acrylates. The potential for inhibitors to produce sensitization and to cross react has also been investigated [17, 171, 176].

Hydroquinone and *p*-methoxyphenol were moderate sensitizers in the FCAT and were almost 100% cross reactive, but there was no direct correlation between the concentration of hydroquinone in acrylic monomers and the incidence of concomitant sensitization [171]. The combination of acrylic monomers with hydroquinone seemed to interfere with the sensitizing potential of the inhibitor. The concomitant sensitization to hydroquinone did not seem to interfere with either the sensitizing potential of the acrylate or the cross reaction pattern. However, in more limited studies, no sensitization to inhibitors (hydroquinone, *p*-methoxyphenol) in guinea pigs was observed [17, 176].

Table II. Sensitizing capacity of monofunctional and multifunctional acrylates in guinea pigs and humans. The number given in the table indicates the reference number.

Acrylate	Method	Guinea Pigs					Human Case Reports
		Non Sensitizing	No Grade	Mild	Sensitizing Moderate	Strong	
MA	GPMT	134					
	Polak	134					
BA	mod. Draize Split Adj.	134			132	24	
	Polak mod. Draize Split Adj.	134					15, 60, 69, 82
EHA	FCAT	178					15
	GPMT	31					
HPA	GPMT	134					
	Polak	31					82, 98
MCA	Polak	134					
BCA	Polak	134					
MMA	FCAT	134				30	33, 56
	GPMT	134					82, 84, 85, 104, 111, 159, 160
EMA	mod. Draize Split Adj.	24, 132, 134					
	FCAT	134				30	33, 82, 104, 109, 111
n-BMA	FCAT	172				30	82, 104
	GPMT	172					
t-BMA	FCAT	134					
	GPMT	172					
PMA	FCAT	172					111
	GPMT	172					
HEMA	FCAT	144, 170					33, 82, 106, 110, 111, 136, 137
	GPMT	134					16, 82
HPMA	GPMT		31				
NPMA	FCAT					176	
OTA-480	GPMT			17			
EEMA	Polak	134					
AMA	Polak	134					
THFMA	Polak	134					
MAA	Polak	134					
TCAA	Polak	134					
AA	Polak		134				57

Table II. Continues...

Acrylate	Method	Guinea Pigs					Human Case Reports
		Non Sensitizing	No Grade	Mild	Sensitizing Moderate	Strong	
EDDA	FCAT GPMIT	173	170	173			
BDDA	FCAT GPMIT	173		173		17	82
HDDA	FCAT GPMIT Polak	173	170 31 134	173		17	52, 53, 82, 126
PDDA	FCAT GPMIT	173		173			
NPDDA	FCAT GPMIT	173		173			
DEGDA	FCAT GPMIT	173		173			50, 82
TPGDA	GPMIT Polak		134			17	57
NRGDA	GPMIT					17	
TEGDA	GPMIT	17		27			14, 50, 82
TTEGDA	GPMIT	17		27			14, 57, 126, 181
PETA	GPMIT Polak		31, 124 134			24	15, 31, 37, 52, 53, 57, 124, 125, 126, 127, 158, 181
TMPTA <sup>1</sup>	GPMIT Polak mod. Draize		31 134 134			24	15, 32, 32, 52, 53, 57, 108, 112, 124, 125, 127
TMPTMA	GPMIT Polak			31			
EDDMA	FCAT GPMIT	173		173			
BDDMA	FCAT GPMIT	17		173			82
HDDMA	FCAT GPMIT	17		173			
PDDMA	GPMIT					173	
EGDMA	GPMIT Polak	17, 31 134	27				12, 33, 50, 82, 166
DEGDMA	FCAT GPMIT		27	173			106
TEGDMA	GPMIT Polak	31 134	27	17			12, 33, 50, 82, 129
TTEGDMA	GPMIT	17	27				106
NPDDMA	FCAT GPMIT			173			
NRPGDMA	GPMIT	17		173			

### Ocular Toxicity

Eye irritation was reported after exposure to acrylate vapors (EA, MA, BA, MMA) [145, 159, 167]. Most of the acrylates are severe eye irritants or corrosive when instilled into the rabbit eye. For example, MMA [159], EA [140], LACA [9], DPOMA [29], and IOA [67] induced eye irritation, inflammation and edema, and/or necrosis. Depending on the severity of the acute exposure most ocular effects were reversible within several days. Acrylate exposure to the eye may represent an acute and/or chronic hazard and should be considered for further investigation in animal and human studies.

### Neurotoxicity

Dermal exposure to monomeric MMA induced dermatitis and was associated with neurotoxicity [83, 174]. Although, motor nerve conduction was not different from control, abnormal muscle responses were observed, suggesting axonal degeneration in motor nerve branches. These limited studies indicate that dermal exposure to acrylates may cause local neuropathies at the site of exposure.

### Teratogenicity

Acrylates were teratogenic in rats either exposed by intraperitoneal injection [156] or by inhalation [122, 128]. Methacrylate esters (MMA; EMA; *n*-BMA; isobutyl methacrylate, IBMA; isodecyl methacrylate, IDMA) and AA produced dose related deleterious effects on the developing embryo and fetus (growth retardation and abnormal development) after injection [156]. At one or more of the doses used, each compound produced some or all of the following effects: skeletal resorptions, gross and skeletal malformations, fetal death, and

decreased fetal size. Methacrylate administration was associated with significant effects on embryonic and fetal development, which were, in general, dose related and compound dependent. AA was acutely toxic to the fetuses of treated rats, while the methacrylate esters were less toxic.

Inhalation exposure to MMA (110,000 mg/m<sup>3</sup> or 26,870 ppm for ~1 h/day) caused an increase in early fetal deaths and a decrease in fetal weight and size [128]. Exposure to same concentration for ~20 min/day decreased fetal size and weight, but did not affect mortality. Hematomas and skeletal abnormalities were observed in the fetuses of both exposure groups. Inhalation exposure to MMA in these concentrations produced significant evidence of embryo and fetal toxicity, and growth retardation. No overt signs of altered behavior or general demeanor in rats exposed to 0 ppm, 50 ppm (205 mg/m<sup>3</sup>), or 150 ppm (615 mg/m<sup>3</sup>) of EA by inhalation for 6 h/day during days 6 through 15 of gestation [122]. However, there was some evidence of maternal toxicity among rats exposed to 150 ppm. No incidence of skeletal resorptions nor adverse effects on the mean litter size were observed. EA was not considered to be embryo lethal and concentrations up to 150 ppm for 6 h/day were not considered to be teratogenic to rats. No studies were found on teratogenic effect on MuFA. Inhalation studies indicate that there may be some potential teratogenic risk if exposed to high enough concentrations of vapors of acrylates.

### Carcinogenicity

In general, alkylating agents, such as the acrylates, have an inherent potential to interact with biomolecules, e.g., cellular proteins and nucleic acids. However, their po-

tential to interact with biomolecules is limited by their molecular size and functional groups and their corresponding log P values [96]. EA is a contact carcinogen, when administered orally by intubation, that causes papillomas and squamous cell carcinomas in the forestomachs of rats and mice [130]. EA is considered a potential human carcinogen [78]. The requirement for sustained administration of EA to induce cellular proliferation preceding induction of forestomach tumors was demonstrated using the same rat strain and exposure conditions [66]. However, EA was not found to be carcinogenic to rats or mice by dermal application [45] or inhalation [115] under the experimental conditions employed. 2-Ethylhexyl acrylate (EHA) [46, 180] and neopentyl glycol diacrylate (NPGDA) [46] induced benign and malignant skin tumors in C3H/HeJ mice when administered topically. Triethylene glycol diacrylate (TEGDA) or tetraethylene glycol diacrylate (TEGDA), but not HDDA, TPGDA, trimethylol propane triacrylate (TMPTA), trimethylol propane trimethacrylate (TMPTMA), or tetraethylene glycol dimethacrylate (TEGDMA), induced a low incidence of either benign or malignant skin tumors in male C3H/HeJ mice (age not stated) when treated 2x/week for 80 weeks (2.5 mg/mouse for acrylates or 25 mg/mouse for methacrylates in mineral oil) [9]. In order to understand the potential carcinogenicity of individual acrylates as well as the acrylates in general, more research is required.

#### Mutagenicity

Based on their chemical structure and molecular reactivity, acrylates have the potential to interact with nucleic acids and/or nucleoproteins and induce mutations at the

gene or chromosomal level [10]. There is a great deal of variability reported *in vitro* and *in vivo* cytogenetic and mutagenicity studies in rodents. In general, evidence for the potential genotoxicity of the acrylates appears to be limited. Salmonella mutation assays (with or without mammalian metabolizing enzymes incorporated) have been negative for acrylates [26, 67, 81, 177]. The MuFA, TMPTA and TMPTMA, were marginally positive in Salmonella (strain TA1535), suggesting clastogenic activity [26]. In contrast, the *in vitro* mouse lymphoma assays (L5178 TK +/- assay) were generally positive [26, 38, 117, 118]. The possible inherent clastogenic activity of acrylates is not strongly supported by other *in vitro* assays. At concentrations one-half of the minimum lethal dose, EA and MA (ip; two consecutive daily doses) induced a 3-4 fold increase in micronuclei relative to the mitomycin C (positive control) in BALB/c mice [141]. However, in a similar study, EA failed to increase the incidence of micronuclei in C57BL/6 Aplk or BALB/c mice in a series of dosing regimens [11]. Finally, EA was toxic *in vitro* and *in vivo* (ip), but failed to induce an increase in sister chromatid exchange (SCE) rate, chromosomal aberrations, or micronuclei in C57BL/6 mice *in vivo* [89]. Only after *in vitro* exposure to splenocytes was chroma-tid-type aberrations observed. Possible differences in assay conditions (dosing regimens, chemical purity, substrain differences, etc.) and/or inability to deliver sufficiently toxic dose *in vivo* were cited as possible explanations in the differences observed. No studies of cytogeneticity carried out with MuFA were found in the literature. No studies were found that used the expected exposure routes for humans, i.e., dermal or inhalation exposure. On the basis

of these studies, no general conclusions can be drawn about the cytogeneticity of acrylates.

#### Mechanisms of Action

There are at least two possible mechanisms for the biological reactivity of acrylates. These are: (1) Michael's addition reaction with nucleophilic centers of biological molecules, e.g., sulfhydryl (-SH), amino (-NH<sub>2</sub>), or hydroxyl (-OH) [11, 43, 47], and/or (2) epoxidation of the vinyl double bond. However, epoxidation of the vinyl double bond does not have experimental support [44]. Most evidence suggests that acrylates react via the Michael's addition reaction mechanism.

For example, compounds which contain a double bond adjacent to an electron withdrawing group, such as the carbonyl moiety of an ester or aldehyde, are particularly reactive with nucleophiles and would be expected to react with and deplete NPSH [47]. The Michael's-type reaction formally involves attack on an electron deficient double bond by a carbon nucleophile (a carbanion) [11]. Attack at this position by -NH<sub>2</sub>, -OH, or -SH substituents would occur in a biological system. The stimulus for the reaction is two-fold. First, an electron withdrawing substituent renders the carbon ( $\beta$ -carbon) of the double bond electron deficient and, thus, prone to nucleophilic (electron rich) attack. In the case of EA, the substituent is -CO<sub>2</sub>C<sub>2</sub>H<sub>5</sub>; a similar activation is observed with acrylamide (-CONH<sub>2</sub>) and acrylonitrile (-CN). Vinyl chloride (-Cl) does not react in this manner since the chloro-substituent is only marginally electron donating. Second, once an attack at the  $\beta$ -carbon has taken place, a negative charge is formally localized on the

$\alpha$ -carbon, prior to its acquisition of a proton. Groups such as -CO<sub>2</sub>C<sub>2</sub>H<sub>5</sub>, -CONH<sub>2</sub>, etc., can stabilize this intermediate carbanion and promote the reaction. The net effect is an alkylation of the attacking nucleophile (e.g., NPSH or DNA) [11, 43].

These molecular mechanisms are supported by observations of toxicity in biological systems. For example, Dixon [47] suggested that sensory irritation and lacrimation may occur as a result of the reaction of chemicals with protein-SH groups of mucous membranes. The presence of a methyl group at the  $\alpha$ -carbon may diminish this electron withdrawing effect with a consequent fall in reactivity towards nucleophiles. The lower sensitizing potential of methacrylates is in good agreement with this theory. Generally, a covalent binding in the skin to a carrier protein is necessary to induce contact sensitization [138, 168].

However, the hydrolytic pathway may be the most important initial reaction for acrylate ester detoxification at low exposure concentrations. Low concentrations of MA and EA, sulfhydryl depletion occurred only in tritriethanolyl phosphate (esterase inhibitor) pretreated animals [155].

The variation in toxicity of acrylates was found to be significantly dependent upon log P or log K (second rate constant for a chemical reaction), and this might indicate the importance of their hydrophobicities in eliciting the acute toxic effects [13, 95, 153, 155, 161].

Although immunological cross-reactions between acrylates were discussed during consideration of dermal toxicity, it should be kept in mind that individual members of a "family" showing similar structural activity correlations must be characterized both

by the type and number of functional groups (i.e., acrylic and methacrylic), and by the central skeleton [31]. The central skeleton confers both bulk and steric properties, which might change the antigenic determinants more than the relatively small volume of a functional group(s). Both sensitization and resulting allergic contact dermatitis to simple chemicals are the result of immune reactions directed towards the hapten combined with a carrier. This can take the form of covalent binding or efficient complexing with endogenous proteins often by reactions with -NH or -SH groups [49, 133, 138]. The actual binding depends on the reactivity of the functional groups as well as the environment of the site of binding, each providing one or more determinants. Another factor is the accessibility of the site, which is governed not only by the bulk and charge of the hapten, but on its ability to cross-link.

The mechanism of acrylate induced tumorigenesis has not been determined. Silver and Murphy [155] found that MA and EA depleted NPSH groups in rats exposed by inhalation. Furthermore, EA and MMA were found to react directly with glutathione [70, 114], presumably via Michael's addition. Delbressine and colleagues [43] detected mercapturic acid metabolites excreted by rats following MA and MMA exposure. These metabolites correspond to Michael's addition to glutathione by the acrylates. When Delbressine and his colleagues [44] investigated metabolites derived from possible vinyl epoxidation reactions of phenyl-substituted MA, none were found. In addition, hydrolysis of acrylate esters can result in the generation of AA, which is toxic by itself [114]. Therefore, the Michael's addition reaction is a direct acting mechanism that

may be responsible for some or all of the genotoxicity of these compounds. The majority of the results in the mouse lymphoma assays support this direct acting mechanism, in which acrylates are usually active without exogenous activation [6, 117]. Chromosomal damage after EA exposure *in vitro* of interphase splenocytes was not observed [89]. However, EA was clastogenic when blast-transformed splenocytes were exposed, indicating that during cell division splenocytes were susceptible. Hypothetically, acrylates may saturate protective mechanisms, e.g., phase two conjugation systems like glutathione, cause irritation, inflammation, apoptosis or cell death, and induce hyperplasia or cell proliferation. Thus, exposing target cells during sensitive stages of the cell cycle to the clastogenic action of acrylates may lead to tumorigenesis [65, 89].

### Biological Effects of Ultraviolet Radiation

The biological effects of ultraviolet radiation may be divided into acute and chronic exposure effects, whose severity depends upon interaction between duration of exposure and spectral distribution of the UVR [79, 92]. The UVR spectrum (invisible to the human eye) is subdivided into near (UVA: 315-400 nm), middle (UVB: 280-315 nm), and far (UVC: 100-280 nm) wavelength regions. Acute effects include sunburn to eyes (photokeratitis) and skin (erythema and inflammation) as well as local and systemic immunosuppression, including suppression of contact hypersensitivity. Chronic effects include cataracts (eyes) and aging of skin (irreversible damage to the dermis), persistent systemic immunosuppression, and cancer (mutation and

clonal expansion of preneoplastic cells leading to skin cancer).

Solar radiation is considered to be carcinogenic to humans [79]. UVB is considered to be the oncogenic factor in solar radiation and is known to penetrate into the supra basal layer of the epidermis, which also contains the dendritic antigen presenting Langerhans cells [169]. UVR induced defects in antigen presentation may be a critical factor in the cascade of immune effects leading to the induction of immunosuppression [36, 79, 185]. UVA may be considered to be a greater cancer promoting factor than UVB, because it penetrates deeper into the epidermal basal cell layer and dermis; effectively inducing reactive oxygen species (ROS) and inflammation [51]. Application of the potent contact hapten, dinitrofluorobenzene (DNFB) to UVB exposed or unexposed normal human skin and the resulting in variation in presence or absence of the development of contact hypersensitivity between individuals suggests that UVB-resistant or UVB-susceptible human phenotypes exist in the general population [185]. Nonmelanoma skin cancer patients (92%) were observed to be of the UVB-susceptible phenotype because they failed to develop contact hypersensitivity after DNFB was applied to the UVB irradiated site. These UVB-resistant or susceptible phenotypes have recently been correlated with polymorphisms in tumor necrosis factor alpha (TNF $\alpha$ ) that affects cutaneous immunity in lipopolysaccharide-sensitive mouse strains [175].

### Exposure Limit Values for Acrylates and UV Radiation

MuFA have a much lower vapor pressure than MoFA. Most MuFA have a vapor pressure <0.01 mmHg at 20°C [4, 7, 8], indicating that the potential inhalation exposure to MuFA should be low under normal work room conditions. Acrylates have a low odor threshold limits, making their presence readily apparent [4, 7, 8]. American Conference of Governmental Industrial Hygienists (ACGIH) has set 8 h time-weighted average threshold limit values (TLV-TWA) to simple MoFA [2]. The American Industrial Hygiene Association (AIHA) recommends 1 mg/m<sup>3</sup> as the 8 h time-weighted average (TWA) workplace environmental exposure level (WEEL) for MuFA. In the United States the Occupational Safety and Health Administration (OSHA) has not set allowable air concentrations for any MuFA.

International Radiation Protection Association/International Non-ionizing Committee (IRPA/INIRC) has published recommendations and guidelines for exposure to UVR to prevent adverse health effects [80]. ACGIH has also recommended the TLV for occupational exposure to UVR in different wavelength regions [3]. In Sweden, Criteria Group for Physical Risk Factors has recently suggested occupational exposure limits to UVR in indoor situations [93]. Exposure limit of 30 J/m<sup>2</sup> as a 8 h TWA and the total dose of 7,000 J/m<sup>2</sup> (biologically weighted in relation to skin erythema action spectrum [113]) over one year period for indoor work are proposed.

Occupational risks related to the use of UVR curable acrylic coatings in the surface coating of wood have not been investigated. It is important to understand the mechanism and the different factors affecting the curing process and what new requirements are made on the work environment and the worker in order to create a safe work environment in the wood surface coating industry. The main goal of this research project was to gain an understanding and to describe the surface coating of wood with UVR curable acrylic coatings and to identify risks and any adverse health effects to workers employed in the UVR surface coating processes.

Determination of risks to wood manufacturing workers using UVR curing processes was conducted by:

1. identifying chemicals involved in UVR coating and the risks in handling these chemicals (lacquers, paints, fillers, pig-

ments, photoinitiators, etc.), during and after the coating process, and during sanding, finishing, and assembling the product.

2. developing methods to evaluate and measure worker exposure.
3. conducting a one day industrial hygiene survey in selected plants.
4. identifying and measuring adverse health effects workers have experienced.
5. identifying and selecting potential toxic chemicals and complex mixtures of UVR curable acrylic coatings for determination of their carcinogenic and mutagenic properties in transgenic mice using short term exposure *in vivo*.
6. identifying and selecting potential toxic chemicals in UVR curable acrylic coatings for determination of cytotoxicity to normal human skin and lung cells *in vitro*.

The research project consisted of three different types of studies: (1) occupational exposure assessment (Papers I-II), (2) survey on adverse health effects using questionnaires and medical examinations of workers at work sites (Papers III-IV), and (3) prospective *in vivo* and *in vitro* toxicity and carcinogenicity studies using a transgenic animal model system and normal human skin and lung cell cultures (Papers V-VII).

## Occupational Exposure Assessment (Papers I-II)

The purpose of the field studies was to identify potential hazards and characterize their association with surface coating of wood with UVR curable acrylic coatings and to develop strategy for risk management. In order to determine potential risks in the manufacture of wood products, which are surface coated with UVR curable acrylic coatings, and to develop strategies to reduce and/or prevent worker exposure to hazardous materials, full scale occupational hygiene surveys were conducted in this industry. This included:

1. measurement of worker exposures, e.g., airborne contaminants (acrylates, dust, ozone, solvents) and UVR.
2. measurement and control of UVR curing units in order to determine curing efficiency.
3. analysis of possible reactive chemicals (monomers) remaining after curing in sanding dust.
4. detection of recurring risks using video filming of workers during the entire work day and using film analysis and

questionnaire as aids for determining risk management procedures.

## Identification and Selection of Industrial Sites and Subjects

Identification and selection of industrial sites and the characterization of the work force have been described in Paper I. Table III shows the general information of the industrial sites surveyed in this study. All workers potentially exposed to uncured acrylic coatings and UVR and/or cured acrylic coatings were identified through employers.

## Manufacturing Processes

The process descriptions for the application of UVR curable wood surface coating included only flat object processing. Any manufacturer processing three dimensional objects with this technology was not found. The UVR surface coating process was comprised of roller coaters and/or filling by hand. Primarily, the manufacturers used one or two application methods and more than one type of UVR curable coatings. Application of the lacquer and/or paint to the product surface was accomplished by a roller coater. Lacquer or paint was continuously fed onto the roller coater by pumping from an adjacent 20-200 liter container. In this manner, a ~0.01 mm thick (5-25 g/m<sup>2</sup>) coating was applied by roller to the substrate during each application. Excess coating from the roller coater was recycled to the container. In parquet floor manufacturing, fillers were also used and were usually applied by hand or by pouring the

Table III. General information on the surveyed UV surface coating manufacturing plants.

Information / Plant ID	1	2	3	4	5	6	7	8	TOTAL
Production	parquet floors	furniture	parquet floors	furniture	furniture	furniture	parquet floors	furniture	
Nr employees	105	95	1100	950	100	105	553	221	3229
Nr production workers	90	90	1000	700	86	95	459	166	2686
Nr UV line workers	28	12	53	3	5	7	6	6	120
Nr post-UV workers	14	15	0	150	22	8	30	20	259
Previous UV workers	0	2	32	0	1	0	0	4	39
Hand sanding	7	0	0	0	0	0	0	1	8
Nr of UV lines	1	2	1	1	1	1	1	1	9
Nr of filling lines	3	0	2	0	0	0	0	0	5
Nr of combined lines	0	0	0	1 UV+solvent	1 UV+solvent	0	0	2 UV+solvent	4
Nr of years UV line(s) used	2	10	9	2	6	2	10	4	5.6**
Nr of days/wk UV line used	5	5	5	4	5	5	5	5	5**
Nr of work shifts	2	2	3	1 (2)*	1 (2)*	1	3	1	
Total amount used / year									
UV lacquer and paint	42,000 kg	17,500 kg	175,000 kg	7,080 kg	18,150 kg	2,300 kg	258,040 kg	6,300 kg	526,370 kg
UV filler	45,400 kg	0	92,000 kg	0	0	0	0	0	137,400 kg
Average amount used / day									
UV lacquer and paint	175 kg	73 kg	730 kg	30 kg	76 kg	10 kg	1,075 kg	26 kg	2,195 kg
UV filler	189 kg	0	380 kg	0	0	0	0	0	569 kg

\* only combi line used in 2 shifts

\*\* average

filler manually onto the roller coater. In one plant the filler was applied by an automatic process but still required manual control and hand filling. If filling was required, it was always performed before coating the product with UVR curable acrylate lacquer or paint. If an additional application of another type of formulation (e.g., water based coating, acid curing coating, or stain) was performed together with a UVR curable acrylate coating, these were applied on top of the UVR cured coating, except stain, which was applied before the coating.

#### Acrylate Coatings and Other Chemicals

Three major UVR surface coating formulations who supply UVR curable acrylate coatings to the wood surface coating industry operate in Sweden. UVR curable coatings are usually supplied in 20 l containers, but in some cases 200 l barrels or 1,000 l plastic containers are used. Altogether, 19 different acrylate coatings were observed to be in use at the 8 surveyed plants. According to the material safety data sheets (MSDS) on file the most frequently observed UVR curable coatings were composed of acrylate prepolymers and TPQDA, but HDDA was often used in these coatings in combination with TPQDA. The acrylate prepolymers were most often stated generally as oligomers, but some MSDS stated the prepolymer used was epoxy acrylate (5/19 coating formulations). Polyester-acrylate was stated to be the prepolymer in 2/19 coating formulations.

TPQDA and HDDA are the most frequently used MuFA in radiation curable coatings [73]. The percentage of MuFA monomers, HDDA and TPQDA, in the coatings used in the surveyed plants, were most often reported in the MSDS reviewed to be between 10-30%. Less often, glycerol

propoxy triacrylate (GPTA), TMPTA, and ethylglycol acrylate (EDGA) were reported. In addition, zinc sulfide, titanium dioxide, talc, silica, methyl ethanol amine, hydroxy alkyl phenone, butyl acetate, and pigments were reported in the MSDS. MoFA (e.g., HEA), may be used in small quantities as reactive diluents to lower the viscosity of some types of radiation curable coatings [1], but none of these were reported in the MSDS reviewed. In three work places hardeners were mixed into UVR curable coatings in order to ensure uniform and maximum curing of the coating. Methyl ketone peroxide, polymeric isocyanate, monomeric hexamethylene diisocyanate, or polyfunctional aziridine were used as hardeners in these work places.

#### Worker Exposure Measurements

Exposure assessment was determined during a 1-day industrial hygiene measuring survey conducted at eight selected plants. Sample collections were conducted with personal sampling equipment placed in the breathing zone of the worker during a complete 8 h work day on a UVR surface coating line or on a station for the finishing of cured products.

Airborne dust samples were collected with a 37 mm glass fiber filter (Whatman, GF/F) or a 37 mm cellulose ester filter (Millipore, pore size 0.8 µm) and a personal sampling pump (flow rate of 2 l/min). All the samples were analyzed gravimetrically and some samples were further analyzed for acrylates and photoinitiators by a gas chromatograph.

Particle size selective sampling was conducted using a personal cascade impactor and a personal sampling pump (flow rate of 2 l/min). The cascade impactor was a 6-stage device with cut points ranging from

0.5-10 µm aerodynamic diameter. Particles larger than the cut point for each stage were collected on a 34 mm Mylar filter and particles smaller than the cut point of the last stage were collected on a 34 mm PVC af-fertiler (pore size 5 µm). The aerosol size distribution was determined through gravi-metric analysis for the sample collected on each filter.

Airborne vapors were collected with a SKC charcoal tube containing 150 mg char-coal (with a 50 mg back-up section) and a personal sampling pump (flow rate of 50 or 200 ml/min). Charcoal tubes were extrac-ted with 2 ml of carbon disulfide for 4 h and analyzed by a gas chromatograph, similar to airborne total dust samples.

Airborne acrylate vapors and aerosols were collected with an OSHA Versatile Sampler (OVS) tube containing a glass fiber filter, Tenax sorbent (140/70 mg), and polyurethane foam and a personal sampling pump (flow rate of 1 l/min). The OVS tubes were extracted with 5 ml of acetone for 4 h and analyzed by a gas chromato-graph.

#### Stationary Measurements

Stationary airborne dust samples were col-lected on a 25 mm Nuclepore® gold coated polycarbonate filter (pore size 0.4 µm) placed in a nonconductive holder using a personal sampling pump (flow rate of 2 l/min) for electron microscopic analysis. The sampling time was ~20 min. The fil-ters were gold coated and analyzed for par-ticle size and shape using a JEOL scanning electron microscope connected to an image analyzer. The smallest particle diameter (D<sub>min</sub>) was measured from 400 particles. The medium diameter and lower and upper quartiles were calculated.

Ozone concentration measurements were determined in the ventilation exhaust pipes of UV lamps during lamp ignition and the first hour of operation and in the work place ambient air. Measurements were conducted using a portable ozone analyzer connected to a printer.

Surface wipe samples were collected us-ing glass fiber filters (Whatman, GF/A, 37 mm) dampened with distilled water suffi-cient to wipe a determined surface area (100 cm<sup>2</sup> or 25 cm<sup>2</sup>) at different places were exposure may occur. The samples were extracted with 5 ml of methanol and analyzed after 4 h by a gas chromatograph.

#### UV Radiation Measurements

##### UV Exposure Measurements

The spectral distribution of UVR was mea-sured using a Photodyne 88 radiometer and an Yvon John grating monochromator or a MSS 2040 spectroradiometer. The UVR intensity measurements were determined with a Photodyne 88 radiometer equipped with an EE&G optical sphere and inter-ference filters with different windows within specific UVR wavelength regions or an IL 1400A radiometer with the UVA detector SL021#193 or the UVB detector SEL240 #3256.

##### Production Parameters

The performance of UV lamps in the curing units was measured using UVIMAP® in-strument. After measurement (data collec-tion) the UVIMAP® was connected to a thermal printer or to a portable personal computer for print out or analysis of the collected data, respectively. Further pro-cessing of the collected data enabled us to calculate the transfer efficiency of the UVR energy in the wavelength region 320-390 nm and analyze the proper mounting and

maintenance of the UV units. The transfer efficiency of the UV lamp was determined as the total measured UVR energy in the given wavelength region (320-390 nm), which impinged the maximal curable sur-face area in percentage of the energy con-sumption of the UV lamp according to manufacturer's specifications. Operation times of the UV lamps were also recorded.

#### Work Practices

In order to record and analyze worker per-formance, work tasks, and used protective clothing and equipment during the work day, video filming was conducted during the 1-day industrial hygiene survey of the plants.

#### Medical Surveillance (Papers III-IV)

Health effects related to the presence of po-tential hazardous chemicals were studied in individuals at work sites where exposure measurements had been conducted as well as at sites that had not been surveyed. All workers exposed to uncured acrylate coat-ings and UVR and/or cured acrylate coat-ings were identified in an occupational hy-giene survey (Paper I). For comparison, workers employed in the manufacture of wood surface coated products by other methods than UVR curing (i.e., acid curing lacquers and paints) and unexposed matched controls were identified in these plants. In addition, a group of 30 office workers, never employed in the wood sur-face coating industry, were included as unexposed matched controls. All investiga-tions were performed in the work places during the working hours.

#### Identification and Selection of Study Populations

For investigation of upper airway and occu-lar symptoms, five groups of subjects were formed from the study population. Group (I) consisted of 26 UVR surface coating line workers (UV line), exposed to uncured and/or cured acrylate coatings and UVR. Group (II) consisted of 24 acid curing sur-face coating line workers (AC line), ex-posed to organic solvents, formaldehyde, and uncured and/or cured acid curing coat-ings. Group (III) consisted of 33 workers employed in the finishing processes of UVR cured acrylate surface coated products (UV finisher), exposed to cured and/or partially cured acrylate coating dust and wood dust. Group (IV) consisted of 35 workers employed in the finishing process-es of both UVR cured acrylate and acid cur-ing surface coated products (UV/AC fin-isher), exposed to cured and/or partially cured acrylate and acid curing coating dust and wood dust. Group (V) was a control group of 30 office workers (control) from the same village, where the majority of sur-veyed plants were located, and who had no history of occupational exposure to acry-lates, organic solvents, and/or wood dust. For dermatological evaluation, seven groups of subjects were formed from the study population of 659 workers. Groups (I) to (IV) were the same employment cate-gories as for the upper airway and ocular symptom investigation, except that more individuals were included and that group (IV) also included workers employed in spray painting with acid curing lacquers and paints. Group (I) consisted of 146 workers, group (II) of 57 workers, group (III) of 54 workers, and group (IV) of 97 workers. Additional groups were formed because of the variety of work tasks in the

wood surface coating industry. Group (V) consisted of 71 workers employed in the surface coating line using both UVR curable acrylate and acid curing coatings (U/VAC line). Group (VI) consisted of 61 workers previously employed in the UV line, but had moved to other work tasks, that did not include exposure to UVR curable acrylate coatings (prior UV). Group (VII) was a control group of 173 office workers (control) from the same work sites and also included the 30 office workers that had never been employed in the wood surface coating industry.

## Questionnaire

Questionnaires were used to clarify how workers experienced their work environment and possible health effects, which workers connect to work with UVR curing process, as well as to independently detect possible irritation or other health problems experienced in airways, eyes, and skin. Questionnaires were distributed to all exposed workers and their matched controls before the medical examination, completed by the participant, and reviewed in detail by the medical examiner before the examination and/or with the participant during the examination. The questionnaire included questions concerning previous and present work tasks, employment time, sex, age, smoking history, airways, nose, and ocular symptoms, allergy and hyperreactivity, previous and current skin problems with special regard to problems with exposure to different working materials and work tasks, and use of personal protective equipment.

## Medical examinations

### Airways, Nose, and Eyes (Paper III)

The medical examinations were performed during November and December in order to minimize the effects of seasonal allergies. The medical examination focused primarily on the nose and the throat. Nasal obstruction was measured with a peak expiratory flow meter (The Right Peak Flow Meter, Airmed, England) connected to an anesthetic face mask by an adapter. The mean value of the two best of three maximal expirations was used for statistical analysis. In a single blind fashion, nasal mucociliary clearance was measured with one-quarter of a saccharin tablet (Hermescetas<sup>®</sup>) applied on the inferior turbinate, 1 cm behind the anterior border of the turbinate. Time was recorded until the subject could describe the associated taste. A time of transport (taste sensation) exceeding 20 min was regarded as pathological. Olfaction was determined by measuring the olfactory threshold [25]. The odorant stimulus was an aqueous dilution of 1-butanol where successive dilutions (step 1-13) differed by a factor of three. The highest concentration was 4% (step 1) and the lowest 0.4x10<sup>-6</sup>% (step 13). The lowest concentration that was repeated correctly detected was marked as threshold level.

Spirometry was performed with a Vitalograph<sup>™</sup> (Buckingham, England). At least three efforts were recorded by each subject, and the best one was used for all calculations. Vital capacity and forced vital capacity in one second (FVC<sub>1.0</sub>) were recorded. All the individual values were compared with the expected normal values (based on age, sex, height, weight and smoking habits) according to Quanjer [142].

### Skin (Paper IV)

Clinical examination of the skin of hands, arms, and face was conducted in all workers that expressed skin problems (eczema) as a child or as an adult. Patch testing was performed using standard panels (TRUE Test<sup>™</sup>, Pharmacia, Uppsala, Sweden), which includes 24 test materials (48 different chemicals, individual or combination; Table IV) and a special test series applied

by chamber test technique used for chemicals included in acrylate coatings with UVR surface coating of wood (Table V). White petrolatum was used as the vehicle for these chemicals. Patch tests were placed on the sides of the upper back. Patch tests were scored (Table VI) according to international standard [55], 72 h after application and 24 h after the removal of the patches.

Table IV) and a special test series applied

Table IV. Standard test series (TRUE Test<sup>™</sup>). Vehicles used for test material: hydroxypropylcellulose, carboxymethylcellulose, or polyvinylpyrrolidone.

1. Nickel Sulfate	13. p-Terz-Butylphenol Formaldehyde Resin
2. Wool Alcohols	14. Parabene Mix
3. Neomycin Sulfate	15. Carba Mix
4. Potassium Dichromate	16. Black Rubber Mix
5. Caine Mix	17. Cr <sup>VI</sup> -Me <sup>2</sup> -Isobutazolinone
6. Fragrance Mix	18. Quaternium-15
7. Colophony	19. Mercaptoenzothiazole
8. Epoxy Resin	20. p-Phenylenediamin (PDD)
9. Quinoline Mix	21. Formaldehyde
10. Balsam of Peru	22. Mercapto Mix
11. Ethylenediamine Dithiochloride	23. Thimerosal
12. Cobalt Chloride	24. Thiuran Mix

Table V. Acrylate patch test series. Vehicle used for test material was white petrolatum. The weight percent of the chemical in the vehicle is expressed in parenthesis.

25. Epoxy Acrylate (0.5%)	30. Trimethylol Propane Triacrylate (TMPTA; 0.1%)
26. Oligo Triacrylate (OTA) = Glyceryl Propoxy Triacrylate (0.1%)	31. Tripropylene Glycol Diacrylate (TPGDA; 0.1%)
27. Methylhydroquinone (1%)	32. 2-Hydroxy-2-methyl-1-phenylpropane (Diacour 1173; 2%)
28. 1,6-Hexanediol Diacrylate (HDDA; 0.1%)	33. 2,2-Dimethoxy-1,2-diphenylethane-1-one (Irgacure 651; 2%)
29. Triethylene Glycol Dimethacrylate (TEGDMA; 2%)	34. 1-Hydroxy-cyclohexyl-phenyl-ketone (Irgacure 184; 2%)

Table VI. Patch test scoring.

-	Negative reaction
~/R	Questionable or irritative reaction, which do not fulfill criteria for positive reaction
+	Weak positive reaction, redness, infiltration, and discrete papules
++	Distinct positive reaction, redness, infiltration, papules, and sometimes vesicles
+++	Strong positive reaction, intensive redness, infiltration, and joining vesicles

### Comparative *In Vivo* and *In Vitro* Toxicity of Selected Acrylates (Papers V-VIII)

The toxicity of TPGDA and two UVR curable acrylate lacquers containing TPGDA was investigated. Short term carcinogenicity and mutagenicity studies using transgenic mice and *in vitro* cytotoxicity studies using normal human skin and lung cells were conducted. In these studies, the toxicity of TPGDA was compared to EA, a monofunctional acrylate, used as a reference acrylate because of the number of published studies on its toxicity, in order to place the significance of the findings in context to the studies conducted.

### Chemicals

EA, a reference acrylate, was a colorless liquid with a pungent odor at 99% purity. TPGDA was a pale-yellow tinted liquid with a mild odor at 80% pure monomer with >18% TPGDA oligomer. Lacquer A (reference lacquer containing only the base components for UVR curable wood surface coating) was a pale-yellow tinted liquid with an acrid odor and according to the manufacturer contained 56.4% TPGDA monomer (remaining components were proprietary ingredients). A commercially available UVR curable wood surface coating lacquer (Lacquer B) was a dark opaque and viscous liquid with a acrid odor and

according to the manufacturer contained 24.8% TPGDA monomer (remaining components were proprietary ingredients). Due to the tendency of the acrylates to polymerize when exposed to light, hydroquinone (<200 ppm) was added as an inhibitor to each test article by the manufacturer. Samples were stored in the dark at 4-6°C. Analyses of the stability of the test compounds were not carried out. All studies were conducted during the manufacturer's warranty for chemical stability. Acetone was used as a vehicle control. For a positive control, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was used.

### Short Term *In Vivo* Toxicology, Carcinogenesis, and Mutagenesis Studies (Papers V-VI)

#### Mice

Female homozygous TG•AC transgenic mice ( $\zeta$ -globin promoter fused with the *Ha-ras* oncogene on the FVB strain) were used. All mice were examined upon receipt and acclimated for two weeks. Mice were housed 3-5 per cage. Based on the body weights, mice were randomly assigned to treatment or control groups; 3-4 female TG•AC mice per dose group were selected for the dose finding studies and 10 female mice per dose group for the 20 week study. These animals were divided into 3 dose groups per test substance and positive and negative (vehicle) control groups. One

positive and one negative (vehicle) control group served as control groups for all the test substances (EA, TPGDA, Lacquer A, and Lacquer B) for data comparison. Prior to the first dose application, all animals were inspected and their backs closely shaved with electric clipper on the site of application, while avoiding the production of abrasions or cuts. On the day before each treatment all animals were inspected for the hair growth and an additional shaving was conducted on the day of treatment, if needed.

The test substances, dissolved in acetone in 200  $\mu$ l total volume were applied to the shaved back of each mouse from the mid-back (interscapular region) to near the base of the tail ( $\approx$ 8 cm<sup>2</sup>) using a calibrated micropipette. Mice were treated once daily with a single dose of 200  $\mu$ l total volume per animal three times a week (Monday, Wednesday, and Friday) over a 2 or 20 week period. At 4, 8, 12, 16, and 20 weeks of treatment, 1-2 mm of the terminal portion of the tail was snipped and 3 drops of blood collected; 1 drop for Single Cell Gel (SCG) assay and 2 drops to prepare blood smears for micronucleus (MN) analysis.

#### Exposure

Two week dose finding studies were conducted by administering EA, TPGDA, Lacquer A, Lacquer B, and TPA in acetone vehicle with doses of 60, 300, or 600  $\mu$ moles (3, 15, or 30 mg/ml acetone), 0.5, 1.0, 5.0, 60, 300, or 600  $\mu$ moles (0.75, 1.5, or 7.5 ml/ml acetone, 90, 450.4, or 900.75 mg/ml acetone), 0.5, 1.0, or 5.0  $\mu$ moles (0.0013, 0.0025, or 0.0125 ml/ml acetone), 0.5, 1.0, or 5.0  $\mu$ moles (0.0029, 0.0057, or 0.0286 ml/ml acetone), or 2  $\mu$ moles (6.25  $\mu$ g/ml acetone), respectively. To evaluate the dose dependency for increasing relative

skin thickness (epidermal keratinocyte hyperplasia), three concentrations of EA (60, 300, or 600  $\mu$ moles), TPGDA (1, 5, or 10  $\mu$ moles), and Lacquer A (1, 5, or 10  $\mu$ moles) in acetone were selected for the short term (20 week) experiment.

#### General observations

Individual body weights were recorded at the beginning of the study, twice weekly through the study, and at study termination. All animals were observed in their cages twice daily for signs of morbidity or mortality. Weekly, clinical observations were performed and the test sites were examined for signs of irritation and local skin lesions as well as the onset and progression of tumor growth.

#### Post-Mortem Procedures

At dose finding study termination, gross necropsies were performed on all mice. A section of skin from the test site was collected in Bouin's fixative and the inguinal lymph nodes were collected in 10% neutral buffered formalin. The test site skin and the inguinal lymph nodes were processed and subjected to histopathological analysis. In addition, the test site skins were graded for epidermal thickness. Relative skin thickness was determined by preparing each mouse's dorsal skin by excision and fixation for 24 h in 10% neutral buffered formalin, processed, and embedded in paraffin. Approximately 6  $\mu$ m sections were bichromically stained with hematoxylin and eosin for histological examination. Morphometric analysis was performed with an ocular micrometer. The number of nucleated epidermal cell layers was counted at five randomly selected locations per slide and averaged. The thickness of the nucleated cell layers of the epidermis was also

measured in a similar manner, and the means  $\pm$  standard errors of the mean were calculated.

In the short term carcinogenesis study, complete necropsies were carried out on mice that died or were sacrificed when moribund. A section of skin from the test site was collected in Bouin's fixative and all gross lesions were placed in 10% buffered formalin.

#### Single Cell Gel Analysis

The extent of DNA damage in nucleated cells of the blood (leukocytes) as a measure of systemic cytotoxicity was measured by the alkaline Single Cell Gel microgel electrophoresis (SCG) assay. This technique is capable of detecting intercellular differences in DNA damage (DNA single strand breaks and alkali labile sites in individual cells) in virtually any eukaryotic cell population and it requires extremely small numbers of cells (1,000 to 10,000 cells) [157]. Cells with increased DNA damage display increased migration of the DNA from the nucleus towards the anode. The parameter used to evaluate DNA damage was the percentage of migrated DNA.

Peripheral blood samples were collected at 4, 8, 12, and 20 weeks of treatment. Each blood sample was placed in a pre-labeled vial containing 1 ml of ice cold Hank's Balanced Salt Solution (HBSS) with 20 mM EDTA and 10% DMSO [165]. The cell suspension was pelleted by centrifugation, mixed with 0.5% low melting point agarose at 37°C and layered onto microscope slides [157], one per mouse. All slides were placed in a lysing solution consisting of 1% sodium sarcosinate, 2.5 M NaCl, 100 mM Na<sub>2</sub>-EDTA, 10 mM Tris (pH 10) with 10% DMSO and 1% Triton X-100, added fresh at 4°C until elec-

trophoresed. After remaining in the lysing solution for at least 1 h, the SCG slides were treated with alkali (1 mM Na<sub>2</sub>•EDTA and 300 mM NaOH; pH > 13) for 60 min to express alkali labile damage, followed by electrophoresis for 20 min at 25 V/300 mA. Following electrophoresis, the alkali was neutralized with 0.4 M Tris (pH 7) and stained with ethidium bromide (0.5 µg/ml). Slides were scored in numerical order from randomly numbered mice to eliminate bias analysis. Observations were made at 250x magnification using a fluorescent microscope equipped with an excitation filter of 515-560 nm and a barrier filter of 590 nm. Twenty five cells per mouse were evaluated for DNA damage (single strand breaks and alkali labile sites) using the Comet Image Analysis System (Kinetic Imaging Ltd., Liverpool, UK).

#### Micronucleus Analysis

For evaluation of the systemic toxicity of EA and TPGDA, the frequency of micronucleated polychromatic erythrocytes (MN-PCE) and the percentage of polychromatic erythrocytes (%PCE) in peripheral blood were evaluated at week 20. The analysis of micronucleated erythrocytes is the approach most commonly used for evaluating the ability of suspect genotoxicants to induce either structural or numerical damage in proliferating *in vivo* cell populations [71, 102, 164]. The assay is conducted in mice by evaluating the frequency of MN in immature erythrocytes (i.e., PCE) scored in bone marrow or in peripheral blood preparations [71, 103, 150]. Peripheral blood sampling is especially useful because it allows for repeat sampling on the same animal and a temporal evaluation of DNA damage induced in bone marrow [100, 101, 103, 150]. In addition, an

analysis of the %PCE in peripheral blood allows an evaluation of the effect of chemical treatment on the rate of erythropoiesis.

Peripheral blood smears were air dried, fixed using absolute methanol, and then stained with acridine orange [163]. Coded slides were scored in numerical order at 1,000x magnification using epi-illuminated fluorescence microscopy (450-490 nm excitation, 520 nm emission). To assess if chemical treatment affected the rate of bone marrow erythropoiesis (signified by a change in the proportion of PCEs within the total erythrocyte population), the number of PCE among a total of 1,000 erythrocytes was determined at 1,000x magnification. For the determination of the frequency of micronucleated cells, 2,000 PCEs were evaluated at 1,000x magnification.

#### Comparative *In Vitro* Cytotoxicity

##### (Paper VIII)

##### Cell Culture

Cryopreserved primary or proliferating secondary normal human epidermal keratinocytes (NHEK), dermal fibroblasts (NHDF), and bronchial epithelial cells (NHBE) from 3-5 different adult female of male individuals were subcultured in growth medium in loosely capped 75 cm<sup>2</sup> tissue culture flasks in a humidified 5% CO<sub>2</sub> incubator at 37°C. Growth medium was changed every 48 h. For chemical exposure, cells were harvested from culture flasks (60-90% confluent) by standard trypsin digestion methods, counted, and seeded onto 96-well flat-bottomed plates at a density of ~2500 cells/well or 60-100 cm<sup>2</sup> at 500 cells/dish for determination of cloning efficiency. At confluence or ~2/3 confluence, depending upon the assay, the culture medium was removed and replaced

with growth medium containing the test chemical. All experiments were performed on cells between the second and fourth passage from primary culture.

##### Chemical Exposure

Cell cultures were exposed to EA, TPGDA, Lacquer A, or acetone dissolved in the required growth mediums for 18 h in a humidified 5% CO<sub>2</sub> incubator at 37°C. Concentrations of EA, TPGDA, or Lacquer A varied from 5 to 0.0024 µmoles by 2 fold increments. Acetone (0.1% in medium) was used as a negative controls. Chemicals were diluted in acetone before serial dilutions in medium to achieve the desired concentration. All exposures were performed with a total volume of 50 µl. All exposures and measurements were performed with two replicate plates for each treatment (N=8 per plate) and were repeated twice (N=32).

##### UV Radiation Exposure

Epidermal keratinocyte cultures (eight 60 cm<sup>2</sup> dishes per treatment; ~90% confluent) were exposed to: (1) 5 J/cm<sup>2</sup> UVA (600 µW/cm<sup>2</sup>) alone, (2) 1 J/cm<sup>2</sup> UVA (600 µW/cm<sup>2</sup>) followed by 3.54, 1.77, or 0.354 µmoles of TPGDA/dish, (3) 3.54 or 0.354 µmoles TPGDA alone, or (4) 0.1% acetone alone at 35-37°C. The experiment was replicated once. UVA radiation was generated using two Sylvania Blacklie Blue lamps. Lamp output was determined using a spectral radiometer. All exposures were performed in sufficient keratinocyte basal medium (KBM) to cover the cell layer and prevent desiccation during exposure. Chemical exposure cultures were sham irradiated under the same conditions minus UVA exposure. Medium was changed immediately to growth medium after radiation or sham irradiation. After 48 h, cells were

subcultured to 3 dishes (500 viable cells/dish) for determination of the cloning efficiency and transformation index. Cytotoxicity (viability) was determined on 3 aliquots from each dish by the MTT assay (see below; 5000 cells/well) and percent viability (relative to control) calculated. Subcultured cells were held at 4°C until plating.

#### *Viability, Cell Growth, and Free Sulfhydryl Content*

The methylthiazol tetrazolium (MTT) assay was used to determine cell viability. MTT is a pale-yellow substrate that produces a dark blue formazan product when incubated with living cells. The MTT ring is cleaved in active mitochondria and the reaction occurs only in living cells [121]. The MTT assay has been used widely to determine cellular proliferation and viability of cells *in vitro* after exposure to toxic agents [61, 86, 97]. After exposure, 15  $\mu$ l 4,5-(dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, was added to each well. After a 4 h incubation at 37°C, 100  $\mu$ l stop/solubilization was added; except for individual wells with cells to serve as background controls, which had stop/solubilization solution added immediately after substrate. Cell cultures were incubated overnight in a humidified atmosphere at room temperature. Absorbance was determined at 570 nm using an ELISA (Enzyme Linked Immuno Sorbent Assay) plate reader. Untreated and acetone vehicle control designated wells containing equal numbers of cells served as controls.

After exposure, free sulfhydryl content of control and treated cells in 96-well plates was determined by modification of Ellman's standard method and reagents. Cell cultures were washed twice with 100  $\mu$ l phosphate buffered saline (PBS) and 10  $\mu$ l 0.1

$\text{Na}_2\text{HPO}_4$  at pH 8.0 added. Culture plates were double wrapped in plastic and stored at -70°C for 24 h. Plates were allowed to equilibrate at room temperature (20-22°C) and 10  $\mu$ l of 5,5'-dithio-bis-(2-nitrobenzoic acid) (4 mg/ml in 0.1  $\text{Na}_2\text{HPO}_4$ ) was added and mixed by pipette. A final volume of 190  $\mu$ l 0.1  $\text{Na}_2\text{HPO}_4$  was added and mixed. After 15 min, absorbance was determined at 412 nm in the ELISA plate reader and the free sulfhydryl concentration determined from a cysteine-HCl standard curve. A molar extinction coefficient of  $E_{412} = 1.36 \times 10^4 \text{ cm}^{-1} \text{ M}^{-1}$  was used.

#### Statistical Analysis

For analysis of the questionnaire and mucociliary clearance odds ratios (OR) with 95% confidence limits (95% CI) were calculated. When OR were not applicable, Fisher's exact test was used. Nasal peak expiratory flow and nasal inflammatory end-points were analyzed with Student's paired one- and two-tailed t-test and olfaction with Fisher's exact test and Mann-Whitney's U test. The differences were classified as significant if the p-value was <0.05.

Student's one- and two-tailed t-test were used for comparison of morphological and physiological variables in the short term toxicity, carcinogenesis, and mutagenesis studies. The 50% inhibitory cell viability dose was estimated by the least squares method using linear regression analysis; differences between mean values were determined by two-tailed Student's t-test. Micronucleus assay data were analyzed by a one-tailed trend test (alpha level set at 0.05) and one-tailed Pearson Chi-Square test. An analysis of variance (ANOVA) test and two-tailed Student's t-test were

used for analysis of PCE. The percentage of migrated DNA was analyzed using a one-tailed trend test (alpha level set at 0.05) and an uncorrected two-tailed Student's t-test.

# Results

## Occupational Exposure Assessment (Papers I-II)

### Worker Exposure

The average 8 h time-weighted (8 h TWA) total dust concentration in the workers' breathing zones was 0.4 mg/m<sup>3</sup> (range 0.02-1.0 mg/m<sup>3</sup>) in all work places surveyed. The average total dust concentration in the breathing zones of the workers who worked on the UV lacquer lines was 0.3 mg/m<sup>3</sup> (range 0.02-0.7 mg/m<sup>3</sup>), 0.5 mg/m<sup>3</sup> (range 0.2-1.0 mg/m<sup>3</sup>) on the UV filling lines, and 0.4 mg/m<sup>3</sup> (range 0.1-0.7 mg/m<sup>3</sup>) during finishing work (Fig. 3). Two out of 26 samples contained trace levels of HDDA

(detection limit, LOD, 2 µg/m<sup>3</sup>). A photoinitiator, BP, concentrations averaged 0.01 mg/m<sup>3</sup> (range 0.003-0.022 mg/m<sup>3</sup>). The average proportion of thoracic (<10 µm) and respirable dust (<5 µm) particle concentrations were 79% (range 62-99%) and 28% (range 12-62%), respectively, from the 8 h TWA total dust concentrations in the 14 samples collected in the breathing zones of workers in the 7 out of 8 work places surveyed (Table VII). No differences were observed between the different job categories (UV line workers, fillers, and finishers). The average 8 h TWA particle mass distribution of the personal cascade impactor samples is shown in Figure 4.

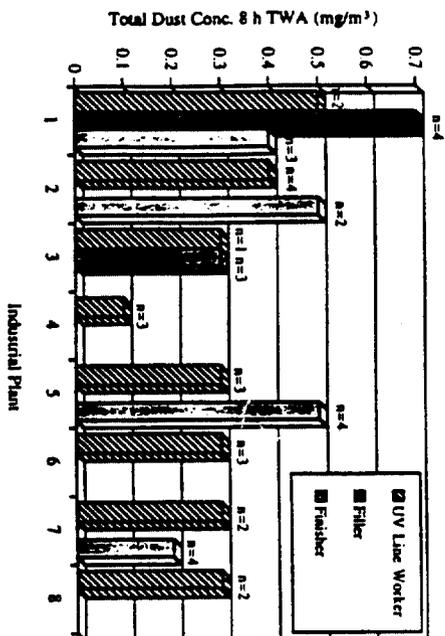


Figure 3. The average total dust concentrations (8 h TWA) in workers' breathing zones in the surveyed work places. The absence of data indicates the absence of a job category at a plant. For example, fillers are used only in parquet floor manufacturing. N equals the number of workers tested.

Table VII. Average thoracic and respirable dust concentrations (mg/m<sup>3</sup>) in the workers' breathing zones in the surveyed UV/R surface coating manufacturing plants.

Plant Worker / Particle Size	1	2	3	4	5	6	8
UV line worker:							
≤ 10 µm	0.386 (85%)*	0.384 (82%)*	0.065 (99%)*	0.112 (75%)*	0.257 (74%)*	0.197 (78%)*	0.184 (68%)*
≤ 5 µm	0.154 (34%)*	0.141 (33%)*	0.040 (62%)*	0.028 (18%)*	0.124 (36%)*	0.072 (25%)*	0.067 (26%)*
≤ 1 µm	0.077 (17%)*	0.106 (16%)*	0.037 (57%)*	0.021 (13%)*	0.028 (8%)*	0.071 (24%)*	0.042 (16%)*
Filler:							
≤ 10 µm	0.483 (93%)*	NA	0.133 (84%)*	NA	NA	NA	NA
≤ 5 µm	0.138 (27%)*	NA	0.018 (12%)*	NA	NA	NA	NA
≤ 1 µm	0.063 (12%)*	NA	0.018 (12%)*	NA	NA	NA	NA
Finisher:							
≤ 10 µm	ND	ND	NA	NA	0.326 (62%)*	NA	NA
≤ 5 µm	ND	ND	NA	NA	0.091 (17%)*	NA	NA
≤ 1 µm	ND	ND	NA	NA	0.044 (8%)*	NA	NA

\* Number in parenthesis indicates the percentage from the total dust concentration.  
 NA = Not determined due to absence of job category at that plant.  
 ND = Not determined

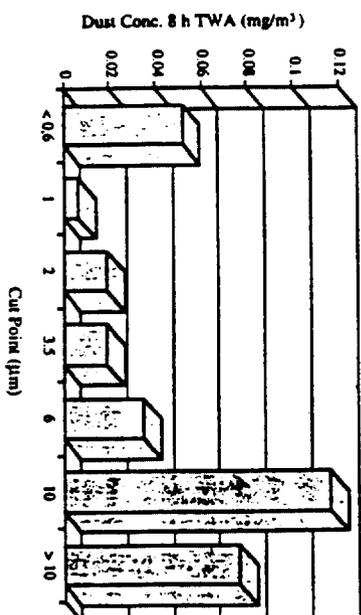


Figure 4. Particle size distribution in the measured breathing zone samples in the surveyed work places.

Worker exposure to TPGDA and HDDA at the UV line was below the detection limit (LOD 2  $\mu\text{g}/\text{m}^3$ ) except for one worker (out of 9), for whom HDDA was detected (15  $\mu\text{g}/\text{m}^3$  8 h TWA). Also, TPGDA was detected in this sample (5  $\mu\text{g}/\text{m}^3$  8 h TWA). The greatest TPGDA concentrations were observed in the breathing zones of the hand fillers (average 11  $\mu\text{g}/\text{m}^3$  8 h TWA, range 8-14  $\mu\text{g}/\text{m}^3$ ). BP was identified in all of the samples (range <1-30  $\mu\text{g}/\text{m}^3$  8 h TWA; LOD 1  $\mu\text{g}/\text{m}^3$ ). The highest BP concentrations (average 26  $\mu\text{g}/\text{m}^3$  8 h TWA) were measured in the hand fillers' breathing zones. In the hand sander's breathing zone TPGDA, HDDA, and BP were below the limit of detection.

Small concentrations of different organic solvents were detected in the workers' breathing zones: toluene, 0.64  $\text{mg}/\text{m}^3$  8 h TWA (0.03-2.03  $\text{mg}/\text{m}^3$ ); xylene, 0.78  $\text{mg}/\text{m}^3$  8 h TWA (0.11-2.79  $\text{mg}/\text{m}^3$ ); butyl acetate, 3.44  $\text{mg}/\text{m}^3$  8 h TWA (0.08-11.68  $\text{mg}/\text{m}^3$ ); terpenes 2.68  $\text{mg}/\text{m}^3$  8 h TWA (0.02-6.34  $\text{mg}/\text{m}^3$ ). The calculated average additive effect of organic solvent exposure was 0.03.

#### Stationary Measurements

A representative particle size distribution in the UVR curing environment is presented in Figure 5. Particle median diameter was between 0.16-0.19  $\mu\text{m}$  (avg. 0.17  $\mu\text{m}$ ); the range of lower and upper quartiles were 0.10-0.13  $\mu\text{m}$  (avg. 0.11  $\mu\text{m}$ ) and 0.25-0.28  $\mu\text{m}$  (avg. 0.27  $\mu\text{m}$ ), respectively. The particles were spherical in shape. Small amounts of TPGDA (0.018-0.04 w-%) were detected in dust samples taken immediately after hand sanding of wood products, which were surface coated with UVR curable acrylic lacquer. Also, the photoinitiators, BP and BDMK were detected in these sam-

ples (0.4-1.6 w-% and 0.4-0.5 w-%, respectively).

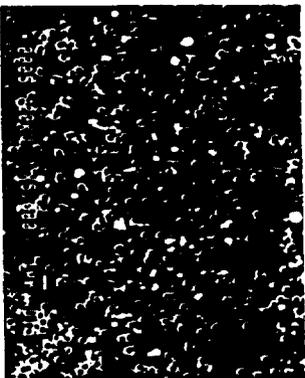


Figure 5. Scanning electron micrograph of the particles typical to the occupational environment, where surface coating of wood is conducted with UVR curable acrylic coatings.

In surface samples (100  $\text{cm}^2$ ), taken from the top of the UV units on the UV lines, traces of TPGDA were detected (<0.02  $\text{mg}/\text{sample}$ ; 2/29). Photoinitiators were also detected in these two samples (BP, <0.005  $\text{mg}/\text{sample}$ ; BDMK, 0.009-0.015  $\text{mg}/\text{sample}$ ).

Ozone concentrations varied in the ventilation exhaust pipes of UV units at lamp ignition (0.05-3.0 ppm), but ozone levels rapidly stabilized (0.01-0.8 ppm) within minutes. Ozone was also measured in the work place ambient air in 4/8 plants surveyed (50.01 ppm).

#### Work Practices

The workers' average duration of employment in the wood surface coating industry was 11 years (range 0.5-45 years). The average time of employment on the UV line

was considerably less, an average of 3 years (range 0.5-10 years).

Preparations for the UV line operation required 2-5% of the work day, including the start and warm up procedures of the UV lamps to reach the optimal curing conditions, which took between 10-20 min. The feeders spent  $\approx$ 70% of the work time feeding products into the process. The receivers spent  $\approx$ 65% by receiving and stacking the cured products at the end of the UV line. The UV line controllers also cleaned the sanding machines on the UV line (1% of the work time).

Duration of potential direct exposure to acrylates varied in accord with occupational group. The UV line controllers spent  $\approx$ 80% of their work time controlling the curing conditions on the UV line and were most likely to have direct skin contact with uncured acrylic coating ( $\approx$ 5% of the work time). The hand fillers were most frequently in direct skin contact with uncured acrylic coating ( $\approx$ 60% of the work time) of all the UV line workers.

The use of personal protective equipment (e.g., gloves, respirators, safety shoes, aprons, disposable overalls, etc.) was inconsistent at the work places surveyed.

Operations during which gloves were worn by workers were feeding and receiving of the products on the UV line, direct contact with coating, and finishing work. The gloves most commonly used by UV line workers and finishers were cloth-leather gloves. None of the gloves worn by the workers at any work place surveyed were sufficient to protect the workers from dermal exposure to acrylic coatings. Actual respirator use was not observed during the survey at any workplace.

Basic worker education and instructions for working with UVR curable acrylic

coatings were inconsistent. Information and education was primarily given by the UVR curable surface coating formulation manufacturers and usually consisted of 1-4 h course. However, only 48% of workers on a UV line had received some form of information and education.

#### UV Radiation Measurements

##### UV Exposure Measurements

The proportion of the UVR between different wavelength ranged from 41-65% UVA, 29-38% UVB, and 7-22% UVC from different UV units. A representative spectral distribution curve of a UV lamp is presented in Figure 6. Unshielded radiation from the UV lamps occasionally reached the environment with intensities of 10-20  $\text{mW}/\text{cm}^2$ . UVR reflected from the entrance and exit openings at the line were usually in the range of 0.05-0.2  $\text{mW}/\text{cm}^2$ . From the transport line, 1-10% of this radiation was reflected to the environment. The intensity and erythema inducing properties of UVR in the surface coating of wood with UVR curable acrylic coatings are presented in Table VIII.

##### Production Parameters

The performance of the UV units was inconsistent. Approximately half of the UV units required some form of alteration (e.g., mounting, cleaning, or lamp change) for optimization of the curing conditions. Only in one of the eight UV lines was the radiation from all the measured UV lamps (4 lamps) focused on the passing substrate. The uniformity of the UVR intensity was observed to vary in different parts (e.g., middle and sides) of the UV lamp (Fig. 7).

Generally, UVR curing of wood surface coating is recommended to be carried out at

the air temperature >50°C [91]. The temperature in the UV units measured varied between 40-100°C. The observed operation times of the UV lamps varied greatly, between 7 and 7222 h. However, the efficiency of the lamp output (transfer efficiency) was observed to be independent of the operation time (Fig. 8).

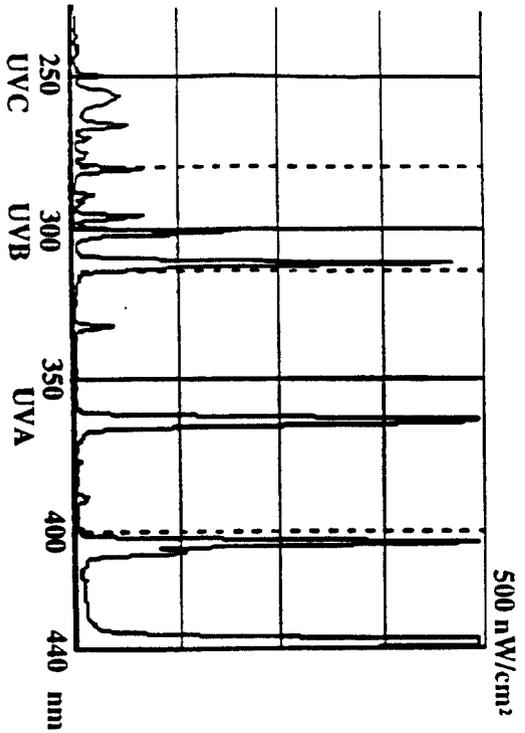


Figure 6. A representative spectral distribution of a UV lamp.

Table VIII. The intensity and erythema inducing properties of ultraviolet radiation in the UVR surface coating environment.

Radiation type	Maximal intensity (mW/cm <sup>2</sup> )	Erythema threshold time
Under the UV curing unit at the substrate passage level	100	< 1 sec
Direct radiation through holes and slots	10	5-10 sec
Reflected radiation through entrance and exit slots	0.05-0.2	5-10 min
Secondary reflection to the work area	0.002	3-5 h

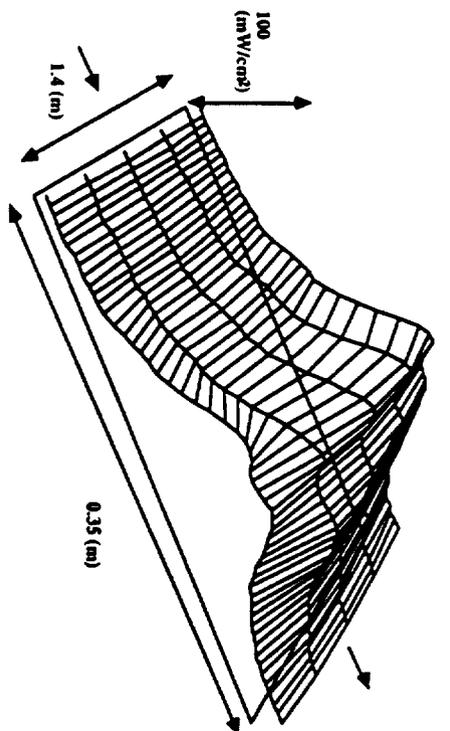


Figure 7. Variation of UVR energy in the different part of the UV lamp.

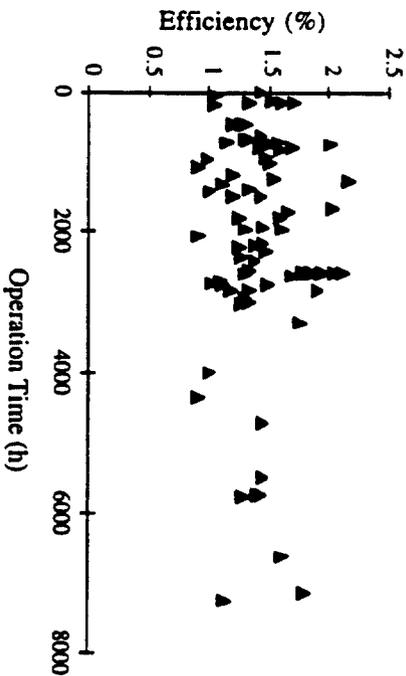


Figure 8. The variation of UV lamp transfer efficiency as a function of operation time of the UV lamp.

## Medical Examinations

### Airways, Nose, and Eyes (Paper III)

Summary statistics of the study population are presented in Table IX. There were no significant differences in tobacco use, age, or frequency of atopy between the study

groups. Nasal, pharyngeal, and ocular symptoms of discomfort, but not complaints about the lower airways, were more common among all the exposed workers than the unexposed control group (Table X). In the four groups of exposed workers nasal complaints were the most frequent in group III (UV finisher) compared to the

Table IX. Summary statistics of the study population according to the type of exposure.

Study Group	I. UV line (N=26)	II. AC line (N=24)	III. UV finisher (N=33)	IV. UV/AC finisher (N=35)	V. Control (N=30)
Sex (females/males)	9/17	4/20	18/15	8/27	19/11
Age	35	46	42	39	42
Employment / Exposure (years) <sup>1</sup>	2.8	10.7	6.7	8.0	20.6
Employment in wood industry (years)	9.6	17.4	13.0	14.3	0
Smokers (%)	50	46	45	23	27
Atopy (%)	19	17	18	23	17

<sup>1</sup> Employment equals the time employed at the present job. Workers were assigned to the corresponding exposure groups according to their present work tasks; thus, employment corresponds to exposure time at the current work environment.

control group. Pharyngeal symptoms were over represented in groups I (UV line), III (UV finisher), and IV (UV/AC finisher), whereas ocular symptoms were significantly more common in groups I (UV line), II (AC line), and III (UV finisher), when compared to the control group. When nasal, pharyngeal, and ocular symptoms were summed in each group and compared, groups I (UV line) and III (UV finisher) were observed to have more symptoms compared to groups II (AC line) and IV (UV/AC finisher). Daily symptoms of discomfort were significantly more often observed in group I (UV line), while more frequent symptoms (a few times per month) were observed more often in all four exposed groups.

Specific nasal symptoms associated with nasal complaints, such as nasal obstruction, runny nose, nose bleeding, dryness, and an impaired sense of smell, were not statistically over represented in any of the exposed groups compared to the control group, except for sneezing. Sneezing was more frequent and significantly different in groups III (OR 14.5, 95% CL 1.74-120.9)

and IV (OR 11.4, 95% CL 1.34-96.19) compared to the control group. Specific pharyngeal symptoms, such as sore throat, dryness, and lump, were not statistically more frequent in the exposed groups compared to the control group. For ocular symptoms, however, itching was significantly more frequent in groups I ( $p < 0.01$ ; Fisher's exact test), II ( $p < 0.05$ ), and III ( $p < 0.05$ ) and ocular secretion was significantly more frequent in groups I ( $p < 0.05$ ) and III ( $p < 0.05$ ) compared to the control group.

A history of sinusitis was more frequent in group III (OR 9.3, 95% CL 1.08-79.40), but pharyngeal infections, bronchitis, and pneumonia were not more often reported in exposed groups than in the control group.

The frequency of hyperreactivity and/or work related allergic symptoms was estimated based on the answers in the questionnaire (discomfort when exposed to odors, cigarette smoke, exhaust, fresh paper, cold air, etc.). Nasal hyperreactivity was significantly more often observed in groups III (OR 2.9, 95% CL 1.01-8.42) and IV (OR 6.0, 95% CL 2.04-17.65) compared

Table X. Summary statistics of systemic discomfort experienced by surface coating industry workers when compared to matched unexposed controls.

Symptom	I. UV line		II. AC line		III. UV finisher		IV. UV/AC finisher		Total OR 95% CL	
	OR	95% CL	OR	95% CL	OR	95% CL	OR	95% CL		
Nasal discomfort	2.1	0.63-7.07	1.7	0.47-5.78	4.8	1.56-14.81	3.0	0.98-9.17	2.8	1.08-7.47
Pharyngeal discomfort	10.7	1.22-93.92	2.6	0.22-30.97	12.6	1.50-105.81	8.6	1.01-73.32	8.6	1.12-66.12
Lower airway discomfort	1.2	0.26-5.28	4.0	0.41-38.70	3.9	0.43-36.12	2.8	0.30-27.16	3.0	0.37-23.94
Ocular discomfort	15.4	1.79-131.94	9.7	1.07-87.00	10.9	1.29-92.03	2.7	0.27-27.62	8.6	1.12-66.12

to the control group, but was not significant in group I (OR 2.8, 95% CL 0.90-8.40). Pharyngeal hyperreactivity was significantly more frequent in all exposed groups ( $p < 0.01$  for groups I, III, and IV;  $p < 0.05$  for group II; Fisher's exact test), while ocular hyperreactivity was most frequent but not statistically significant in group I (OR 3.4; 95% CL 0.91-12.97). For lower airway hyperreactivity, there was no difference between the exposed groups and the control group.

A great number of exposed workers had signs of nasal inflammation, such as redness of the nasal mucosa, dryness of the nasal lining, crusting, and blood. A few cases with nasal polyps and septal perforation were also observed. In the control group these signs were more infrequent.

Nasal peak expiratory flow (NPEF) showed no significant differences, except for a lower mean value in group III compared to the control group (Table XI).

The olfaction test disclosed the lowest threshold values in the control group (Table XI). In all exposed groups, the sense of smell was significantly deteriorated compared to the control group except in group III. The duration of employment in the particular work tasks in the wood surface

coating industry did not affect significantly the olfaction. Olfaction was significantly decreased in the older workers (groups divided into two by the average age of the group) in groups I, II, and IV but in the younger workers only in group IV. A tendency of impaired olfaction was observed in all groups with increasing age. Smoking did not affect the sense of smell in any of the study groups.

The number of subjects with impaired nasal mucociliary clearance was greater in all four exposed groups compared to the control group (Table XI). The difference for group I. The mean value of clearance time among subjects within normal transport time (below 20 min) did not differ statistically between the exposed groups and the control group. Nasal complaints were not more frequent among subjects with impaired mucociliary clearance compared to those with a value in the normal range. Mucociliary clearance was not significantly different in the exposed groups when adjusted for duration of employment in the present work task or smoking. However, mucociliary clearance was significantly impaired ( $p < 0.05$ ; Fisher's exact test) in young UV line workers (535 years of age)

Table XI. Results from the nasal peak expiratory flow (NPEF) test, olfactory test, and nasal clearance test in the exposure and the control groups. Nasal clearance in different exposure groups is compared statistically (odds ratios and 95% confidence limits) to the control group.

Exposure Group	NPEF		Olfactory test		Clearance	
	Mean ± STD	Mean ± STD	OR	95% CI.	(%)*	
I. UV line (N=26)	324 ± 18.3	4.9 ± 1.3**	9.2	1.02-82.21	23	
II. AC line (N=24)	315 ± 22.9	4.7 ± 1.8**	6.4	0.67-62.31	17	
III. UV finisher (N=33)	252 ± 17.9	5.6 ± 1.9	2.9	0.28-29.51	9	
IV. UV/AC finisher (N=35)	318 ± 16.6	4.8 ± 1.7***	3.7	0.39-35.47	11	
V. Control (N=30)	294 ± 15.7	6.4 ± 1.9			3	

\* percent of subjects in the study group with impaired mucociliary clearance

\*\* p<0.01

\*\*\* p<0.001

when compared to the young controls (≤42 years of ages).

Pirometry measurements indicated that vital capacity and FEV<sub>1.0</sub> were close to expected values in all exposed groups as well as in the control group.

#### Skin (Paper IV)

Altogether, 659 workers, 496 men (75.3%) and 163 women (24.7%), participated in the study. Summary statistics of the study population are presented in Table XII. The age distribution between the study groups was similar (avg. 40 ± 11 years, N=659) as was the employment time in the wood industry (avg. 13 ± 10 years) in the exposed groups. Most of the workers at the UV lines had been employed 5-5 years (83%) in their present work. More than half of these workers (66%) had been working on the UV lines between 1-5 years. The group of UV line workers investigated represents a majority of the Swedish UV line workers with a particular occupational exposure conditions. For every UV line worker included in the study an attempt was made to obtain a control individual matched for sex

and age among workers employed on acid curing surface coating lines, finishers employed in UV surface coated products, and office workers. However, too few acid curing line workers as well as UV finishers made it difficult to obtain a satisfactory match. Therefore, finishers with mixed exposures were also included, but are presented separately.

From the questionnaire, a total of 132 workers (19%) were determined to be atopic. The highest frequency of atopy (34%) was observed in group VI (prior UV) and the lowest (13%) in group II (AC line). Group I (UV line) had a similar frequency of atopy compared to the control group (22% and 23%, respectively).

Dermatologic symptoms such as erythema, itching, and dryness experienced by individuals in different exposed groups were determined and analyzed from the questionnaires. Workers in groups I (UV line) and III (UV finisher) experienced skin irritation, itching, and dryness from UV acrylate coating and coating dust. Workers in group II (AC line) reported itching and dryness, but not erythema from acid curing coatings and coating dust. Itching and dry-

Table XII. Summary statistics of the study population according to the type of exposure.

Study Group	I (N=146)	II (N=57)	III (N=54)	IV (N=97)	V (N=71)	VI (N=61)	VII (N=173)
Sex (female/male)	24/122	3/54	29/25	26/71	7/64	13/48	61/112
Age (average)	38	42	40	39	39	39	42
Employment in UVR surface coating (years)	3.6	0	5.0	4.1	3.8	2.3	0
Employment in wood industry (years)	11.4	16.0	9.8	15.0	12.4	13.8	14.8
Smokers (%)	52	30	41	28	32	20	18
Atopy (%)	22	13	26	17	17	34	23

ness were also reported from individuals in all exposed groups when exposed to wood dust. Few individuals reported dermatologic symptoms from UV lamps. The most reported skin symptoms from UVR were erythema, edema, and pain after accidental exposure during lamp service. Erythema and smarting was reported by hand fillers, who worked in the front of poorly shielded UV units.

Altogether, 144 individuals with history of past or present dermatitis were investigated clinically and were patch tested. Forty individuals with a history of eczema were interviewed by telephone. Telephone interviews were efficient because of the distant location of the industrial plant and very few individuals (usually one or two) with skin disease(s) were involved at each plant. The distribution of investigated in-

dividuals with skin disease in the different study groups are presented in Table XIII.

This clinical investigation disclosed an expected panorama of noneczematous skin disease among individuals investigated. Different types of eczema were diagnosed in 168 individuals (Table XIV). Irritant contact dermatitis was more common among workers in groups I (UV line; OR 3.77, 95% CL 1.86-7.63) and II (AC line; OR 2.85, 95% CL 1.16-7.02) than in the control group. Distribution of eczematous disease and relation to work tasks in all exposed groups was significantly different from the control group (p<0.05; Table XV). However, in group I the total eczema frequency was the greatest of the exposed groups and significantly different (OR 2.07, 95% CL 1.24-3.43) from the control group. Twenty of the exposed workers (13%) judged the work with the UV acrylate coat-

Table XIII. Number of individuals investigated with a history of eczematous skin disease according to the study group.

Study Group	I (N=146)	II (N=57)	III (N=54)	IV (N=97)	V (N=71)	VI (N=61)	VII (N=173)
Clinical investigation	44	12	18	13	11	14	32
Telephone interview	9	3	1	12	8	1	6

ings to be the main cause of their disease. No cases of skin cancer were observed in this study.

Altogether, 49 positive patch test reactions were observed in 31 individuals (Table XIV). Only one individual was allergic to acrylates (1+ reaction to patch tests nr. 25, 26, 28, 29, 30, 31; see Table V). This individual left the UV line work due to eczematous skin disease, which healed after leaving. Except for methyl hydroquinone (an inhibitor), which gave 18 irritant reactions of erythematous type, the acrylate test series presented few irritant reactions. No other patch test reaction had a direct association to the work with UVR curable coatings. The three reactions to colophony were associated with previous or present contact with wood. The strongest reaction was observed in an office worker who had had hand eczema when working in wood finishing. Four of the nickel reactions were potentially work related since they occurred in individuals with hand eczema who assembled furniture with nickel plated com-

ponents. Two reactions, one to formaldehyde and one to *p*-*t*-phenolformaldehyde resin observed were considered to be related to worker exposure to acid curing coatings or to hard board dust. Most of the remaining positive patch test reactions were related to past or present work activities (e.g., chromium – construction work) or leisure activities.

### Comparative *In Vivo* and *In Vitro* Toxicity of Selected Acrylates

Both immunotoxic (induced hypersensitivity) and toxicity (including potential mutagenic and/or carcinogenic responses over long exposure time) are possible consequences of exposure to chemicals associated with the manufacture of wood products, which are surface coated with UVR curable acrylate coatings. In order to identify and determine the potential toxic chemicals and/or chemical mixtures *in vivo* and *in vitro* toxicity studies were conducted.

Table XIV. The number of individuals working in the wood surface coating industry and the type of eczema observed according to exposed groups (I-VI) and the matched controls (VII). Those with a positive patch test are indicated by parentheses.

Eczema / Study Group	I.	II.	III.	IV.	V.	VI.	VII.
Atopic dermatitis	6 (1)	2	6 (1)	9 (2)	2	2	13 (2)
Contact dermatitis	32† (3)	10† (3)	7 (1)	6 (2)	10 (1)	7 (3)	12 (5)
Nummular eczema	1	-	1	1	2	1	1
Schoenleichen dermatitis	9 (1)	4	2 (1)	5	-	1	3
Typhoid eczema	-	-	-	1	-	-	-
Vesiculosis	1	-	1	-	-	1	3 (2)
Other eczema	-	-	-	1	3	-	2
Total	49††	16	17	23	17	12*	34*

\* One individual with psoriasis had a positive patch test reaction.  
† Significantly different from the control group.

Table XV. Eczematous disease among workers employed in the wood surface coating industry and the unexposed controls. Results are expressed as the number of workers, whose disease was not work related compared to those, whose disease was determined to be work related to the previous or present occupation.

Eczema / Study Group	I.	II.	III.	IV.	V.	VI.	VII.
Hand eczema							
- past work	17	-	12	01	01	10	40
- present work	1/3	1/2	-	1/1	2/0	1/1	3/0
Other eczema localization							
- past work	6/6	1/3	3/1	4/1	4/1	1/0	8/0
- present work	3/4	1/1	3/1	2/3	1/1	0/3	7/0
Hands and other eczema localization							
- past work	2/3	2/1	0/1	3/1	2/4	0/3	3/0
- present work	1/3	-	1/2	0/1	1/0	0/1	6/0
Total	1426	57	87	108	107	38	310
Total % in the group	27.4	21.1	27.8	18.6	23.9	18.0	17.9

### Short Term *In Vivo* Toxicology, Carcinogenesis, and Mutagenesis Studies (Papers V-VII)

The toxicity of TPGDA was unknown, except for its strong sensitizing properties in the GPMT [17]. First, the acute toxicity was determined. The dose required to kill 50% of the mice that received a single topical application of TPGDA was estimated at 120  $\mu$ moles per mouse (4 mmoles or 1.2 g/kg bw). The minimum lethal dose of EA was greater than 600  $\mu$ moles per mouse (>20 mmoles or >2 g/kg bw) under the conditions of this study. Pozzani *et al.* [140] reported the LD<sub>50</sub> value in rabbits (strain not cited) to be ~1.8 g or 20 mmoles EA/kg bw. EA at 600  $\mu$ moles per mouse was observed to induce a similar level of relative skin thickness or hyperkeratosis when compared to 5  $\mu$ moles TPGDA per mouse (both significantly different from the

control at  $p < 0.05$ ; Fig. 9). A no effect level for increasing relative skin thickness was observed at 300  $\mu$ moles EA per mouse or 1  $\mu$ mole TPGDA alone or in Lacquer A or Lacquer B (Fig. 9). No treatment related differences were observed in histopathological examination of the inguinal lymph nodes.

Doses of 60, 300, or 600  $\mu$ moles EA per mouse or 1, 5, or 10  $\mu$ moles TPGDA alone or in Lacquer A were selected for the short term carcinogenesis studies by skin paint. Because differences in acute toxicity were not observed between the two lacquer formulations, further studies with Lacquer B were not conducted. After repeated application of TPA, TPGDA or TPGDA in lacquer formulation A, three times a week for 20 weeks, focal areas of hyperkeratosis developed into papillomas. TPGDA alone showed a dramatic increase in papillomas per mouse over time when compared to the

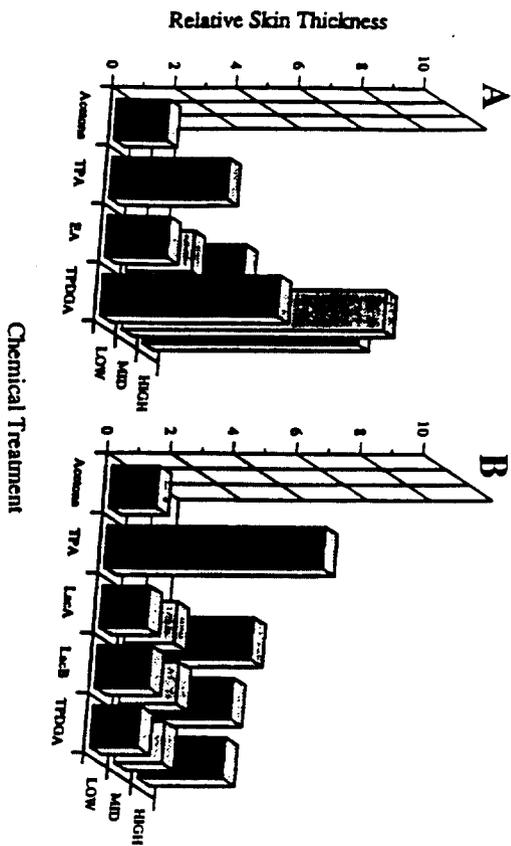


Figure 9. Relative skin thickness (number of epidermal cell layers) after chemical treatment in the acute dose finding studies. A. Doses ( $\mu\text{moles}/\text{mouse}$ ). TPA: 0.002, EA: LOW, 60; MID, 300; HIGH, 600. TPGDA: LOW, 12; MID, 60; HIGH, 120. B. Doses ( $\mu\text{moles}/\text{mouse}$ ). TPA: 0.002, Lacquer A (Lac A), Lacquer B (Lac B), or TPGDA: LOW, 0.5; MID, 1.0; HIGH, 5.0. Acetone vehicle control and all doses were delivered in 200  $\mu\text{l}$  acetone.

vehicle control (acetone) or EA (Fig. 10) that was dose related. Similar effects were observed with Lacquer A (adjusted equimolar to TPGDA alone based on percent TPGDA) at 5 or 10  $\mu\text{moles}$  (Fig. 11). No effects were observed at 1  $\mu\text{mole}$  TPGDA, 1  $\mu\text{mole}$  Lacquer A (adjusted to equimolar TPGDA), or at even the highest dose of 600  $\mu\text{moles}$  EA (Fig. 11; lower dose EA data not shown).

In the TPA treated (positive control) group, early mortality, possibly due to tumor burden and other treatment effects, resulted in a dramatic decrease in the maximum number of papillomas per mouse (Fig. 10 or 11) in surviving mice. The rate of tumor occurrence was less in the acrylate

treated groups (Fig. 10 and 11) compared to the TPA treated group.

Tumor incidence varied between the different treatment and control groups, but did not appear to be dose related (TableXVI). No significant differences were observed in mean survival time between any treatment group and the negative control. Mean latency (no. days  $\pm$  std dev) until each mouse developed the first papilloma) was reduced between the negative control and TPA, 5 or 10  $\mu\text{moles}$  TPGDA, or 1, 5 or 10  $\mu\text{moles}$  Lacquer A. The mean number of papillomas per mouse was significantly different ( $p < 0.01$ ) between the negative control and the TPA positive control, 5 or 10  $\mu\text{moles}$  TPGDA or Lacquer A.

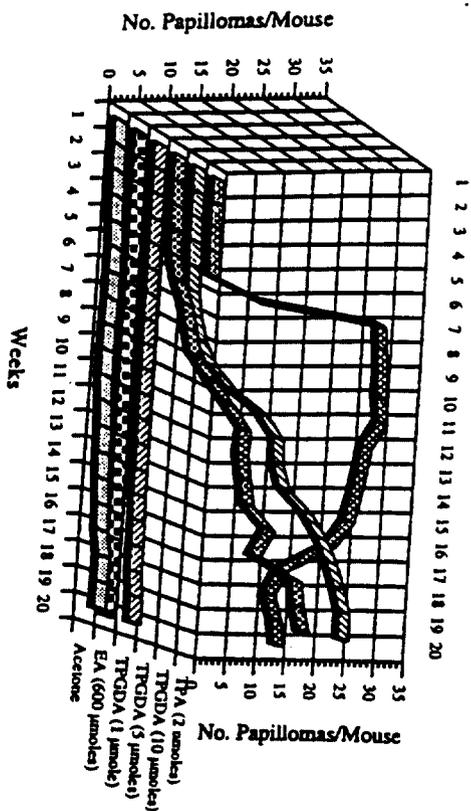


Figure 10. Mean number of papillomas per mouse per week during a 20 week treatment period with ethyl acrylate and tripropylene glycol diacrylate.

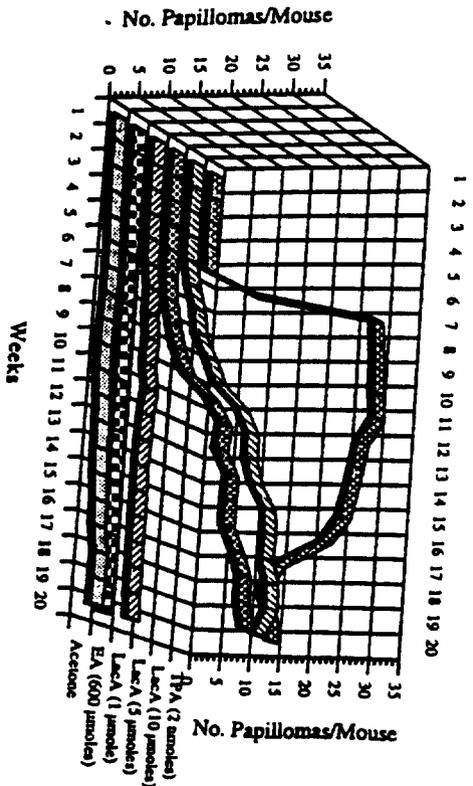


Figure 11. Mean number of papillomas per mouse per week during a 20 week treatment period with Lacquer A.

Table XVI. Effect of topical application of acrylates to the skin of TG<sup>+</sup>AC mice: incidence, survival, induction of papillomas, and tumor latency.

Chemical	Incidence <sup>1</sup>	Mean Survival Time (days ± SD)	Maximum Papillomas/ Mouse (Mean ± SD)	Latency (days ± SD)
TPA (2 nmoles)	5/10	169.1 ± 39.4	31.7 ± 0.9	34.6 ± 2.8
Acetone (200 µl)	10/10	200.1 ± 46.7	5.0 ± 6.8	120.4 ± 92.3
EA (60 µmoles)	2/10	217.1 ± 39.1	7.5 ± 3.5	107.5 ± 84.1
(300 µmoles)	5/10	227.2 ± 23.8	4.4 ± 5.9	103.6 ± 56.1
(600 µmoles)	2/9	186.2 ± 75.8	2.0 ± 1.4	123.5 ± 46.0
TPGDA (1 µmole)	9/9	217.9 ± 30.0	5.4 ± 5.7	136.9 ± 41.5
(5 µmoles)	9/9	166.9 ± 62.6	29.2 ± 5.7	43.9 ± 16.8
(10 µmoles)	10/10	219.3 ± 30.6	30.4 ± 5.1	47.8 ± 12.1
Lac A (1 µmole)	10/10	194.4 ± 60.5	5.8 ± 5.2	43.3 ± 20.0
(5 µmoles)	10/10	209.0 ± 31.8	18.3 ± 11.4	50.9 ± 10.0
(10 µmoles)	10/10	200.5 ± 38.7	23.1 ± 10.1	43.2 ± 11.3

<sup>1</sup> Incidence equals the number of tumor bearing mice over the total number of mice per group surviving more than 10 weeks of treatment.

Body weights were depressed in the EA high dose group (Fig. 12A), but a dose response relationship was not observed in the TPGDA or TPGDA in lacquer formulation treatment groups (Fig. 12B and 12C).

EA at 60, 300, or 600 µmoles/mouse did not induce a significant increase in the percentage of migrated DNA in leukocytes, either by a trend test or by a pairwise comparison of each treatment dose against the concurrent vehicle control at any sample time. Similarly, at 20 weeks, the frequency of MN-PCE was not significantly elevated and the %PCE was not significantly altered. The percentage of migrated DNA and the frequency of MN-PCE was not increased in the mice treated with 2 nmoles TPA. A marginally nonsignificant increase in %PCE was observed in these mice (p=0.054) at 20 weeks treatment.

The percentage of migrated DNA in leukocytes of the mice treated with TPGDA at 1, 5, or 10 µmoles/mouse was not significantly increased, either by a trend test or by a pairwise comparison of each treatment dose against the concurrent vehicle control, at any sample time. Similarly, at 20 weeks, the frequency of MN-PCE was not significantly elevated. However, a highly significant, dose dependent increase in the %PCE was observed in TPGDA treated mice (Table XVII). The lowest effective dose inducing a significant change was 5 µmoles/mouse.

The percentage of migrated DNA in leukocytes of the mice treated with Lacquer A, equimolar for TPGDA, at 1, 5, or 10 µmoles/mouse was not significantly increased, either by a trend test or by a pairwise comparison of each treatment dose against the concurrent vehicle control, at

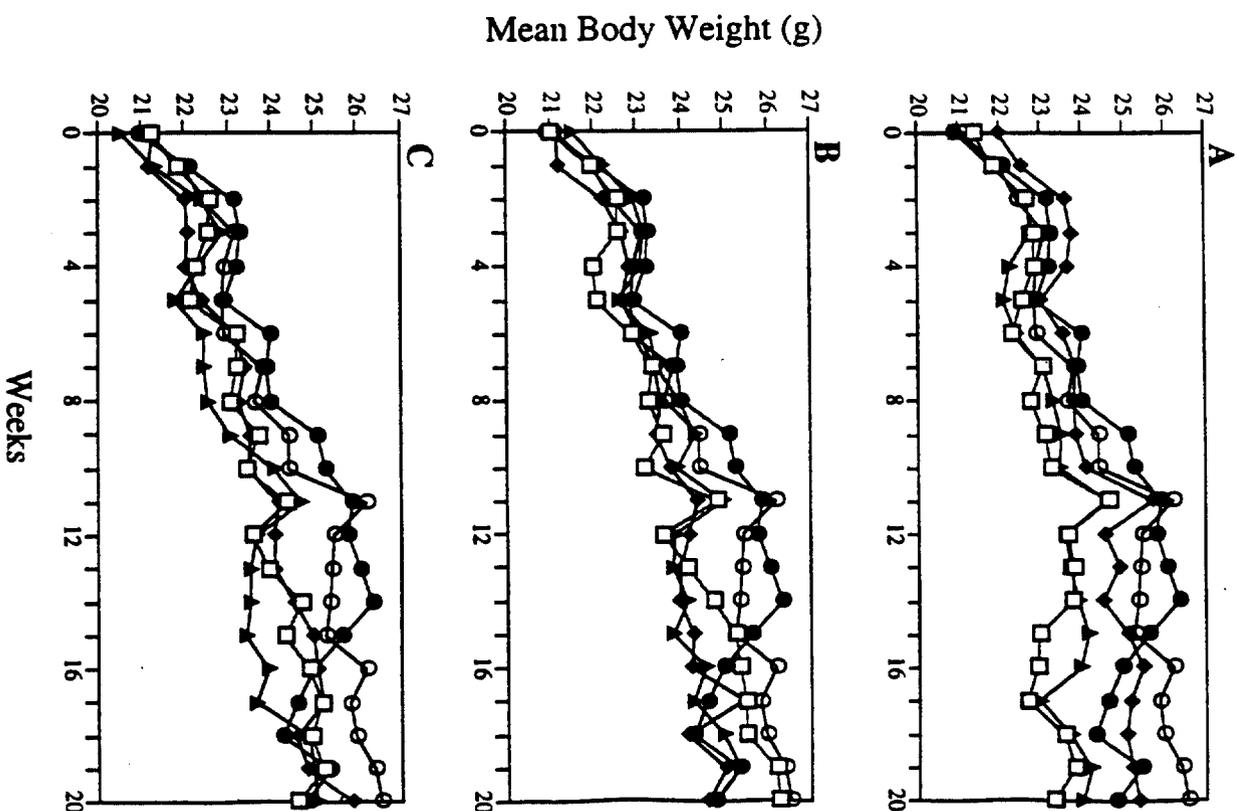


Figure 12. Mean individual mouse body weights per week per dose group during a 20 week treatment period. A. EA, TPA, and acetone controls. B. TPGDA, TPA, and acetone controls. C. Lacquer A (Lac A), TPA, and acetone controls. Acetone (○); TPA (●); LOW (◐); MID (▲); HIGH (◑) for EA, TPGDA (■), and Lac A (Δ).

Table XVII. Micronucleated polychromatic erythrocytes (MN-PCE) and the percentage of polychromatic erythrocytes (%PCE) in female TG-A/C mice treated with tripropylene glycol diacrylate.

Dose μmol/mouse	MN-PCE <sup>a</sup>		%PCE <sup>b</sup>	
	Mean	SEM	Mean	SEM
TPA, 0.002	2.1	0.7	7	10.7
				3.9
0	1.4	0.4	9	3.5
				0.5
1	1.8	0.4	10	3.4
				0.3
5	1.9	0.6	5	7.5*
				0.8
10	2.0	0.3	9	9.7*
				1.3
p-value <sup>#</sup>	0.142		<0.001	

<sup>a</sup> Group mean frequency of MN-PCE per 1,000 PCE and standard error of the mean (SEM) among N mice. Data based on 2,000 PCE scored per mouse.

<sup>b</sup> Group mean percentage of PCE and standard error of the mean among N mice. Data based on 1,000 erythrocytes scored per mouse.

<sup>#</sup> One-tailed trend test p-value.

\* Significantly different from the concurrent control at p<0.05.

any sample time. Similarly, at 20 weeks, the frequency of MN-PCE was not significantly elevated and, while increased in an apparent dose dependent manner, the %PCE was not significantly altered.

#### Comparative *In Vitro* Cytotoxicity (Paper VIII)

Comparative cytotoxicity of EA, TPGDA, or TPGDA in a reference UVR curable lac-

Table XVIII. Acute toxicity of selected acrylates *in vitro* using normal human cells<sup>#</sup>. Estimated ID<sub>50</sub> concentration (μmole/cm<sup>2</sup>).

	NHEK μmole/cm <sup>2</sup>	NHDF μmole/cm <sup>2</sup>	NHBE μmole/cm <sup>2</sup>
TPGDA	0.6	0.2	0.2
Lacquer A	1.3	0.3	0.2
Ethyl acrylate	1.0	20.0 <sup>a</sup>	15.0 <sup>a</sup>

<sup>#</sup> Three to five different individual adult human early passage secondary cell cultures were used under the same conditions to calculate these estimates.

<sup>a</sup> Significantly different from other ID<sub>50</sub> values (p<0.01), but not to other values denoted the same.

corrected to equimolar TPGDA concentration) were more cytotoxic to NHDF and NHBE than EA (Fig. 14 and 15). TPGDA is ≈100 times more cytotoxic than EA to NHDF and NHBE (Table XVIII). Both log-phase and stationary growth population of NHEK and NHDF were equally sensitive to these acrylates.

Equimolar concentrations of TPGDA (81.5%) alone or reference Lacquer A (70.5% TPGDA and proprietary ingredients) yield similar ID<sub>50</sub> concentrations. This suggests that ingredients other than the TPGDA are not present in sufficient concentration to exacerbate or potentiate toxicity and that TPGDA is the cytotoxic agent (Fig. 13-15, Table XVIII). Low doses of TPGDA or EA may be slightly mitogenic to both NHEK and NHDF (Fig. 3 and 4); TPGDA, but not EA, may be mitogenic at low doses to NHBE (Fig. 15). The free

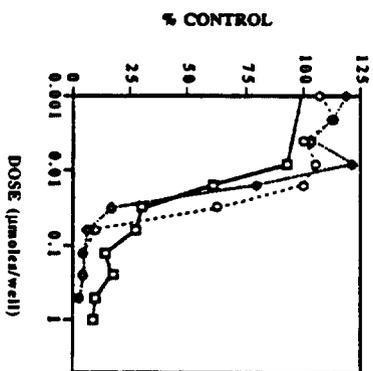


Figure 13. Confluent cultures of normal human epidermal keratinocytes (NHEK) were exposed to ethyl acrylate (□), tripropylene glycol diacrylate (○), or Lacquer A (○) in 50.1% acetone for 18 h and cytotoxicity determined by measurement of MTT assay as an index of cytotoxicity (% control; N=32).

sulphydryl concentrations in acrylate exposed human cells decreased with increasing acrylate dose (Fig. 16 and 17) and paralleled the cytotoxicity curves (Fig. 13 and 15).

UVA exposure alone (1 J/cm<sup>2</sup> with 0.06% UVB) stimulated NHEK activity, whereas 0.06 μmole/cm<sup>2</sup> TPGDA was not cytotoxic (Fig. 18). UVA exposure (1 J/cm<sup>2</sup> with 0.06% UVB) followed by exposure to 6 nmole/cm<sup>2</sup> TPGDA resulted in a synergistic toxicity that was similar in magnitude to exposure to 60 nmole/cm<sup>2</sup> TPGDA (Fig. 18). Neither UVA and/or TPGDA increased cloning efficiency or were observed to induce transformed colonies. An approximately 1% cloning efficiency (as expected for secondary passage diploid human cells) was observed in control or UVA treated NHEK populations.

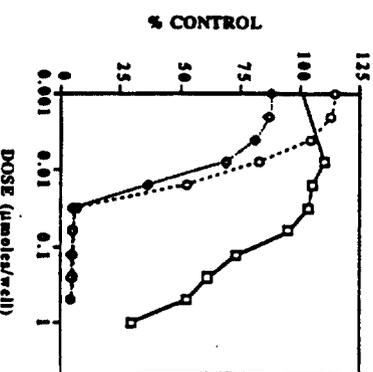


Figure 14. Confluent cultures of normal human dermal fibroblasts (NHDF) were exposed to ethyl acrylate (□), tripropylene glycol diacrylate (○), or Lacquer A (○) in 50.1% acetone for 18 h and cytotoxicity determined by measurement of MTT assay as an index of cytotoxicity (% control; N=32).

# Discussion

Technology for radiation curing of coated surfaces is about 20 years old, but not until the past decade has the use of formulations containing acrylates for UVR curable surface coating applications increased remarkably. This is, at least in part, due to the necessary reduction of organic solvents in the work place and to the environment because of health concerns as well as desired economic benefits. UVR curing provides important advantages over conventional curing systems, such as eliminating emissions of volatile organic compounds (VOC) to the ambient air, saving of raw materials (organic solvent free systems) since conventional systems contain between 50-85% organic solvents, and accelerated curing which allows immediate further processing of the coated product. In addition, it provides lower energy consumption because it eliminates the use of the organic solvents, low thermal stressing of substrate material, minimal space requirements, and is readily adapted into existing production facilities.

The extensive process changes in the surface coating of wood have decreased conventional worker exposures to e.g., organic solvents, but at the same time have introduced potential risks for other health hazards, e.g., reactive airborne dust, skin irritation and sensitization, and eye irritation. Evaluation of new materials and/or processes for potential health effects before inclusion or substitution in the work place is difficult because of the unforeseen ways in which use and work practices evolve. However, it is highly desirable to prospectively identify and evaluate potential health risks to new materials and work practices in the UVR surface coating industry as soon

as possible in order to prevent or minimize unforeseen health risks that work to the disadvantage of both the worker and the industry.

This study was designed to determine (identify and document) worker exposure to acrylates and other chemical and physical agents and to evaluate health effects and work practices in the Swedish UVR wood surface coating industry. This study was also designed to provide a basis for determining and recommending modifications for manufacturing processes and protective equipment in order to better control hazardous exposures to workers in this industry through technical solutions and improved work practices. Because the toxicology of the main UVR curable acrylate coating and the principal active ingredient, TPGDA, was unknown, limited rodent and *in vitro* toxicology studies were conducted to determine the potential toxicity, possible mechanisms of toxicity, and existing data gaps.

## Worker Exposure

The results indicate that workers in the UVR wood surface coating industry in Sweden are exposed to small amounts of potentially harmful acrylate containing aerosols, vapors, and/or dusts and organic solvents. The most significant findings are: (1) an estimated 350 of the 8,500 workers are directly exposed to acrylates (uncured coating, aerosols and vapors, and/or dusts) and UVR, (2) respirable dust particles (28% of the total dust concentration) are present even though overall dust levels are low

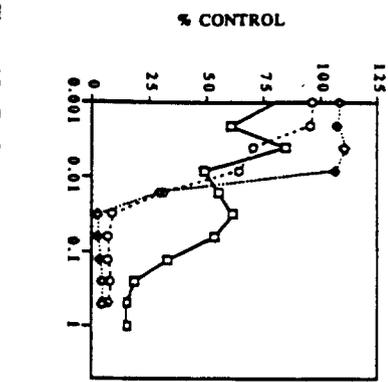


Figure 15. Confluent cultures of normal human bronchiolar epithelium (NHBE) were exposed to ethyl acrylate (□), tripropylene glycol diacrylate (○), or Lacquer A (○) in 50.1% acetone for 18 h and cytotoxicity determined by measurement of MTT assay as an index of cytotoxicity (% control; N=32).

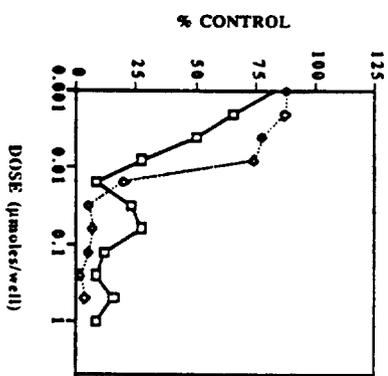


Figure 16. Confluent cultures of normal human epidermal keratinocytes (NHEK) were exposed to ethyl acrylate (□) or tripropylene glycol diacrylate (○) in 50.1% acetone for 18 h and concentration of free sulphydryls were determined by Ellman's method using cysteine for generation of a standard curve (% control; N=16).

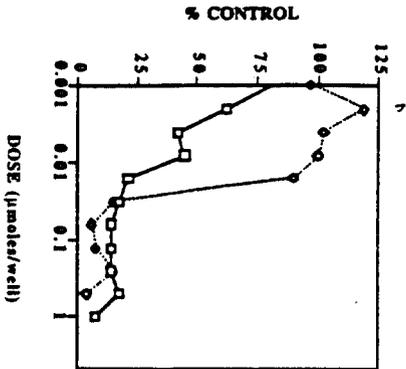


Figure 17. Confluent cultures of normal human bronchiolar epithelium (NHBE) were exposed to ethyl acrylate (□) or tripropylene glycol diacrylate (○) in 50.1% acetone for 18 h and concentration of free sulphydryls were determined by Ellman's method using cysteine for generation of a standard curve (% control; N=16).

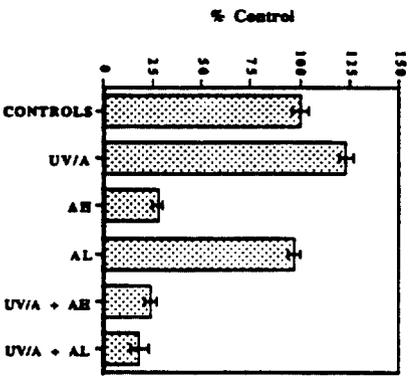


Figure 18. Confluent cultures of normal human epidermal keratinocytes (NHEK) were sequentially exposed to UV (5 J/cm<sup>2</sup>) and tripropylene glycol diacrylate (AH = 60 or AL = 6 mmol/cm<sup>2</sup>; 50.1% acetone) for 2 h and cytotoxicity determined by MTT assay for cytotoxicity (% control; N=8).

(=0.4 mg/m<sup>3</sup>), (3) aerosols, vapors, and dusts containing sensitizing chemicals (TPGDA, HDDA, BP, etc.) were determined to be present in the work places surveyed, (4) release of organic solvents to the ambient environment and concomitant worker exposure is reduced when compared to the acid curing surface coating, (5) in contradiction to coating manufacturers' written published recommendations protective devices for respirable dust were not consistently used (2/8 plants surveyed provided protective devices; however no device was ever observed in use), (6) potential for UVA, UVB, and UVC exposure to unprotected skin at biologically effective dose levels, (7) potential for direct skin exposure to acrylate contaminated surfaces and development of contact dermatitis, and (8) lack and inconsistency of educational programs for workers.

## Health Effects

### Always, Nose, and Eyes

A similar pattern of prevalence of symptoms and dysfunction were observed within the given job categories of all exposed workers when compared to unexposed matched controls. Nasal, pharyngeal and ocular discomfort were cited most often by surface coating line and finishing workers using either or both UVR and acid curing processes. This pattern of prevalence is consistent with the analysis of site specific complaints and their medical evaluation. Symptoms of hyperreactivity were specific for nasal passages but not the lower airways. Although complaints concerning lower airway discomfort were not significant and exposure to dust was low (~30% respirable), the absence of significant com-

plaint is opposite to that observed in formaldehyde-wood dust exposed workers [75]. A nonspecific nasal hyperreactivity was observed in exposed groups, which may explain a greater frequency of complaints. This nonspecific nasal hyperreactivity was also reported in formaldehyde-wood dust exposed workers [182] and was not more frequently observed in atopics.

Mucociliary clearance was impaired in all exposed groups, but that was significantly impaired only in the UV line workers. Olfaction was significantly impaired in the UV line and the AC line workers and UV/AC finishers. These workers may be exposed to low levels of uncured acrylate aerosols and vapors that deposit and act at site of contact in the anterior nasal passage. This effect may be distinct from mechanical irritation by fine dust that may be the major cause of the observed symptoms and complaints.

Changes in mucociliary clearance may be result of either altered rheological properties of the mucous covering the ciliated epithelial cells or altered function of the ciliated cells due to a lowered cilia beat frequency or loss of cilia. These changes may be caused by exposure to dust and/or toxins, including carcinogenic chemicals [5, 20, 23, 119, 120, 183, 184].

Impaired olfaction may be a result of a toxic effect of a chemical or of a blocked nose. In this study, using NPEF, we did not observe significant signs of nasal obstruction in any exposure groups compared to the control group. However, there are some difficulties in interpreting these results since nasal flow is related to the height of the subject tested. Females were over represented in groups III and V, and differences in male and female average height

may be related to the lower values in these groups.

No signs of airway restriction or airway obstruction were observed with Vitalograph. However, determination of hidden pulmonary obstruction cannot be excluded, since any test for reversibility of pulmonary resistance was not performed.

The observed symptoms may be independent of work place chemical exposures (acid curing versus UVR curing). A common exposure between the two different work groups is coating dusts (composed, at least, in part of wood dust) present in concentrations below the Swedish occupational exposure limit value of 3 mg/m<sup>3</sup>. The actual chemical composition of these work place dusts is unknown, but a significant portion is in the respirable range. More information is required on composition of dust and exposure levels for other chemical hazards (e.g., formaldehyde) in the respective work environments in order to determine the etiology of the observed symptoms.

### Skin

Skin exposure to UVR curable coatings varies, but a significant exposure to UV line workers occurred in all plants when cleaning the UV line equipment. Surprisingly, only one individual was positive for an allergic reaction to a acrylate patch test series. Minor erythema and pigmentation was observed in workers employed at stations with direct exposure to UVR from poorly shielded entrance and exit slits of UV units. The special acrylate patch test series included most commonly used acrylates and other proprietary ingredients used in the UV curable coatings for wood. During the investigation some coatings were observed to contain acrylates (e.g., glycerol

propoxy triacrylate and ethylidiglycol acrylate) and/or hardeners (e.g., methyl ketone peroxide, polymeric isocyanate, monomeric isocyanate, polyfunctional aziridine) that were not included in this patch test series. However, the use of these chemicals was restricted to a few plants.

This investigation of dermatologic symptoms indicated irritant problems with the skin connected to acrylate and/or UVR exposures on UV lines. The workers on the UV lines reported three different clinical types of irritant dermatitis: (1) localized well demarcated distinct "burns" on back of hands and arms at the site of accidental contact with uncured UV coatings; (2) dry, fissuring, skin with fine scaling observed in all types of irritant work; and (3) diffusely erythematous swelling of the hands and arms followed by scaling, which persisted during work on the UV line and disappeared within a few days during absence from the UV line, but reappeared with repeated exposure; these symptoms were reported mainly with UVR curable coatings no longer in common use.

## Comparative *In Vivo* and *In Vitro* Toxicity of Selected Acrylates

### Short Term *In Vivo* Toxicology, Carcinogenesis, and Mutagenesis Studies

TPGDA or TPGDA in the UVR curable lacquer formulations at 5  $\mu$ moles/mouse (surface area ~8 cm<sup>2</sup>) induced a level of relative skin thickness (hyperplasia) similar to 600  $\mu$ moles/mouse of EA (~100 fold difference in dose) in acute toxicity studies. Apparent thresholds were observed for induction of epidermal hyperplasia. For TPGDA the threshold appeared between 1 and 5  $\mu$ moles/mouse. A significant in-

crease in dose was required for EA to achieve a similar increase in hyperplasia (between 300 and 600  $\mu\text{moles/mouse}$ ). No differences existed in the induction of hyperplasia between TPGDA alone, or TPGDA in Lacquer A or Lacquer B (when applied equimolar doses, based on their respective concentration). Thus, toxicity may be attributed to the TPGDA and is apparently not significantly affected by the other lacquer components at the concentrations used in the lacquer.

TPGDA applied at a dose and dose rates above the threshold level of induction of hyperplasia for 20 weeks (alone or in lacquer formulation) induced a maximum number of papillomas per mouse similar to the TPA positive control (2  $\text{nmoles/mouse}$ ). Under the same exposure conditions, TPGDA applied at a dose below the threshold for induction of hyperplasia did not induce papillomas. EA applied at dose and dose rates that induced hyperplasia similar to TPGDA (5  $\mu\text{moles/mouse}$ ) did not induce any papillomas. However, hyperkeratosis and a suppression of a gain in body weight was observed in the 20 week study indicating systemic toxicity. These results suggest that properties inherent of TPGDA other than the number of functional acrylate groups may play a role in tumor induction when applied to the skin of these mice. In the only available report [6], TPGDA (8  $\mu\text{moles/mouse}$  in mineral oil) did not induce an increase in skin tumors in female C3H/HeJ mice after 80 weeks of treatment (2x/week). Differences in mouse strain, vehicle, surface area, and dose rate of application may have contributed to the negative finding in this study.

Dermal application of EA or TPGDA to female TG $\bullet$ AC mice over a 20 week period failed to induce a significant systemic

genotoxicity as evidenced by an increase in DNA damage in circulating leukocytes or in proliferating bone marrow cells as measured by MN frequency in peripheral blood PCE. However, a systemic toxicity was observed in the form of a significant increase in %PCE in mice treated with TPGDA (5 or 10  $\mu\text{moles/mouse}$ ) or TPA (2  $\text{nmoles/mouse}$ ) and a marginal (nonsignificant) increase with Lacquer A (10  $\mu\text{moles/mouse}$ ).

The absence of DNA damage and micronuclei suggests that these two acrylates are not systemically genotoxic. However, these acrylates are contact haptens and potential carcinogens and genotoxicity may only occur at the site of contact [45, 65, 66, 130]. The increased rate of erythropoiesis correlated with increased tumor incidence may reflect either systemic homeostatic mechanisms compensating for increased tumor burden or dose related toxicity to epidermal keratinocytes and induced cytokines (e.g., tumor necrosis factor alpha, TNF $\alpha$ ; granulocyte-macrophage colony stimulating factor, GM-CSF; etc.) [149]. The significant difference in dose (equimolar consideration for the number of functional groups) observed in these studies suggest that other inherent characteristics of TPGDA may be responsible for the increased toxicity relative to EA. TPGDA effects may be both qualitatively and quantitatively different from the known tumor promoter TPA, but the systemic effect on the %PCE as it reflects bone marrow activity is similar. The threshold observed for TPGDA toxicity indicates that the protection mechanisms of the skin can be saturated. Glutathione and glutathione-S-transferase and hydrolysis of the conjugated product is believed to be the main source of protection from acrylate toxicity [34, 39,

43, 63, 114, 155] and conjugation may be expected to be proportional to the number of functional groups. Thus, toxicity and the potential of acrylates to promote skin tumors may depend upon the number of functional groups, molecular structure, and molecular weight differences that affect their ability to be absorbed through and interact with the skin and influence the potential systemic genotoxicity of these electrophiles. Additional studies will be required to determine the significance of the role of TPGDA induced cellular proliferation in the induction of papillomas.

#### Comparative *In Vitro* Cytotoxicity

Acrylates were cytotoxic *in vitro* to human epidermal keratinocytes, dermal fibroblasts, and bronchiolar epithelium that are potential target cells from occupational exposure. The approximate 100 fold difference between EA (20  $\mu\text{moles/cm}^2$ ) and TPGDA (0.2  $\mu\text{moles/cm}^2$ ) cytotoxicity observed *in vitro* for human cells is greater than the difference between the LD<sub>50</sub> skin paint dose for EA ( $\approx 600 \mu\text{moles/8 cm}^2$ ) and TPGDA (120  $\mu\text{moles/8 cm}^2$ ) in TG $\bullet$ AC mice when adjusted for the surface area exposed. However, the similarity in acrylate toxicity between human cells *in vitro* and mouse cells *in vivo* supports a contact site toxicity and the *in vitro* and *in vivo* comparison.

Acrylate toxicity to human cells *in vitro* exhibited biphasic dose response curves, which were similar to the free sulphydryl depletion curves. This similarity suggests that free sulphydryls, like cysteine, glutathione, and sulphydryl containing cellular proteins or polypeptides are reduced proportionate to the acrylate dose *in vitro*. Glutathione is known to react with and detoxify acrylates through Michael's addition reactions [58, 64]. The potential of acrylates to react with biomolecules, as well as through single species or multispecies chemical polymerization, demonstrates their toxic potential.

The potential for synergistic toxicity between low levels of UVR and TPGDA to human cells was demonstrated. The potential exists for exposure to UVR (environmental or occupational) and MofA in the radiation curing surface coating industry. UVA radiation is known to induce reactive oxygen species (ROS), which results in the reduction of superoxide dismutase, catalase, and glutathione [9, 59]. Acrylates are known to reduce glutathione upon contact and induce inflammation *in vivo*, which can also induce a prooxidant state with ROS [116]. Sequential *in vitro* exposure to nontoxic dose levels of UVA and TPGDA resulted in induction of ROS and acrylate depletion of glutathione may be sufficient to induce cytotoxicity.

# Conclusions

A thorough understanding of the hazards associated with work using UVR curing on surface coating of wood is necessary for development of strategies for education and reduction of potential risks to workers. To date, no research on the possible effects to humans from changes in manufacturing processes, procedures, and chemicals used in wood surface coating industry has been published. This project was aimed to study and clarify the potential occupational risks related to wood surface coating with UVR curing and to provide a basis for determining and recommending modifications in order to continue to improve safety and health of workers in this industry.

- Work place air contains uncured acrylates as aerosols, vapor, and/or a part of respirable sanding dust created in the UVR curing process and in the process of finishing. Poorly shielded UV units at some installations also resulted in a potentially increased exposure to UVR and minor erythema and pigmentation in workers. Ozone is produced during the ignition and use of a UV lamp, but concentration levels were not observed to exceed the general background concentration of ozone in the work place air. Work practices were inconsistent (e.g., routines for changing and washing of clothes, lack of use of personal protection equipment) and portend a significant risk for skin exposure.
- Clinical investigation of the upper airway and eyes indicated that nasal, pharyngeal, and ocular symptoms of discomfort, but not for the lower airway, were common among all work-

ers in the wood surface coating industry. These symptoms were most frequent in UV line workers and finishers of UVR surface coated wood products. Mucociliary clearance and olfaction were impaired in UV line workers indicating changes in the nasal cavity, which may be the result of toxicity.

- Investigation of dermatologic symptoms disclosed problems with irritant dermatitis of the skin connected to acrylate and/or UVR exposures. However, a low frequency of contact allergy was observed in workers employed in UVR surface coating industry.

Evidence of toxicity and potential carcinogenicity of acrylates used in UVR curable coatings (i.e., TPGDA or HDDA), which are chemical electrophiles and potent contact sensitizers are limited.

- TPGDA has the potential to induce or promote epithelial tumors when topically applied to the skin of mice. TPGDA is toxic to normal human skin and lung cells *in vitro*. Together, these studies indicate that exposure to certain acrylates, at least, may pose a risk to workers and that this risk must be carefully evaluated and managed. Due to the potent sensitizing potential of TPGDA in guinea pigs and humans and tumorigenicity in mice, TPGDA may be a carcinogen at the site of application.
- Estimation of the relative comparative cytotoxicity to human target cells suggests that toxicity to some acry-

lates may be greater than can be accounted for by the numbers of functional acrylate groups, possibly involving other molecular characteristics. MuFA are inherently more complex than MoFA and their potential toxicity difficult to predict. The relative difference in vapor pressure must be considered to contribute to the relative differences in cytotoxicity observed. The synergistic toxicity between UVA and TPGDA *in vitro* warrants further investigation *in vivo* and *in vivo* to determine the potential importance to occupational exposure.

- Toxicity and the potential of acrylates to promote skin tumors may depend upon the number of functional groups, molecular structure, and molecular weight differences that affect their ability to be absorbed through and interact with the skin and influence the potential systemic genotoxicity of these electrophiles. Additional studies will be required to determine the significance of the role of TPGDA induced cellular proliferation, mutagenicity at the application site, and in the induction of skin tumors.

Surface coating of wood with UVR curable acrylate coatings does involve a potential health risk for workers. Uncured or partially cured acrylate coatings must be consid-

ered as potentially harmful to eyes and skin. Exposure to respirable aerosols of partially or uncured acrylates and UVR arc factors involving special risk that must be considered more extensively and in more detail. The overall significance of the health effects observed from the exposures described and investigated in this study are not thoroughly understood. There is insufficient information at present to estimate either risk for cancer or noncancer endpoints to workers.

The use of UVR curable acrylate coatings is increasing. Future research on exposure assessment, risk assessment, and managing risk by process, emission, and exposure control technology is required and urgent. It is advisable to treat the UVR curable acrylate coatings with great care and consideration. Recommendations given to users by the coating manufacturers in Sweden indicate the necessity to use special protective gloves, disposable overalls, separate areas for work cloths and private clothes, etc., when working with UVR curable acrylate coatings. Control of the UVR curing process in respect to complete curing and shielding of UV units are also important measures to reduce potential risk of exposure. Finally, regular industrial hygiene assessment focusing on aerosol and vapor exposure to airways and skin, UVR and efficiency of curing, and monitoring of work practices to reduce potential to exposure are mandated.

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# Assessment of worker exposure in the processing of ultraviolet radiation cured acrylate lacquer coated wood products

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Manufacturing of wood products coated with ultraviolet radiation curable acrylate coatings has increased in recent years and, to our knowledge, an assessment of worker exposure has not been conducted. To assess worker exposure we identified manufacturing plants in Sweden using this technology and evaluated work force and production by questionnaire. From this data base we selected eight plants that we believed were representative of the industry. At each plant, an industrial hygiene survey was conducted. Worker exposure to acrylate vapors and aerosols, dust, solvents, ozone, UV radiation (including operational performance of UV lamps), and work practices were investigated. We found that ~350 of the 8,500 production workers are potentially directly exposed to acrylates and UV radiation. Although, dust levels were low ( $\approx 0.4$  mg/m<sup>3</sup>, 8 h TWA),  $\approx 30\%$  of dust particles were respirable ( $< 5$   $\mu$ m dia). Exposure to potential sensitizing agents (acrylates and photoinitiators) was indicated. Our knowledge of potential health risks associated with the measured exposure levels of sensitizing agents is limited, and therefore, we have no basis at present for estimating risk. Potential exposure to UV

radiation (UV<sub>A</sub>, UV<sub>B</sub>, and UV<sub>C</sub> wavelength ranges) was observed to reach intensities of  $\approx 10$ -20 mW/cm<sup>2</sup>. The observed use of protective equipment was often insufficient and of incorrect type to prevent or minimize exposure. In conclusion, these results indicate that worker exposure to harmful agents may occur and additional investigations are warranted to estimate risk and to improve methods for evaluation of worker exposure by multiple routes.

## INTRODUCTION

In recent years, ultraviolet radiation (UVR) curing of surface coatings has greatly increased in importance and the coating materials, the methods of application, and the curing processes have been undergoing significant changes. This technology is about 20 years old, but not until the past decade has the use of formulations containing acrylates for UVR cured coating applications increased remarkably. This is, at least in part, due to the mandated requirements to reduce solvent emissions from solvent based curing systems for coated surfaces. UVR curing provides important advantages over conventional curing sys-

tems, such as eliminating emissions of volatile organic compounds (VOCs) to the ambient air, saving of raw materials (solvent free systems) since conventional systems contain between 50-85% solvent, and accelerated curing which allows immediate further processing of the coated product. In addition, it provides lower energy consumption because it eliminates the use of the solvents, low thermal stressing of substrate material, minimal space requirements, and is readily adapted into existing production facilities.

Acrylates are ester derivatives of acrylic acid that are formed by reacting an alcohol with one of the acids. They polymerize readily and are widely used in the chemical industry to make polymeric resins for applications including paints and coatings, printing inks, adhesives, textiles, molded plastic objects, polishes, and leather treatment.<sup>(1)</sup> Acrylates may be classified as either monofunctional or multifunctional. Monofunctional acrylates may be presented by generic formula  $\text{CH}_2=\text{CHCOO}-\text{R}$ , where R presents the respective alcohol group in the ester (e.g., methyl, ethyl, *n*-butyl, 2-ethylhexyl, hydroxyethyl). Multifunctional acrylates (MuFA) are defined as those containing more than one acrylate group, respectively (e.g., diethylene glycol diacrylate, trimethylol propane triacrylate). MuFA are generally used as monomers in formulations for cross linking the polymer to provide bulk properties such as hardness, abrasion resistance, flexibility, elasticity, and resistance in the cured film.<sup>(2)</sup> They may also be used as reactive diluents to adjust the desired viscosity of radiation curable formulations in much the same way as solvents in conventional systems. The UVR curable acrylate lacquers, paints, and fillers (UV coatings) used in the surface

coating industry are normally composed of three basic components: an acrylated prepolymer (urethane acrylate, polyester acrylate, etc.), a MuFA monomer (e.g., tripropylene glycol diacrylate, TPGDA; 1,6-hexanediol diacrylate, HDDA), and a photoinitiator system (e.g., benzophenone, BP; benzil dimethylketal, BDMK).<sup>(2)</sup>

The average annual growth of production of radiation curable acrylate formulations, ~20% in Europe, has been steady for the past 10 years. The total European market for 1990 was estimated at 21,000 metric tons<sup>(3)</sup> and is expected to grow to 50,000 metric tons by the year 1995.<sup>(4)</sup> The total global market for radiation curable formulations have been evaluated to 85,000 metric tons per year (paints 57%, printing inks 10%, and adhesives 3%).<sup>(5)</sup> The volume growth in the near future is estimated at 15-20% per annum to the year 2000 with a projected rise in the number of radiation curable applications, in which MuFA are predominantly used. Main users are the United States (33%), Western Europe (30%), and Japan (20%).

MuFA have a much lower vapor pressure than those in the monofunctional category. Most MuFA have a vapor pressure <0.01 mmHg at 20°C,<sup>(6-8)</sup> indicating that the potential for inhalation exposure to the MuFA should be low under normal work room conditions. Acrylates have extremely low odor threshold limits, making their presence readily apparent.<sup>(6-8)</sup> Few methods have been published on the sampling and analysis of MuFA.<sup>(8, 9)</sup> There are significant technical problems associated with sampling and analyzing for MuFA.<sup>(8)</sup> The American Industrial Hygiene Association (AIHA) recommends 1 mg/m<sup>3</sup> with a skin notation as the 8 h time-weighted average (TWA) workplace environmental exposure

level (WEEL) for MuFA.<sup>(10)</sup> In the United States the Occupational Safety and Health Administration (OSHA) has not set allowable air concentrations for any MuFA.

Potential occupational risks related to the use of UVR curable acrylate coatings (UV coatings) in wood surface coating industry have not been investigated. Our study was designed to determine (identify and document) potential worker exposure to acrylates and other chemical contaminants via inhalation and/or dermal routes and to evaluate work practices in Swedish UVR wood surface coating industry. In Sweden, there are 48 known facilities employing the application of UV coatings on wood products. The principal type of application method for transferring the formulation onto the product is a direct roller coater. Also, a manual application of filler is used. A total of 8 plant surveys were conducted. Our objectives for the surveys were: 1) identify, describe, and measure specific tasks where exposures may occur, and 2) describe current industrial hygiene and safety practices, including engineering controls, personal protective equipment used, and work practices. This study was also designed to provide a basis for determining and recommending modifications for manufacturing processes in order to better control chemical exposures to workers in the UVR wood surface coating industry through technical solutions and improved work practices. We report and discuss here the potential exposure to hazards at the plants surveyed, and our conclusion and recommendations to limit the potential for dermal and inhalation exposure to acrylates, in particular.

## MATERIALS AND METHODS

### Identification of Manufacturers and Site Selection Criteria

Swedish UVR wood surface coating producing plants were identified for the survey by contacting the Swedish UV coating formulators and requesting information on their customers. The plants identified in this manner were surveyed in two phases. The first phase included contact with all plants using UVR wood surface coating technique and a request to complete a questionnaire focusing on their work force and production. The first phase survey was used to obtain additional information on the manufacturing plants and allow identification and selection of representative plants for the second phase industrial hygiene survey. Site visits to 2 plants were conducted to obtain information necessary to design the second phase investigation procedures. Selection criteria were to include diverse types of plants and considered were: plant size and diversity, processed products, coating formulations used, UVR curing systems, geographical location, etc. Eight plants were considered representative of the Swedish UVR wood surface coating producing plants.

### Plant Surveys

Plant surveys were conducted in 8 UVR wood surface coating manufacturing plants during the period between October 1992 and April 1993. The surveys included preliminary contact with the plant management and employee representatives to explain the scope and objectives of the study and to collect data pertaining to the use of acrylates and other chemicals at that plant. Exposure assessment was determined during a 1-day industrial hygiene measuring

survey conducted at each 8 selected plants. Worker exposure measurements and observations were made during normal process operations and employee activities.

A detailed form, developed for collecting information during the survey, was completed. This information included the plant description (e.g., physical description of the plant, history of the plant, types of products produced), the process description (e.g., production process, type and use volume of UV coatings and other production related chemicals, potential emission sources and contact areas), the description of the work force (e.g., number of workers, work schedule and number of workers on each shift, job categories, worker activities, duration of exposure, work histories), and the description of industrial hygiene and safety measures employed.

Plant descriptions were useful in identifying the length of time the plants had been using UV coatings for wood surface coating. Process description data were gathered in order to determine conditions for the curing process and to assess operations or areas where the potential for inhalation and dermal exposure exists. Data on engineering controls and personal protective equipment were collected to determine the effectiveness of different types of controls and different types of personal protective equipment worn by operators in reducing the potential for worker exposure. The characterization of the work force allowed the determination of the number and types of operators, who are potentially, directly exposed, and the length of time they were potentially exposed.

#### Exposure Measurements and Analysis of the Samples

Sample collections were conducted during a complete 8 h work day on a UVR surface coating line (UV line) or on a station for the finishing of cured products; each worker was performing a set of defined work tasks during the entire work day. The workers wore the sampler affixed to the right side of their work apparel lapel, or if two samples were collected on the same worker, samplers were attached to opposite apparel lapels.

#### Dust

Airborne dust samples were collected with a 37 mm glass fiber filter (Whatman, GF/F) or a 37 mm cellulose ester filter (Millipore, 0.8 µm) and a personal sampling pump (DuPont P 2500, E.I. du Pont de Nemours and Co. Inc., Wilmington, DE, USA) operating at a nominal flow rate of 2 l/min in the worker's breathing zone. All the samples were analyzed gravimetrically (Mettler ME2236, Mettler Instrument AG, Zurich, Switzerland) and some samples were further analyzed for acrylates and photoinitiators by a gas chromatograph. (11)

Particle size selective sampling was conducted using a personal cascade impactor (Marple Personal Cascade Impactor, SE296, Gracely Andersen, Atlanta, GA, USA) and a personal sampling pump (DuPont P 2500) operating at a nominal flow rate of 2 l/min in the worker's breathing zone. The cascade impactor was a 6-stage device with cut points ranging from 0.5-10 µm aerodynamic diameter. Particles larger than the cut point for each stage were collected on a 34 mm Mylar filter and particles smaller than the cut point of the last stage were collected on a 34 mm PVC acetate filter (pore size 5 µm). The aerosol size

distribution was determined through gravimetric analysis (Cahn 28, Cahn Instruments Inc., Cerritos, CA, USA) for the sample collected on each filter.

At 3/8 plants stationary airborne dust samples were collected on a 25 mm Nuclepore® gold coated polycarbonate filter (pore size 0.4 µm) placed in a nonconductive holder using a personal sampling pump (DuPont P 2500) operating at a nominal flow rate of 2 l/min for electron microscopic analysis. The sampling time was ~20 min. The filters were gold coated and analyzed for particle size and shape using a JEOL scanning electron microscope (JSM-840 A, Japan) connected to an image analyzer (KONTRON, SEM-IPS, Germany). The smallest particle diameter (D<sub>min</sub>) was measured from 400 particles. The medium diameter and lower and upper quantities were calculated (StatView, Abacus Concepts Inc., Berkeley, CA, USA).

Sanding dust was collected into a clean brown glass jar from a sanding table immediately after sanding of surface coated boards. UVR cured coating dust sample (100 mg) was extracted with 10 ml of either acetone, methylene chloride, or methanol (1:100), shaken in a SML-automatic shaker for 4 h and analyzed within 24 h by a gas chromatograph. (11)

#### Vapors and Aerosols

Airborne vapors were collected with a SKC charcoal tube (Cat. No. 226-01, SKC Inc., Eighty Four, PA, USA) containing 150 mg charcoal (with a 50 mg back-up section) and a personal sampling pump (Sipin SP 1/SP 15, Anatole J. Sipin Co., New York, NY, USA) operating at a nominal flow rate of 50 or 200 ml/min in the worker's breathing zone. Charcoal tubes were

extracted with 2 ml of carbon disulfide for 4 h and analyzed by a gas chromatograph, similar to airborne total dust samples. (11)

An additional monitoring of worker exposure to airborne MufA was conducted at 2 of the 8 plants surveyed, approximately 6 months later, because we observed trace levels of MufA in the dust samples and an absence in the charcoal tube samples. The following method was developed and tested under laboratory conditions for airborne MufA aerosols and vapors prior to field use. (11) Airborne acrylate vapors and aerosols were collected with an OSHA Versatile Sampler tube (OVS; Cat. No. 226-56, SKC Inc., Eighty Four, PA, USA) containing a glass fiber filter, tenax sorbent (140/70 mg), and polyurethane foam and a personal sampling pump (DuPont P 2500) operating at a nominal flow rate of 1 l/min in the worker's breathing zone. The OVS tubes were extracted with 5 ml of acetone for 4 h and analyzed by a gas chromatograph.

#### Surface Contamination

Surface wipe samples were collected using glass fiber filters (Whatman, GF/A, 37 mm) dampened with distilled water sufficient to wipe a determined surface area (100 cm<sup>2</sup> or 25 cm<sup>2</sup>) at different places where exposure may occur. The samples were extracted with 5 ml of methanol and analyzed after 4 h by a gas chromatograph. (11)

#### Ozone

Ozone concentration measurements were determined in the ventilation exhaust pipes of UV lamps during lamp ignition and the first hour of operation. In 4/8 plants ozone was measured in the work place ambient air. Measurements were conducted using a

Table XI. Results from the nasal peak expiratory flow (NPEF) test, olfactory test, and nasal clearance test in the exposure and the control groups. Nasal clearance in different exposure groups is compared statistically (odds ratios and 95% confidence limits) to the control group.

Exposure Group	NPEF Mean $\pm$ STD	Olfactory test Mean $\pm$ STD	OR	Clearance 95% CI.	(%)*
I. UV line (N=26)	324 $\pm$ 18.3	4.9 $\pm$ 1.3**	9.2	1.02-82.21	23
II. AC line (N=24)	315 $\pm$ 22.9	4.7 $\pm$ 1.8**	6.4	0.67-62.31	17
III. UV finisher (N=33)	252 $\pm$ 17.9	5.6 $\pm$ 1.9	2.9	0.28-29.51	9
IV. UV/VAC finisher (N=35)	318 $\pm$ 16.6	4.8 $\pm$ 1.7***	3.7	0.39-35.47	11
V. Control (N=30)	294 $\pm$ 15.7	6.4 $\pm$ 1.9			3

\* percent of subjects in the study group with impaired mucociliary clearance

\*\* p<0.01

\*\*\* p<0.001

when compared to the young controls (542 years of ages).

Spirometry measurements indicated that vital capacity and FEV<sub>1.0</sub> were close to expected values in all exposed groups as well as in the control group.

#### Skin (Paper IV)

Altogether, 659 workers, 496 men (75.3%) and 163 women (24.7%), participated in the study. Summary statistics of the study population are presented in Table XII. The age distribution between the study groups was similar (avg. 40  $\pm$  11 years, N=659) as was the employment time in the wood industry (avg. 13  $\pm$  10 years) in the exposed groups. Most of the workers at the UV lines had been employed 55 years (83%) in their present work. More than half of these workers (66%) had been working on the UV lines between 1-5 years. The group of UV line workers investigated represents a majority of the Swedish UV line workers with a particular occupational exposure conditions. For every UV line worker included in the study an attempt was made to obtain a control individual matched for sex

and age among workers employed on acid curing surface coating lines, finishers employed in UV surface coated products, and office workers. However, too few acid curing line workers as well as UV finishers made it difficult to obtain a satisfactory match. Therefore, finishers with mixed exposures were also included, but are presented separately.

From the questionnaire, a total of 132 workers (19%) were determined to be atopic. The highest frequency of atopy (34%) was observed in group VI (prior UV) and the lowest (13%) in group II (AC line). Group I (UV line) had a similar frequency of atopy compared to the control group (22% and 23%, respectively).

Dermatologic symptoms such as erythema, itching, and dryness experienced by individuals in different exposed groups were determined and analyzed from the questionnaires. Workers in groups I (UV line) and III (UV finisher) experienced skin irritation, itching, and dryness from UV acrylate coating and coating dust. Workers in group II (AC line) reported itching and dryness, but not erythema from acid curing coatings and coating dust. Itching and dry-

Table XII. Summary statistics of the study population according to the type of exposure.

Study Group	I (N=146)	II (N=57)	III (N=54)	IV (N=97)	V (N=71)	VI (N=61)	VII (N=173)
Sex (female/male)	24/122	3/54	29/25	26/71	7/64	13/48	61/112
Age (average)	38	42	40	39	39	39	42
Employment in UVR surface coating (years)	3.6	0	5.0	4.1	3.8	2.3	0
Employment in wood industry (years)	11.4	16.0	9.8	15.0	12.4	13.8	14.8
Smokers (%)	52	30	41	28	32	20	18
Atopy (%)	22	13	26	17	17	34	23

ness were also reported from individuals in all exposed groups when exposed to wood dust. Few individuals reported dermatologic symptoms from UV lamps. The most reported skin symptoms from UVR were erythema, edema, and pain after accidental exposure during lamp service. Erythema and smarting was reported by hand fillers, who worked in the front of poorly shielded UV units.

Altogether, 144 individuals with history of past or present dermatitis were investigated clinically and were patch tested. Forty individuals with a history of eczema were interviewed by telephone. Telephone interviews were efficient because of the distant location of the industrial plant and very few individuals (usually one or two) with skin disease(s) were involved at each plant. The distribution of investigated in-

dividuals with skin disease in the different study groups are presented in Table XIII.

This clinical investigation disclosed an expected panorama of noneczematous skin disease among individuals investigated. Different types of eczema were diagnosed in 168 individuals (Table XIV). Irritant contact dermatitis was more common among workers in groups I (UV line; OR 3.77, 95% CL 1.86-7.63) and II (AC line; OR 2.85, 95% CL 1.16-7.02) than in the control group. Distribution of eczematous disease and relation to work tasks in all exposed groups was significantly different from the control group (p<0.05; Table XV). However, in group I the total eczema frequency was the greatest of the exposed groups and significantly different (OR 2.07, 95% CL 1.24-3.43) from the control group. Twenty of the exposed workers (13%) judged the work with the UV acrylate coat-

Table XIII. Number of individuals investigated with a history of eczematous skin disease according to the study group.

Study Group	I (N=146)	II (N=57)	III (N=54)	IV (N=97)	V (N=71)	VI (N=61)	VII (N=173)
Clinical investigation	44	12	18	13	11	14	32
Telephone interview	9	3	1	12	8	1	6

ings to be the main cause of their disease. No cases of skin cancer were observed in this study.

Altogether, 49 positive patch test reactions were observed in 31 individuals (Table XIV). Only one individual was allergic to acrylates (1+ reaction to patch tests nr. 25, 26, 28, 29, 30, 31; see Table V). This individual left the UV line work due to eczematous skin disease, which healed after leaving. Except for methyl hydroquinone (an inhibitor), which gave 18 irritant reactions of erythematous type, the acrylate test series presented few irritant reactions. No other patch test reaction had a direct association to the work with UVR curable coatings. The three reactions to colophony were associated with previous or present contact with wood. The strongest reaction was observed in an office worker who had had hand eczema when working in wood finishing. Four of the nickel reactions were potentially work related since they occurred in individuals with hand eczema who assembled furniture with nickel plated com-

ponents. Two reactions, one to formaldehyde and one to *p*-*t*-phenolformaldehyde resin observed were considered to be related to worker exposure to acid curing coatings or to hard board dust. Most of the remaining positive patch test reactions were related to past or present work activities (e.g., chromium - construction work) or leisure activities.

### Comparative *In Vivo* and *In Vitro* Toxicity of Selected Acrylates

Both immunotoxic (induced hypersensitivity) and toxicity (including potential mutagenic and/or carcinogenic responses over long exposure time) are possible consequences of exposure to chemicals associated with the manufacture of wood products, which are surface coated with UVR curable acrylate coatings. In order to identify and determine the potential toxic chemicals and/or chemical mixtures *in vivo* and *in vitro* toxicity studies were conducted.

Table XIV. The number of individuals working in the wood surface coating industry and the type of eczema observed according to exposed groups (I-VI) and the matched controls (VII). Those with a positive patch test are indicated by parentheses.

Eczema / Study Group	I.	II.	III.	IV.	V.	VI.	VII.
Allergic dermatitis	6 (1)	2	6 (1)	9 (2)	2	2	13 (2)
Contact dermatitis	32† (3)	10† (3)	7 (1)	6 (2)	10 (1)	7 (3)	12 (5)
Nummular eczema	1	-	1	1	2	1	1
Seborrheic dermatitis	9 (1)	4	2 (1)	5	-	1	3
Tyloitic eczema	-	-	-	1	-	-	-
Vesiculosis	1	-	1	-	-	1	3 (2)
Other eczema	-	-	-	1	3	-	2
Total	49*†	16	17	23	17	12*	34*

\* One individual with psoriasis had a positive patch test reaction.  
† Significantly different from the control group.

Table XV. Eczematous disease among workers employed in the wood surface coating industry and the unexposed controls. Results are expressed as the number of workers, whose disease was not work related compared to those, whose disease was determined to be work related to the previous or present occupation.

Eczema / Study Group	I.	II.	III.	IV.	V.	VI.	VII.
Hand eczema							
- past work	1/7	-	1/2	0/1	0/1	1/0	4/0
- present work	1/3	1/2	-	1/1	2/0	1/1	3/0
Other eczema localization							
- past work	6/6	1/3	3/1	4/1	4/1	1/0	8/0
- present work	3/4	1/1	3/1	2/3	1/1	0/3	7/0
Hands and other eczema localization							
- past work	2/3	2/1	0/1	3/1	2/4	0/3	3/0
- present work	1/3	-	1/2	0/1	1/0	0/1	6/0
Total	14/26	5/7	8/7	10/8	10/7	3/8	31/0
Total % in the group	27.4	21.1	27.8	18.6	23.9	18.0	17.9

### Short Term *In Vivo* Toxicology, Carcinogenesis, and Mutagenesis Studies (Papers V-VI)

The toxicity of TPGDA was unknown, except for its strong sensitizing properties in the GPMT [17]. First, the acute toxicity was determined. The dose required to kill 50% of the mice that received a single topical application of TPGDA was estimated at 120 μmoles per mouse (4 mmoles or 1.2 g/kg bw). The minimum lethal dose of EA was greater than 600 μmoles per mouse (>20 mmoles or >2 g/kg bw) under the conditions of this study. Pozzani *et al.* [140] reported the LD<sub>50</sub> value in rabbits (strain not cited) to be =1.8 g or 20 mmoles EA/kg bw. EA at 600 μmoles per mouse was observed to induce a similar level of relative skin thickness or hyperkeratosis when compared to 5 μmoles TPGDA per mouse (both significantly different from the

control at  $p < 0.05$ ; Fig. 9). A no effect level for increasing relative skin thickness was observed at 300 μmoles EA per mouse or 1 μmole TPGDA alone or in Lacquer A or Lacquer B (Fig. 9). No treatment related differences were observed in histopathological examination of the inguinal lymph nodes.

Doses of 60, 300, or 600 μmoles EA per mouse or 1, 5, or 10 μmoles TPGDA alone or in Lacquer A were selected for the short term carcinogenesis studies by skin paint. Because differences in acute toxicity were not observed between the two lacquer formulations, further studies with Lacquer B were not conducted. After repeated application of TPA, TPGDA or TPGDA in lacquer formulation A, three times a week for 20 weeks, focal areas of hyperkeratosis developed into papillomas. TPGDA alone showed a dramatic increase in papillomas per mouse over time when compared to the

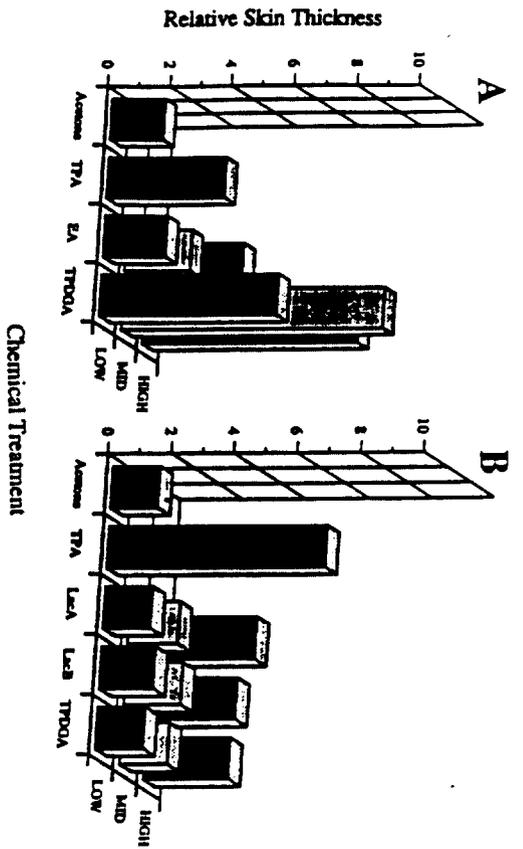


Figure 9. Relative skin thickness (number of epidermal cell layers) after chemical treatment in the acute dose finding studies. A. Doses ( $\mu\text{moles}/\text{mouse}$ ): TPA: 0.002, EA: LOW, 60; MID, 300; HIGH, 600. TPGDA: LOW, 12; MID, 60; HIGH, 120. B. Doses ( $\mu\text{moles}/\text{mouse}$ ): TPA: 0.002, Lacquer A (Lac A), Lacquer B (Lac B), or TPGDA: LOW, 0.5; MID, 1.0; HIGH, 5.0. Acetone vehicle control and all doses were delivered in 200  $\mu\text{l}$  acetone.

vehicle control (acetone) or EA (Fig. 10) that was dose related. Similar effects were observed with Lacquer A (adjusted equimolar to TPGDA alone based on percent TPGDA) at 5 or 10  $\mu\text{moles}$  (Fig. 11). No effects were observed at 1  $\mu\text{mole}$  TPGDA, 1  $\mu\text{mole}$  Lacquer A (adjusted to equimolar TPGDA), or at even the highest dose of 600  $\mu\text{moles}$  EA (Fig. 11; lower dose EA data not shown).

In the TPA treated (positive control) group, early mortality, possibly due to tumor burden and other treatment effects, resulted in a dramatic decrease in the maximum number of papillomas per mouse (Fig. 10 or 11) in surviving mice. The rate of tumor occurrence was less in the acrylate

treated groups (Fig. 10 and 11) compared to the TPA treated group.

Tumor incidence varied between the different treatment and control groups, but did not appear to be dose related (Table XVI). No significant differences were observed in mean survival time between any treatment group and the negative control. Mean latency (no. days  $\pm$  std dev until each mouse developed the first papilloma) was reduced between the negative control and TPA, 5 or 10  $\mu\text{moles}$  TPGDA, or 1, 5 or 10  $\mu\text{moles}$  Lacquer A. The mean number of papillomas per mouse was significantly different ( $p < 0.01$ ) between the negative control and the TPA positive control, 5 or 10  $\mu\text{moles}$  TPGDA or Lacquer A.

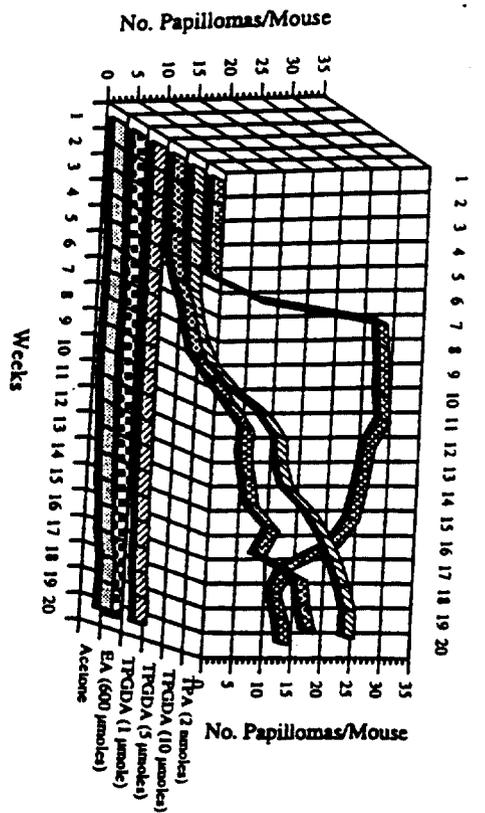


Figure 10. Mean number of papillomas per mouse per week during a 20 week treatment period with ethyl acrylate and tripropylene glycol diacrylate.

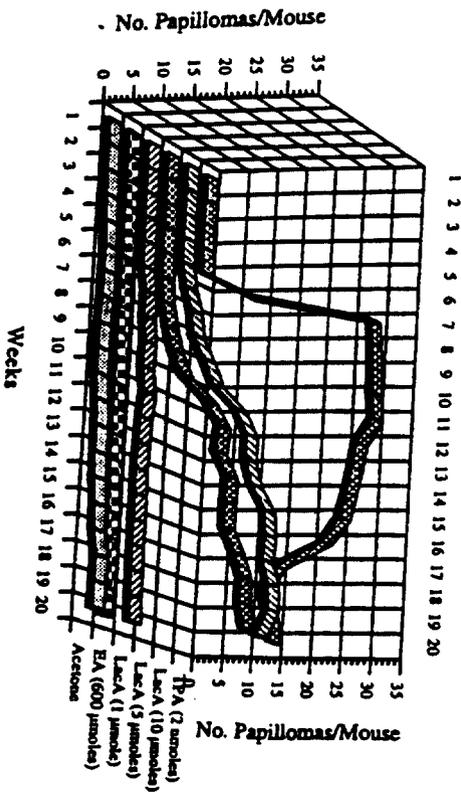


Figure 11. Mean number of papillomas per mouse per week during a 20 week treatment period with Lacquer A.

Table XVI. Effect of topical application of acrylates to the skin of TC1<sup>a</sup>AC mice: incidence, survival, induction of papillomas, and tumor latency.

Chemical	Incidence <sup>1</sup>	Mean Survival Time (days ± SD)	Maximum Papillomas/ Mouse (Mean ± SD)	Latency (days ± SD)
TPA (2 nmoles)	5/10	169.1 ± 59.4	31.7 ± 0.9	34.6 ± 2.8
Acetone (200 µl)	10/10	200.1 ± 46.7	5.0 ± 6.8	120.4 ± 92.3
EA (60 µmoles)	2/10	217.1 ± 39.1	7.5 ± 3.5	107.5 ± 84.1
(300 µmoles)	5/10	227.2 ± 23.8	4.4 ± 5.9	103.6 ± 56.1
(600 µmoles)	2/9	186.2 ± 75.8	2.0 ± 1.4	123.5 ± 46.0
TPGDA (1 µmole)	9/9	217.9 ± 30.0	5.4 ± 5.7	136.9 ± 41.5
(5 µmoles)	9/9	166.9 ± 62.6	29.2 ± 5.7	43.9 ± 16.8
(10 µmoles)	10/10	219.3 ± 30.6	30.4 ± 5.1	47.8 ± 12.1
Lac A (1 µmole)	10/10	194.4 ± 60.5	5.8 ± 5.2	43.3 ± 20.0
(5 µmoles)	10/10	209.0 ± 31.8	18.3 ± 11.4	50.9 ± 10.0
(10 µmoles)	10/10	200.5 ± 38.7	23.1 ± 10.1	43.2 ± 11.3

<sup>1</sup> Incidence equals the number of tumor bearing mice over the total number of mice per group surviving more than 10 weeks of treatment.

Body weights were depressed in the EA high dose group (Fig. 12A), but a dose response relationship was not observed in the TPGDA or TPGDA in lacquer formulation treatment groups (Fig. 12B and 12C).

EA at 60, 300, or 600 µmoles/mouse did not induce a significant increase in the percentage of migrated DNA in leukocytes, either by a trend test or by a pairwise comparison of each treatment dose against the concurrent vehicle control at any sample time. Similarly, at 20 weeks, the frequency of MN-PCE was not significantly elevated and the %PCE was not significantly altered. The percentage of migrated DNA and the frequency of MN-PCE was not increased in the mice treated with 2 nmoles TPA. A marginally nonsignificant increase in %PCE was observed in these mice ( $p=0.054$ ) at 20 weeks treatment.

The percentage of migrated DNA in leukocytes of the mice treated with TPGDA at 1, 5, or 10 µmoles/mouse was not significantly increased, either by a trend test or by a pairwise comparison of each treatment dose against the concurrent vehicle control, at any sample time. Similarly, at 20 weeks, the frequency of MN-PCE was not significantly elevated. However, a highly significant, dose dependent increase in the %PCE was observed in TPGDA treated mice (Table XVIII). The lowest effective dose inducing a significant change was 5 µmoles/mouse.

The percentage of migrated DNA in leukocytes of the mice treated with Lacquer A, equimolar for TPGDA, at 1, 5, or 10 µmoles/mouse was not significantly increased, either by a trend test or by a pairwise comparison of each treatment dose against the concurrent vehicle control, at

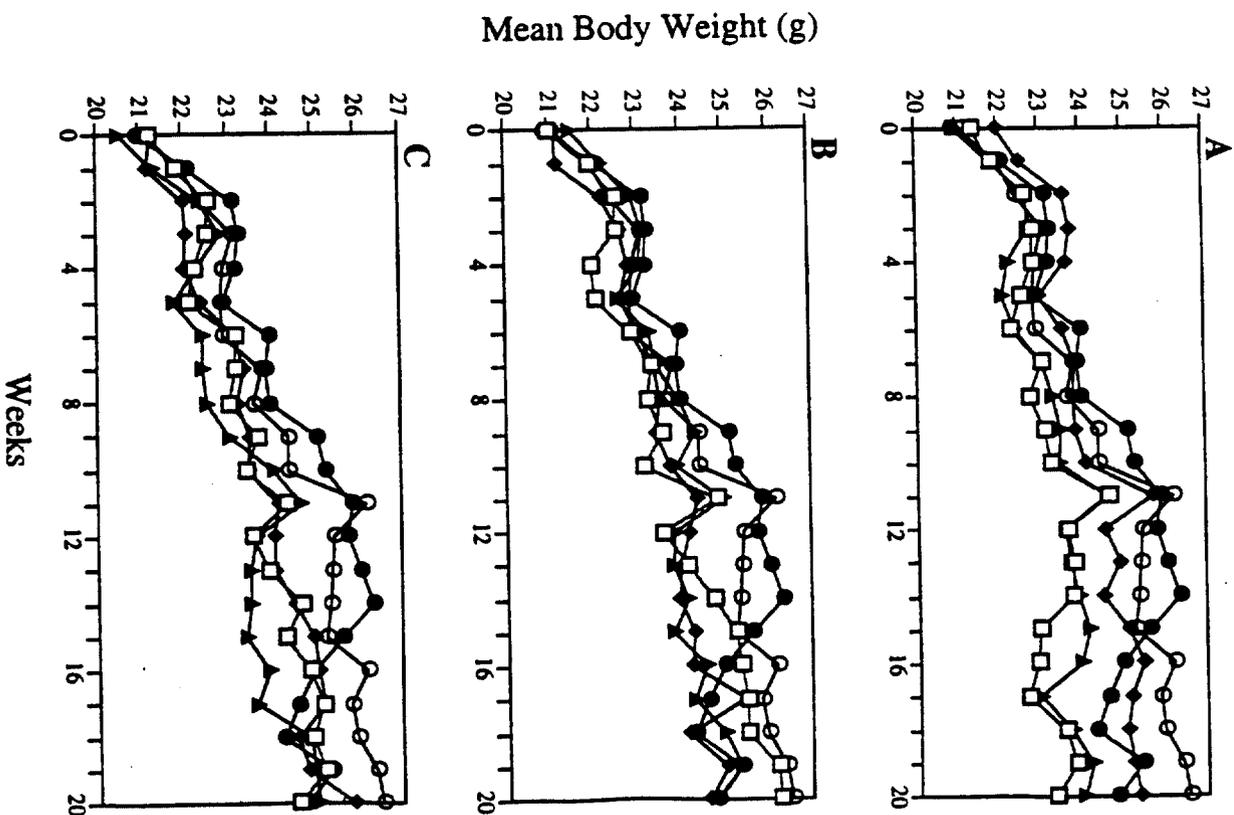


Figure 12. Mean individual mouse body weights per week per dose group during a 20 week treatment period. A. EA, TPA, and acetone controls. B. TPGDA, TPA, and acetone controls. C. Lacquer A (Lac A), TPA, and acetone controls. Acetone (○); TPA (●); LOW (◆); MID (▲); HIGH (◇) for EA (○), TPGDA (■), and Lac A (Δ).

Table XVII. Micronucleated polychromatic erythrocytes (MN-PCE) and the percentage of polychromatic erythrocytes (%PCE) in female TG $\times$ AC mice treated with tripropylene glycol diacrylate.

Dose $\mu$ mol/mouse	MN-PCE <sup>a</sup>		N	%PCE <sup>b</sup>		N
	Mean	SEM		Mean	SEM	
TPA, 0.002	2.1	0.7	7	10.7	3.9	7
0	1.4	0.4	9	3.5	0.5	9
1	1.8	0.4	10	3.4	0.3	10
5	1.9	0.6	5	7.5*	0.8	5
10	2.0	0.3	9	9.7*	1.3	9
				0.142		
				<0.001		

<sup>a</sup> Group mean frequency of MN-PCE per 1,000 PCE and standard error of the mean (SEM) among N mice. Data based on 2,000 PCE scored per mouse.

<sup>b</sup> Group mean percentage of PCE and standard error of the mean among N mice. Data based on 1,000 erythrocytes scored per mouse.

\* One-tailed t-test p-value.

• Significantly different from the concurrent control at  $p < 0.05$ .

any sample time. Similarly, at 20 weeks, the frequency of MN-PCE was not significantly elevated and, while increased in an apparent dose dependent manner, the %PCE was not significantly altered.

#### Comparative *In Vitro* Cytotoxicity (Paper VIII)

Comparative cytotoxicity of EA, TPGDA, or TPGDA in a reference UVR curable lac-

quer (Laquer A) to normal human cells *in vitro* (NHEK, NHDF, or NHBE) was investigated using a methylthiazol tetrazolium (MTT) assay as a measure of cellular viability and/or proliferation after acute exposure. The results indicate that EA, TPGDA, or Laquer A were equally cytotoxic to NHEK at equimolar dose levels (Fig. 13). TPGDA and Laquer A (81.5 and 70.5% TPGDA monomer, respectively;

Table XVIII. Acute toxicity of selected acrylates *in vitro* using normal human cells<sup>#</sup>. Estimated ID<sub>50</sub> concentration ( $\mu$ mole/cm<sup>2</sup>).

	NHEK $\mu$ mole/cm <sup>2</sup>	NHDF $\mu$ mole/cm <sup>2</sup>	NHBE $\mu$ mole/cm <sup>2</sup>
TPGDA	0.6	0.2	0.2
Laquer A	1.3	0.3	0.2
Ethyl acrylate	1.0	20.0 <sup>#</sup>	15.0 <sup>#</sup>

<sup>#</sup> Three to five different individual adult human early passage secondary cell cultures were used under the same conditions to calculate these estimates.

• Significantly different from other ID<sub>50</sub> values ( $p < 0.01$ ), but not to other values denoted the same.

corrected to equimolar TPGDA concentration) were more cytotoxic to NHDF and NHBE than EA (Fig. 14 and 15). TPGDA is  $\approx 100$  times more cytotoxic than EA to NHDF and NHBE (Table XVIII). Both log-phase and stationary growth population of NHEK and NHDF were equally sensitive to these acrylates.

Equimolar concentrations of TPGDA (81.5%) alone or reference Laquer A (70.5% TPGDA and proprietary ingredients) yield similar ID<sub>50</sub> concentrations. This suggests that ingredients other than the TPGDA are not present in sufficient concentration to exacerbate or potentiate toxicity and that TPGDA is the cytotoxic agent (Fig. 13-15, Table XVIII). Low doses of TPGDA or EA may be slightly mitogenic to both NHEK and NHDF (Fig. 3 and 4); TPGDA, but not EA, may be mitogenic at low doses to NHBE (Fig. 15). The free

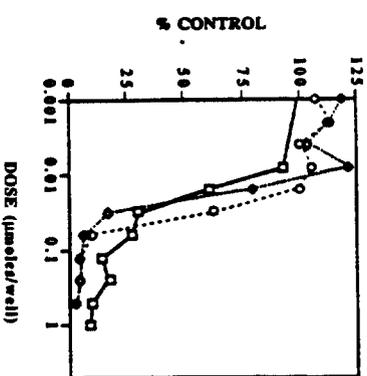


Figure 13. Confluent cultures of normal human epidermal keratinocytes (NHEK) were exposed to ethyl acrylate ( $\square$ ), tripropylene glycol diacrylate ( $\diamond$ ), or Laquer A ( $\circ$ ) in 50.1% acetone for 18 h and cytotoxicity determined by measurement of MTT assay as an index of cytotoxicity (% control;  $N=32$ ).

sulphydryl concentrations in acrylate exposed human cells decreased with increasing acrylate dose (Fig. 16 and 17) and paralleled the cytotoxicity curves (Fig. 13 and 15).

UVA exposure alone (1 J/cm<sup>2</sup> with 0.06% UVB) stimulated NHEK activity, whereas 0.06  $\mu$ mole/cm<sup>2</sup> TPGDA was not cytotoxic (Fig. 18). UVA exposure (1 J/cm<sup>2</sup> with 0.06% UVB) followed by exposure to 6  $\mu$ mole/cm<sup>2</sup> TPGDA resulted in a synergistic toxicity that was similar in magnitude to exposure to 60  $\mu$ mole/cm<sup>2</sup> TPGDA (Fig. 18). Neither UVA and/or TPGDA increased cloning efficiency or were observed to induce transformed colonies. An approximately 1% cloning efficiency (as expected for secondary passage diploid human cells) was observed in control or UVA treated NHEK populations.

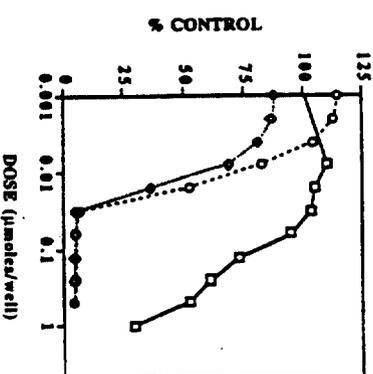


Figure 14. Confluent cultures of normal human dermal fibroblasts (NHDF) were exposed to ethyl acrylate ( $\square$ ), tripropylene glycol diacrylate ( $\diamond$ ), or Laquer A ( $\circ$ ) in 50.1% acetone for 18 h and cytotoxicity determined by measurement of MTT assay as an index of cytotoxicity (% control;  $N=32$ ).

## Discussion

Technology for radiation curing of coated surfaces is about 20 years old, but not until the past decade has the use of formulations containing acrylates for UVR curable surface coating applications increased remarkably. This is, at least in part, due to the necessary reduction of organic solvents in the work place and to the environment because of health concerns as well as desired economic benefits. UVR curing provides important advantages over conventional curing systems, such as eliminating emissions of volatile organic compounds (VOC) to the ambient air, saving of raw materials (organic solvent free systems) since conventional systems contain between 50-85% organic solvents, and accelerated curing which allows immediate further processing of the coated product. In addition, it provides lower energy consumption because it eliminates the use of the organic solvents, low thermal stressing of substrate material, minimal space requirements, and is readily adapted into existing production facilities.

The extensive process changes in the surface coating of wood have decreased conventional worker exposures to e.g., organic solvents, but at the same time have introduced potential risks for other health hazards, e.g., reactive airborne dust, skin irritation and sensitization, and eye irritation. Evaluation of new materials and/or processes for potential health effects before inclusion or substitution in the work place is difficult because of the unforeseen ways in which use and work practices evolve. However, it is highly desirable to prospectively identify and evaluate potential health risks to new materials and work practices in the UVR surface coating industry as soon

as possible in order to prevent or minimize unforeseen health risks that work to the disadvantage of both the worker and the industry.

This study was designed to determine (identity and document) worker exposure to acrylates and other chemical and physical agents and to evaluate health effects and work practices in the Swedish UVR wood surface coating industry. This study was also designed to provide a basis for determining and recommending modifications for manufacturing processes and protective equipment in order to better control hazardous exposures to workers in this industry through technical solutions and improved work practices. Because the toxicology of the main UVR curable acrylate coating and the principal active ingredient, TPGDA, was unknown, limited rodent and *in vitro* toxicology studies were conducted to determine the potential toxicity, possible mechanisms of toxicity, and existing data gaps.

### Worker Exposure

The results indicate that workers in the UVR wood surface coating industry in Sweden are exposed to small amounts of potentially harmful acrylate containing aerosols, vapors, and/or dusts and organic solvents. The most significant findings are: (1) an estimated 350 of the 8,500 workers are directly exposed to acrylates (uncured coating, aerosols and vapors, and/or dusts) and UVR, (2) respirable dust particles (28% of the total dust concentration) are present even though overall dust levels are low

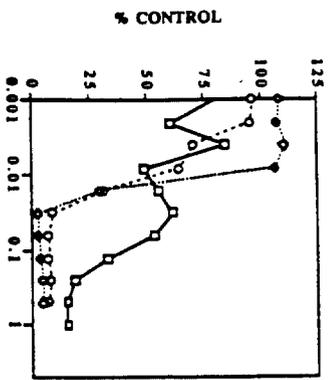


Figure 15. Confluent cultures of normal human bronchiolar epithelium (NHBE) were exposed to ethyl acrylate (□), tripropylene glycol diacrylate (◇), or Lacquer A (○) in 50.1% acetone for 18 h and cytotoxicity determined by measurement of MTT assay as an index of cytotoxicity (% control; N=32).

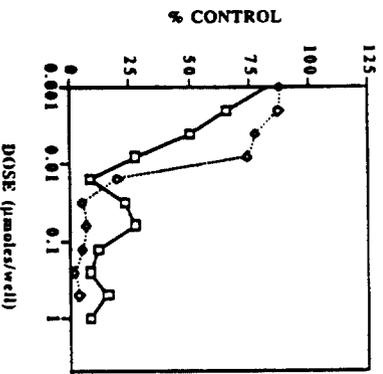


Figure 16. Confluent cultures of normal human epidermal keratinocytes (NHEK) were exposed to ethyl acrylate (□) or tripropylene glycol diacrylate (◇) in  $\le 0.1\%$  acetone for 18 h and concentration of free sulfhydryls were determined by Ellman's method using cysteine for generation of a standard curve (% control; N=16).

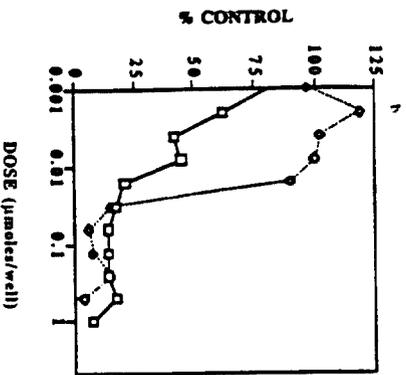


Figure 17. Confluent cultures of normal human bronchiolar epithelium (NHBE) were exposed to ethyl acrylate (□) or tripropylene glycol diacrylate (◇) in 50.1% acetone for 18 h and concentration of free sulfhydryls were determined by Ellman's method using cysteine for generation of a standard curve (% control; N=16).

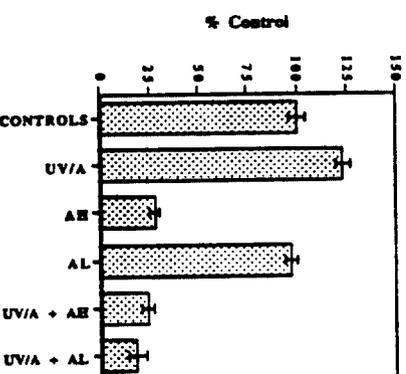


Figure 18. Confluent cultures of normal human epidermal keratinocytes (NHEK) were sequentially exposed to UV (5 J/cm<sup>2</sup>) and tripropylene glycol diacrylate (AH = 60 or AL = 6 mmolels/cm<sup>2</sup>;  $\le 0.1\%$  acetone) and cytotoxicity determined by MTT assay for cytotoxicity (% control; N=8).

(=0.4 mg/m<sup>3</sup>), (3) aerosols, vapors, and dusts containing sensitizing chemicals (TPGDA, HDDA, BP, etc.) were determined to be present in the work places surveyed, (4) release of organic solvents to the ambient environment and concomitant worker exposure is reduced when compared to the acid curing surface coating, (5) in contradiction to coating manufacturers' written published recommendations protection devices for respirable dust were not consistently used (2/8 plants surveyed provided protective devices; however no device was ever observed in use), (6) potential for UVA, UVB, and UVC exposure to unprotected skin at biologically effective dose levels, (7) potential for direct skin exposure to acrylate contaminated surfaces and development of contact dermatitis, and (8) lack and inconsistency of educational programs for workers.

## Health Effects

### Always, Nose, and Eyes

A similar pattern of prevalence of symptoms and dysfunction were observed within the given job categories of all exposed workers when compared to unexposed matched controls. Nasal, pharyngeal, and ocular discomfort were cited most often by surface coating line and finishing workers using either or both UVR and acid curing processes. This pattern of prevalence is consistent with the analysis of site specific complaints and their medical evaluation. Symptoms of hyperreactivity were specific for nasal passages but not the lower airways. Although complaints concerning lower airway discomfort were not significant and exposure to dust was low (~30% respirable), the absence of significant com-

plaint is opposite to that observed in formaldehyde-wood dust exposed workers [75]. A nonspecific nasal hyperreactivity was observed in exposed groups, which may explain a greater frequency of complaints. This nonspecific nasal hyperreactivity was also reported in formaldehyde-wood dust exposed workers [182] and was not more frequently observed in atopics.

Mucociliary clearance was impaired in all exposed groups, but that was significantly impaired only in the UV line workers. Olfaction was significantly impaired in the UV line and the AC line workers and UV/AC finishers. These workers may be exposed to low levels of uncured acrylate aerosols and vapors that deposit and act at site of contact in the anterior nasal passage. This effect may be distinct from mechanical irritation by fine dust that may be the major cause of the observed symptoms and complaints.

Changes in mucociliary clearance may be result of either altered rheological properties of the mucous covering the ciliated epithelial cells or altered function of the ciliated cells due to a lowered cilia beat frequency or loss of cilia. These changes may be caused by exposure to dust and/or toxins, including carcinogenic chemicals [5, 20, 23, 119, 120, 183, 184].

Impaired olfaction may be a result of a toxic effect of a chemical or of a blocked nose. In this study, using NPEF, we did not observe significant signs of nasal obstruction in any exposure groups compared to the control group. However, there are some difficulties in interpreting these results since nasal flow is related to the height of the subject tested. Females were over represented in groups III and V, and differences in male and female average height

may be related to the lower values in these groups.

No signs of airway restriction or airway obstruction were observed with Vitalograph. However, determination of hidden pulmonary obstruction cannot be excluded, since any test for reversibility of pulmonary resistance was not performed.

The observed symptoms may be independent of work place chemical exposures (acid curing versus UVR curing). A common exposure between the two different work groups is coating dusts (composed, at least in part of wood dust) present in concentrations below the Swedish occupational exposure limit value of 3 mg/m<sup>3</sup>. The actual chemical composition of these work place dusts is unknown, but a significant portion is in the respirable range. More information is required on composition of dust and exposure levels for other chemical hazards (e.g., formaldehyde) in the respective work environments in order to determine the etiology of the observed symptoms.

### Skin

Skin exposure to UVR curable coatings varies, but a significant exposure to UV line workers occurred in all plants when cleaning the UV line equipment. Surprisingly, only one individual was positive for an allergic reaction to a acrylate patch test series. Minor erythema and pigmentation was observed in workers employed at stations with direct exposure to UVR from poorly shielded entrance and exit slits of UV units. The special acrylate patch test series included most commonly used acrylates and other proprietary ingredients used in the UV curable coatings for wood. During the investigation some coatings were observed to contain acrylates (e.g., glycerol

propoxy triacrylate and ethylidiglycol acrylate) and/or hardeners (e.g., methyl ketone peroxide, polymeric isocyanate, monomeric isocyanate, polyfunctional aziridine) that were not included in this patch test series. However, the use of these chemicals was restricted to a few plants.

This investigation of dermatologic symptoms indicated irritant problems with the skin connected to acrylate and/or UVR exposures on UV lines. The workers on the UV lines reported three different clinical types of irritant dermatitis: (1) localized well demarcated distinct "burns" on back of hands and arms at the site of accidental contact with uncured UV coatings; (2) dry, fissuring, skin with fine scaling observed in all types of irritant work; and (3) diffuse itchy erythematous swelling of the hands and arms followed by scaling, which persisted during work on the UV line and disappeared within a few days during absence from the UV line, but reappeared with repeated exposure; these symptoms were reported mainly with UVR curable coatings no longer in common use.

## Comparative *In Vivo* and *In Vitro* Toxicity of Selected Acrylates

### Short Term *In Vivo* Toxicology, Carcinogenesis, and Mutagenesis Studies

TPGDA or TPGDA in the UVR curable lacquer formulations at 5 μmoles/mouse (surface area ~8 cm<sup>2</sup>) induced a level of relative skin thickness (hyperplasia) similar to 600 μmoles/mouse of EA (~100 fold difference in dose) in acute toxicity studies. Apparent thresholds were observed for induction of epidermal hyperplasia. For TPGDA the threshold appeared between 1 and 5 μmoles/mouse. A significant in-

crease in dose was required for EA to achieve a similar increase in hyperplasia (between 300 and 600  $\mu\text{moles}/\text{mouse}$ ). No differences existed in the induction of hyperplasia between TPGDA alone, or TPGDA in Lacquer A or Lacquer B (when applied equimolar doses, based on their respective concentration). Thus, toxicity may be attributed to the TPGDA and is apparently not significantly affected by the other lacquer components at the concentrations used in the lacquer.

TPGDA applied at a dose and dose rates above the threshold level of induction of hyperplasia for 20 weeks (alone or in lacquer formulation) induced a maximum number of papillomas per mouse similar to the TPA positive control (2  $\text{mmoles}/\text{mouse}$ ). Under the same exposure conditions, TPGDA applied at a dose below the threshold for induction of hyperplasia did not induce papillomas. EA applied at dose and dose rates that induced hyperplasia similar to TPGDA (5  $\mu\text{moles}/\text{mouse}$ ) did not induce any papillomas. However, hyperkeratosis and a suppression of a gain in body weight was observed in the 20 week study indicating systemic toxicity. These results suggest that properties inherent of TPGDA other than the number of functional acrylate groups may play a role in tumor induction when applied to the skin of these mice. In the only available report [6], TPGDA (8  $\mu\text{moles}/\text{mouse}$  in mineral oil) did not induce an increase in skin tumors in male C3H/HeJ mice after 80 weeks of treatment (2x/week). Differences in mouse strain, vehicle, surface area, and dose rate of application may have contributed to the negative finding in this study.

Dermal application of EA or TPGDA to female TG $\cdot$ AC mice over a 20 week period failed to induce a significant systemic

genotoxicity as evidenced by an increase in DNA damage in circulating leukocytes or in proliferating bone marrow cells as measured by MN frequency in peripheral blood PCE. However, a systemic toxicity was observed in the form of a significant increase in %PCE in mice treated with TPGDA (5 or 10  $\mu\text{moles}/\text{mouse}$ ) or TPA (2  $\mu\text{moles}/\text{mouse}$ ) and a marginal (nonsignificant) increase with Lacquer A (10  $\mu\text{moles}/\text{mouse}$ ).

The absence of DNA damage and micronuclei suggests that these two acrylates are not systemically genotoxic. However, these acrylates are contact haptens and potential carcinogens and genotoxicity may only occur at the site of contact [45, 65, 66, 130]. The increased rate of erythropoiesis correlated with increased tumor incidence may reflect either systemic homeostatic mechanisms compensating for increased tumor burden or dose related toxicity to epidermal keratinocytes and induced cytokines (e.g., tumor necrosis factor alpha, TNF $\alpha$ ; granulocyte-macrophage colony stimulating factor, GM-CSF; etc.) [149]. The significant difference in dose (equimolar consideration for the number of functional groups) observed in these studies suggest that other inherent characteristics of TPGDA may be responsible for the increased toxicity relative to EA. TPGDA effects may be both qualitatively and quantitatively different from the known tumor promoter TPA, but the systemic effect on the %PCE as it reflects bone marrow activity is similar. The threshold observed for TPGDA toxicity indicates that the protection mechanisms of the skin can be saturated. Glutathione and glutathione-S-transferase and hydrolysis of the conjugated product is believed to be the main source of protection from acrylate toxicity [34, 39,

43, 63, 114, 155] and conjugation may be expected to be proportional to the number of functional groups. Thus, toxicity and the potential of acrylates to promote skin tumors may depend upon the number of functional groups, molecular structure, and molecular weight differences that affect their ability to be absorbed through and interact with the skin and influence the potential systemic genotoxicity of these electrophiles. Additional studies will be required to determine the significance of the role of TPGDA induced cellular proliferation in the induction of papillomas.

#### Comparative *In Vitro* Cytotoxicity

Acrylates were cytotoxic *in vitro* to human epidermal keratinocytes, dermal fibroblasts, and bronchial epithelium that are potential target cells from occupational exposure. The approximate 100 fold difference between EA (20  $\mu\text{moles}/\text{cm}^2$ ) and TPGDA (0.2  $\mu\text{moles}/\text{cm}^2$ ) cytotoxicity observed *in vitro* for human cells is greater than the difference between the LD<sub>50</sub> skin paint dose for EA ( $\geq 600$   $\mu\text{moles}/8$   $\text{cm}^2$ ) and TPGDA (120  $\mu\text{moles}/8$   $\text{cm}^2$ ) in TG $\cdot$ AC mice when adjusted for the surface area exposed. However, the similarity in acrylate toxicity between human cells *in vitro* and mouse cells *in vivo* supports a contact site toxicity and the *in vitro* and *in vivo* comparison.

Acrylate toxicity to human cells *in vitro* exhibited biphasic dose response curves, which were similar to the free sulfhydryl depletion curves. This similarity suggests that free sulfhydryls, like cysteine, glutathione, and sulfhydryl containing cellular proteins or polypeptides are reduced proportionate to the acrylate dose *in vitro*. Glutathione is known to react with and detoxify acrylates through Michael's addition reactions [58, 64]. The potential of acrylates to react with biomolecules, as well as through single species or multispecies chemical polymerization, demonstrates their toxic potential.

The potential for synergistic toxicity between low levels of UVR and TPGDA to human cells was demonstrated. The potential exists for exposure to UVR (environmental or occupational) and MuFA in the radiation curing surface coating industry. UVA radiation is known to induce reactive oxygen species (ROS), which results in the reduction of superoxide dismutase, catalase, and glutathione [19, 59]. Acrylates are known to reduce glutathione upon contact and induce inflammation *in vivo*, which can also induce a prooxidant state with ROS [116]. Sequential *in vitro* exposure to nontoxic dose levels of UVA and TPGDA resulted in induction of ROS and acrylate depletion of glutathione may be sufficient to induce cytotoxicity.

# Conclusions

A thorough understanding of the hazards associated with work using UVR curing on surface coating of wood is necessary for development of strategies for education and reduction of potential risks to workers. To date, no research on the possible effects to humans from changes in manufacturing processes, procedures, and chemicals used in wood surface coating industry has been published. This project was aimed to study and clarify the potential occupational risks related to wood surface coating with UVR curing and to provide a basis for determining and recommending modifications in order to continue to improve safety and health of workers in this industry.

- Work place air contains uncured acrylates as aerosols, vapor, and/or a part of respirable sanding dust created in the UVR curing process and in the process of finishing. Poorly shielded UV units at some installations also resulted in a potentially increased exposure to UVR and minor erythema and pigmentation in workers. Ozone is produced during the ignition and use of a UV lamp, but concentration levels were not observed to exceed the general background concentration of ozone in the work place air. Work practices were inconsistent (e.g., routines for changing and washing of clothes, lack of use of personal protection equipment) and ported a significant risk for skin exposure.
- Clinical investigation of the upper airway and eyes indicated that nasal, pharyngeal, and ocular symptoms of discomfort, but not for the lower airway, were common among all work-

ers in the wood surface coating industry. These symptoms were most frequent in UV line workers and finishers of UVR surface coated wood products. Mucociliary clearance and olfaction were impaired in UV line workers indicating changes in the nasal cavity, which may be the result of toxicity.

- Investigation of dermatologic symptoms disclosed problems with irritant dermatitis of the skin connected to acrylate and/or UVR exposures. However, a low frequency of contact allergy was observed in workers employed in UVR surface coating industry.

Evidence of toxicity and potential carcinogenicity of acrylates used in UVR curable coatings (i.e., TPGDA or HDDA), which are chemical electrophiles and potent contact sensitizers are limited.

- TPGDA has the potential to induce or promote epithelial tumors when topically applied to the skin of mice. TPGDA is toxic to normal human skin and lung cells *in vitro*. Together, these studies indicate that exposure to certain acrylates, at least, may pose a risk to workers and that this risk must be carefully evaluated and managed. Due to the potent sensitizing potential of TPGDA in guinea pigs and humans and tumorigenicity in mice, TPGDA may be a carcinogen at the site of application.
- Estimation of the relative comparative cytotoxicity to human target cells suggests that toxicity to some acry-

lates may be greater than can be accounted for by the numbers of functional acrylate groups, possibly involving other molecular characteristics. MuFA are inherently more complex than MoFA and their potential toxicity difficult to predict. The relative difference in vapor pressure must be considered to contribute to the relative differences in cytotoxicity observed. The synergistic toxicity between UVA and TPGDA *in vitro* warrants further investigation *in vivo* and *in vivo* to determine the potential importance to occupational exposure.- Toxicity and the potential of acrylates to promote skin tumors may depend upon the number of functional groups, molecular structure, and molecular weight differences that affect their ability to be absorbed through and interact with the skin and influence the potential systemic genotoxicity of these electrophiles. Additional studies will be required to determine the significance of the role of TPGDA induced cellular proliferation, mutagenicity at the application site, and in the induction of skin tumors.

Surface coating of wood with UVR curable acrylate coatings does involve a potential health risk for workers. Uncured or partially cured acrylate coatings must be consid-

ered as potentially harmful to eyes and skin. Exposure to respirable aerosols of partially or uncured acrylates and UVR are factors involving special risk that must be considered more extensively and in more detail. The overall significance of the health effects observed from the exposures described and investigated in this study are not thoroughly understood. There is insufficient information at present to estimate either risk for cancer or noncancer endpoints to workers.

The use of UVR curable acrylate coatings is increasing. Future research on exposure assessment, risk assessment, and managing risk by process, emission, and exposure control technology is required and urgent. It is advisable to treat the UVR curable acrylate coatings with great care and consideration. Recommendations given to users by the coating manufacturers in Sweden indicate the necessity to use special protective gloves, disposable overalls, separate areas for work clothes and private clothes, etc., when working with UVR curable acrylate coatings. Control of the UVR curing process in respect to complete curing and shielding of UV units are also important measures to reduce potential risk of exposure. Finally, regular industrial hygiene assessment focusing on aerosol and vapor exposure to airways and skin, UVR and efficiency of curing, and monitoring of work practices to reduce potential to exposure are mandated.

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# Assessment of worker exposure in the processing of ultraviolet radiation cured acrylate lacquer coated wood products

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Manufacturing of wood products coated with ultraviolet radiation curable acrylate coatings has increased in recent years and, to our knowledge, an assessment of worker exposure has not been conducted. To assess worker exposure we identified manufacturing plants in Sweden using this technology and evaluated work force and production by questionnaire. From this data base we selected eight plants that we believed were representative of the industry. At each plant, an industrial hygiene survey was conducted. Worker exposure to acrylate vapors and aerosols, dust, solvents, ozone, UV radiation (including operational performance of UV lamps), and work practices were investigated. We found that ~350 of the 8,500 production workers are potentially directly exposed to acrylates and UV radiation. Although, dust levels were low ( $\approx 0.4$  mg/m<sup>3</sup>, 8 h TWA), ~30% of dust particles were respirable ( $< 5$   $\mu$ m dia). Exposure to potential sensitizing agents (acrylates and photoinitiators) was indicated. Our knowledge of potential health risks associated with the measured exposure levels of sensitizing agents is limited, and therefore, we have no basis at present for estimating risk. Potential exposure to UV radiation (UV A, UV B, and UV C wavelength ranges) was observed to reach intensities of  $\approx 10$ -20 mW/cm<sup>2</sup>. The observed use of protective equipment was often insufficient and of incorrect type to prevent or minimize exposure. In conclusion, these results indicate that worker exposure to harmful agents may occur and additional investigations are warranted to estimate risk and to improve methods for evaluation of worker exposure by multiple routes.

## INTRODUCTION

In recent years, ultraviolet radiation (UVR) curing of surface coatings has greatly increased in importance and the coating materials, the methods of application, and the curing processes have been undergoing significant changes. This technology is about 20 years old, but not until the past decade has the use of formulations containing acrylates for UVR cured coating applications increased remarkably. This is, at least in part, due to the mandated requirements to reduce solvent emissions from solvent based curing systems for coated surfaces. UVR curing provides important advantages over conventional curing sys-

tems, such as eliminating emissions of volatile organic compounds (VOCs) to the ambient air, saving of raw materials (solvent free systems) since conventional systems contain between 50-85% solvent, and accelerated curing which allows immediate further processing of the coated product. In addition, it provides lower energy consumption because it eliminates the use of the solvents, low thermal stressing of substrate material, minimal space requirements, and is readily adapted into existing production facilities.

Acrylates are ester derivatives of acrylic acid that are formed by reacting an alcohol with one of the acids. They polymerize readily and are widely used in the chemical industry to make polymeric resins for applications including paints and coatings, printing inks, adhesives, textiles, molded plastic objects, polishes, and leather treatment.<sup>(1)</sup> Acrylates may be classified as either monofunctional or multifunctional. Monofunctional acrylates may be presented by generic formula  $\text{CH}_2=\text{CHCOO-R}$ , where R presents the respective alcohol group in the ester (e.g., methyl, ethyl, *n*-butyl, 2-ethylhexyl, hydroxyethyl). Multifunctional acrylates (MuFA) are defined as those containing more than one acrylate group, respectively (e.g., diethylene glycol diacrylate, trimethylol propane triacrylate). MuFA are generally used as monomers in formulations for cross linking the polymer to provide bulk properties such as hardness, abrasion resistance, flexibility, elasticity, and resistance in the cured film.<sup>(2)</sup> They may also be used as reactive diluents to adjust the desired viscosity of radiation curable formulations in much the same way as solvents in conventional systems. The UV curable acrylate lacquers, paints, and fillers (UV coatings) used in the surface

coating industry are normally composed of three basic components: an acrylated prepolymer (urethane acrylate, polyester acrylate, etc.), a MuFA monomer (e.g., tripropylene glycol diacrylate, TPGDA; 1,6-hexanediol diacrylate, HDDA), and a photoinitiator system (e.g., benzophenone, BP; benzil dimethylketal, BDMK).<sup>(2)</sup>

The average annual growth of production of radiation curable acrylate formulations, ~20% in Europe, has been steady for the past 10 years. The total European market for 1990 was estimated at 21,000 metric tons<sup>(3)</sup> and is expected to grow to 50,000 metric tons by the year 1995.<sup>(4)</sup> The total global market for radiation curable formulations have been evaluated to 85,000 metric tons per year (paints 57%, printing inks 10%, and adhesives 3%).<sup>(5)</sup> The volume growth in the near future is estimated at 15-20% per annum to the year 2000 with a projected rise in the number of radiation curable applications, in which MuFA are predominantly used. Main users are the United States (33%), Western Europe (30%), and Japan (20%).

MuFA have a much lower vapor pressure than those in the monofunctional category. Most MuFA have a vapor pressure <0.01 mmHg at 20°C,<sup>(6-8)</sup> indicating that the potential for inhalation exposure to the MuFA should be low under normal work room conditions. Acrylates have extremely low odor threshold limits, making their presence readily apparent.<sup>(6-8)</sup> Few methods have been published on the sampling and analysis of MuFA.<sup>(8, 9)</sup> There are significant technical problems associated with sampling and analyzing for MuFA.<sup>(8)</sup> The American Industrial Hygiene Association (AIHA) recommends 1 mg/m<sup>3</sup> with a skin notation as the 8 h time-weighted average (TWA) workplace environmental exposure

level (WEEL) for MuFA.<sup>(10)</sup> In the United States the Occupational Safety and Health Administration (OSHA) has not set allowable air concentrations for any MuFA.

Potential occupational risks related to the use of UVR curable acrylate coatings (UV coatings) in wood surface coating industry have not been investigated. Our study was designed to determine (identify and document) potential worker exposure to acrylates and other chemical contaminants via inhalation and/or dermal routes and to evaluate work practices in Swedish UVR wood surface coating industry. In Sweden, there are 48 known facilities employing the application of UV coatings on wood products. The principal type of application method for transferring the formulation onto the product is a direct roller coater. Also, a manual application of filler is used. A total of 8 plant surveys were conducted. Our objectives for the surveys were: 1) identify, describe, and measure specific tasks where exposures may occur, and 2) describe current industrial hygiene and safety practices, including engineering controls, personal protective equipment used, and work practices. This study was also designed to provide a basis for determining and recommending modifications for manufacturing processes in order to better control chemical exposures to workers in the UVR wood surface coating industry through technical solutions and improved work practices. We report and discuss here the potential exposure to hazards at the plants surveyed, and our conclusion and recommendations to limit the potential for dermal and inhalation exposure to acrylates, in particular.

## MATERIALS AND METHODS

### Identification of Manufacturers and Site Selection Criteria

Swedish UVR wood surface coating processing plants were identified for the survey by contacting the Swedish UV coating formulators and requesting information on their customers. The plants identified in this manner were surveyed in two phases. The first phase included contact with all plants using UVR wood surface coating technique and a request to complete a questionnaire focusing on their work force and production. The first phase survey was used to obtain additional information on the manufacturing plants and allow identification and selection of representative plants for the second phase industrial hygiene survey. Site visits to 2 plants were conducted to obtain information necessary to design the second phase investigation procedures. Selection criteria were to include diverse types of plants and considered were: plant size and diversity, processed products, coating formulations used, UVR curing systems, geographical location, etc. Eight plants were considered representative of the Swedish UVR wood surface coating processing plants.

### Plant Surveys

Plant surveys were conducted in 8 UVR wood surface coating manufacturing plants during the period between October 1992 and April 1993. The surveys included preliminary contact with the plant management and employee representatives to explain the scope and objectives of the study and to collect data pertaining to the use of acrylates and other chemicals at that plant. Exposure assessment was determined during a 1-day industrial hygiene measuring

survey conducted at each 8 selected plants. Worker exposure measurements and observations were made during normal process operations and employee activities.

A detailed form, developed for collecting information during the survey, was completed. This information included the plant description (e.g., physical description of the plant, history of the plant, types of products produced), the process description (e.g., production process, type and use volume of UV coatings and other production related chemicals, potential emission sources and contact areas), the description of the work force (e.g., number of workers, work schedule and number of workers on each shift, job categories, worker activities, duration of exposure, work histories), and the description of industrial hygiene and safety measures employed.

Plant descriptions were useful in identifying the length of time the plants had been using UV coatings for wood surface coating. Process description data were gathered in order to determine conditions for the curing process and to assess operations or areas where the potential for inhalation and dermal exposure exists. Data on engineering controls and personal protective equipment were collected to determine the effectiveness of different types of controls and different types of personal protective equipment worn by operators in reducing the potential for worker exposure. The characterization of the work force allowed the determination of the number and types of operators, who are potentially, directly exposed, and the length of time they were potentially exposed.

#### Exposure Measurements and Analysis of the Samples

Sample collections were conducted during a complete 8 h work day on a UVR surface coating line (UV line) or on a station for the finishing of cured products; each worker was performing a set of defined work tasks during the entire work day. The workers wore the sampler affixed to the right side of their work apparel label, or if two samples were collected on the same worker, samplers were attached to opposite apparel labels.

#### Dust

Airborne dust samples were collected with a 37 mm glass fiber filter (Whatman, GF/F) or a 37 mm cellulose ester filter (Millipore, 0.8 µm) and a personal sampling pump (DuPont P 2500, E.I. du Pont de Nemours and Co. Inc., Wilmington, DE, USA) operating at a nominal flow rate of 2 l/min in the worker's breathing zone. All the samples were analyzed gravimetrically (Mettler ME22/36, Mettler Instrument AG, Zurich, Switzerland) and some samples were further analyzed for acrylates and photoinitiators by a gas chromatograph. (11)

Particle size selective sampling was conducted using a personal cascade impactor (Marple Personal Cascade Impactor, SE296, Graceby Andersen, Atlanta, GA, USA) and a personal sampling pump (DuPont P 2500) operating at a nominal flow rate of 2 l/min in the worker's breathing zone. The cascade impactor was a 6-stage device with cut points ranging from 0.5-10 µm aerodynamic diameter. Particles larger than the cut point for each stage were collected on a 34 mm Mylar filter and particles smaller than the cut point of the last stage were collected on a 34 mm PVC afterfilter (pore size 5 µm). The aerosol size

distribution was determined through gravimetric analysis (Cahn 28, Cahn Instruments Inc., Cerritos, CA, USA) for the sample collected on each filter.

At 3/8 plants stationary airborne dust samples were collected on a 25 mm Nuclepore® gold coated polycarbonate filter (pore size 0.4 µm) placed in a nonconductive holder using a personal sampling pump (DuPont P 2500) operating at a nominal flow rate of 2 l/min for electron microscopic analysis. The sampling time was ~20 min. The filters were gold coated and analyzed for particle size and shape using a JEOL scanning electron microscope (JSM-840 A, Japan) connected to an image analyzer (KONTRON, SEM-IPS, Germany). The smallest particle diameter (D<sub>min</sub>) was measured from 400 particles. The medium diameter and lower and upper quartiles were calculated (StatView, Abacus Concepts Inc., Berkeley, CA, USA).

Sanding dust was collected into a clean brown glass jar from a sanding table immediately after sanding of surface coated boards. UVR cured coating dust sample (100 mg) was extracted with 10 ml of either acetone, methylene chloride, or methanol (1:100), shaken in a SMI-automatic shaker for 4 h and analyzed within 24 h by a gas chromatograph. (11)

#### Vapors and Aerosols

Airborne vapors were collected with a SKC charcoal tube (Cat. No. 226-01, SKC Inc., Eighty Four, PA, USA) containing 150 mg charcoal (with a 50 mg back-up section) and a personal sampling pump (Sipin SP 1/SP 15, Anatole J. Sipin Co., New York, NY, USA) operating at a nominal flow rate of 50 or 200 ml/min in the worker's breathing zone. Charcoal tubes were

extracted with 2 ml of carbon disulfide for 4 h and analyzed by a gas chromatograph, similar to airborne total dust samples. (11)

An additional monitoring of worker exposure to airborne MuFA was conducted at 2 of the 8 plants surveyed, approximately 6 months later, because we observed trace levels of MuFA in the dust samples and an absence in the charcoal tube samples. The following method was developed and tested under laboratory conditions for airborne MuFA aerosols and vapors prior to field use. (11) Airborne acrylate vapors and aerosols were collected with an OSHA Versatile Sampler tube (OVS; Cat. No. 226-56, SKC Inc., Eighty Four, PA, USA) containing a glass fiber filter, tenax sorbent (140/70 mg), and polyurethane foam and a personal sampling pump (DuPont P 2500) operating at a nominal flow rate of 1 l/min in the worker's breathing zone. The OVS tubes were extracted with 5 ml of acetone for 4 h and analyzed by a gas chromatograph.

#### Surface Contamination

Surface wipe samples were collected using glass fiber filters (Whatman, GF/A, 37 mm) dampened with distilled water sufficient to wipe a determined surface area (100 cm<sup>2</sup> or 25 cm<sup>2</sup>) at different places where exposure may occur. The samples were extracted with 5 ml of methanol and analyzed after 4 h by a gas chromatograph. (11)

#### Ozone

Ozone concentration measurements were determined in the ventilation exhaust pipes of UV lamps during lamp ignition and the first hour of operation. In 4/8 plants ozone was measured in the work place ambient air. Measurements were conducted using a

portable ozone analyzer (Model 560, Serial 206275, Analytical Instrument Development Inc., Avondale, PA, USA) connected to a printer (Servogor S, BBC Goertz, Germany).

#### *UV Radiation*

Different measuring devices were used and tested in order to evaluate UVR both qualitatively and quantitatively at the UV lines. Both the spectral distribution and intensity of direct and reflected UVR to the work environment were measured. The spectral distribution was measured using a Photodyne 88 radiometer and an Yvon Jobin grating monochromator (Model H.20, I.S.A. Jobin-Yvon, France) or a MSS 2040 spectroradiometer (A.D.N. Elektronic GmbH, Germany). The UVR intensity measurements were determined with a Photodyne 88 radiometer equipped with an EE&G optical sphere (EE&G Gamma Scientific, San Diego, Ca, USA) and interference filters with different windows within specific UV wavelength regions or an IL 1400A radiometer with the UV A detector SL021#193 or the UV B detector SEL 240#3256 (International Light, Newburyport, MA, USA).

#### *Technical Measurements*

##### *UV Lamps*

The performance of ultraviolet lamps in the curing units was measured using UVIMAP® instrument (model UM365H-S, Electronic Instrumentation and Technology Inc., Sterling, VA, USA). The UVIMAP® is a battery powered instrument with a datalogger that measures the UVR energy and temperature in the actual curing environment in relation to time. This instrument is composed of a detector with an optical filter, which transmits UVR in the wavelength

range 320-390 nm and a thermocouple. It is calibrated against a standard mercury vapor lamp curing oven by Electronic Instrumentation and Technology Inc.

Measurements were determined during the normal manufacturing process. The sample collection rate used in the measurements was 40 samples per sec. After measurement (data collection) the UVIMAP® was connected to a thermal printer (DPU-411, Type II, Seiko Instruments Inc., Japan) or to a portable personal computer for print out or analysis of the collected data, respectively. The collected data included the total amount of UVR energy ( $\mu\text{J}/\text{cm}^2$ ) impinged on the device as well as the peak intensity (the highest UVR intensity,  $\text{W}/\text{cm}^2$ ) during the passage under the lamp unit. The average intensity of UVR energy ( $\text{W}/\text{cm}^2$ ) was calculated. Further processing of the collected data enabled us to calculate the transfer efficiency of the UVR energy of this wavelength region and analyze the proper mounting and maintenance of the UV units. The transfer efficiency of the UV lamp was determined as the total measured UVR energy in the given wavelength region (320-390 nm), which impinged the maximal curable surface area in percentage of the energy consumption of the UV lamp according to manufacturer's specifications.

##### *Environmental Conditions*

Temperature and relative humidity were measured several times during the work day using Assman-psiychrometer (Adolf Thies 56037, Göttingen-Geismar, Germany). Air velocity was measured using thermomemometer (Alnor Compuflow GGA-65P, Finland).

#### *Work Practices*

In order to record and analyze worker performance and work tasks during the work day, video filming was conducted during the 1-day industrial hygiene survey of the plants.

#### **RESULTS**

##### **Summary of the Swedish UVR Wood Surface Coating Manufacturing Industry**

In Sweden ~8,500 workers are employed in 48 plants producing UVR cured acrylic surface coated wood products. Approximately 350 employees work on UV lines and are potentially exposed to uncured UV coatings and UVR. Another 700 workers are involved in finishing processes on UVR cured products and have a potential for contact with cured or partially cured acrylic coatings. Furniture manufacturing represents the single largest component of the industry. Other manufactured products include parquet floors, doors, coating of building materials (e.g., strips, MDF boards, plywood), and packing materials.

Thirty-eight of the 48 plants provided detailed information on the work force and production. Production consisted of furniture (74%), parquet floor (16%), building material (5%), and door (3%) manufacturing. One of the plants manufactured both parquet floors and furniture. Altogether, ~6,700 workers were employed in these plants, of which, 5,400 were production workers, 270 were working in the UV lines, and 570 were working in the finishing process of UVR cured products. The plants operated 1 to 3 shifts (27 one shift, 9 two shifts, 2 three shifts), 5 days per week. The work shifts at plants were typically 8 h long. The number of UVR curing lacquer lines reported in these plants was 32 and

UVR curing filling lines 5. Also, UV surface coating and another type of surface coating combined in the same line (combination) were reported. Nine UV lines were connected together with acid curing surface coating process. 1 with water based coating, 2 with electron beam coating, and 1 with acid curing staining. UV lines have been in use for the surface coating of wood in Sweden for an average of 5 years (range 1-12 years). The total amount of UV coatings used varied between 550-267,000 kg per annum (avg. 24,800 kg per plant). The average amount of UV coatings used daily was calculated to vary between 3-1,100 kg (avg. 100 kg per plant).

##### **Summary of the Surveyed Work Places**

The eight plants surveyed represented 17% of the Swedish UVR wood surface coating manufacturing industry and employed 34% of the workers directly exposed to acrylates and UVR (Table 1). Five were furniture manufacturers and 3 were parquet floor manufacturers. The total number of workers per plant at the 8 facilities surveyed varied between 95-1,100 workers and the number of UV line workers per plant varied between 3-53. The figures for the total work force include routinely exposed employees, such as UV line workers as well as other plant employees (e.g., foremen, supervisors, engineers, and administrative personnel). Only a part of the total work force at each plant, however, was either directly or indirectly exposed to acrylates and UVR. A total of 120 workers (4% of production workers in the plants surveyed) were estimated to be directly exposed to acrylates and UVR. Approximately 260 workers (10% of production workers in the plants surveyed) had

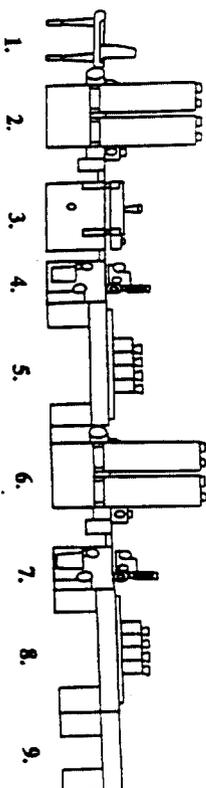
Table I. General information on the surveyed UV surface coating manufacturing plants.

Information / Plant ID	1	2	3	4	5	6	7	8	TOTAL
Production	parquet floors	furniture	parquet floors	furniture	furniture	furniture	parquet floors	furniture	
Nr employees	105	95	1100	950	100	105	553	221	3229
Nr production workers	90	90	1000	700	86	95	459	166	2686
Nr UV line workers	28	12	53	3	5	7	6	6	120
Nr post-UV workers	14	15	0	150	22	8	30	20	259
Previous UV workers	0	2	32	0	1	0	0	4	39
Hand sanding	7	0	0	0	0	0	0	1	8
Nr of UV lines	1	2	1	1	1	1	1	1	9
Nr of filling lines	3	0	2	0	0	0	0	0	5
Nr of combined lines	0	0	0	1 UV+solvent	1 UV+solvent	0	0	2 UV+solvent	4
Nr of years UV line(s) used	2	10	9	2	6	2	10	4	5.6**
Nr of days/wk UV line used	5	5	5	4	5	5	5	5	5**
Nr of work shifts	2	2	3	1 (2)*	1 (2)*	1	3	1	
Total amount used / year									
UV lacquer and paint	42,000 kg	17,500 kg	175,000 kg	7,080 kg	18,150 kg	2,300 kg	258,040 kg	6,300 kg	526,370 kg
UV filler	45,400 kg	0	92,000 kg	0	0	0	0	0	137,400 kg
Average amount used / day									
UV lacquer and paint	175 kg	73 kg	730 kg	30 kg	76 kg	10 kg	1,075 kg	26 kg	2,195 kg
UV filler	189 kg	0	380 kg	0	0	0	0	0	569 kg

\* only combi line used in 2 shifts

\*\* average

FIGURE 1. A schematic illustration of a wood surface coating line for UV coating: 1. Feeding table, 2. Sanding machine, 3. Cleaner with brushes and air suction, 4. Roller coater machine, 5. UV curing unit with 4 UV lamps, 6. Sanding machine, 7. Roller coater machine, 8. UV curing unit with 4 UV lamps, 9. Receiving table.



daily contact with UVR cured products (e.g., sanders, finishing workers, packers). Forty-four workers from these 8 plants were selected for personal air sampling monitoring (ranging from 3 to 9 monitored workers per plant). Thirty UV line workers (25% of UV line workers) and 14 workers (5%) who came into contact with UVR cured products were monitored. The number of UVR curing lacquer lines in these plants was 9, UVR curing filling lines 5, and acid curing surface coating combi lines 3. One of the UVR curing lacquer lines had an acid curing staining unit connected in the front of the line. These manufacturing plants have used UV lines for an average of 5.6 years (range 2-10 years) and are believed to be representative of the industry. However, the average amount of UV coatings used varied between 2,300-267,000 kg per annum (avg. 83,000 kg per plant), which was higher than estimated industry average. The average amount used daily was calculated to vary between 10-1,100 kg (avg. 350 kg per plant).

#### Manufacturing Processes

The process descriptions for the application of UVR wood surface coating included only flat object processing. We did not find any manufacturer processing three dimensional objects with this method. A basic type of UVR curing wood surface coating line is described in Figure 1. A typical manufacturing process began with feeding the boards onto the conveyor; conveyor speed varied between 9-25 m/min. On the conveyor line the wood board passed first through an enclosed sander with local exhaust ventilation and then was cleaned by roller brushes with local exhaust ventilation before coating application. The first application of the base coat was applied by a roller coater and cured by a UV unit, which usually consisted of medium pressure mercury arc lamps (rated at 80 W/cm each) and elliptical reflectors. Application of the UV coating was usually carried out in a series of coating applications, UVR curing, and sanding of the cured surface. In some

cases the first base coating was intentionally only partially cured. This "gelling" produced a tacky surface which was considered to be a better surface for application of the principal surface coatings (top coat). Application of the base and top coatings were usually carried out 2-3 times with UVR curing and sanding performed between applications. If an additional application of another type of coating (e.g., water based, acid curing, or stain) was performed together with a UV coating, these were applied on top of the UVR cured coating, except for stain, which was applied before the UVR cured coating.

The UVR surface coating process was comprised of roller coaters and/or filling by hand. Primarily, the facilities used one or two application methods and more than one type of UV coating. Application of the lacquer to the product surface was primarily carried out by a roller coater, onto which lacquer was fed by pumping from a 20-200 liter container located beside the roller coater. A  $\approx 0.01$  mm thick (5-25 g/m<sup>2</sup>) coating was applied by a roller to the substrate during each application. Excess coating from the roller coater was collected and returned to the process. In parquet floor manufacturing, fillers (UVR curable acrylic paste) were also used and were usually applied by hand or by pouring the filler manually onto the roller coater. In one plant the filler was applied by an automatic process but still required manual control and hand filling. If filling was required, it was always performed before coating the product with UV lacquer.

### UV Coatings and Other Chemicals

Three major UVR curable surface coating formulators who supply UV coatings to the wood surface coating industry operate

in Sweden. UV coatings are usually supplied in 20 l containers, but in some cases 200 l barrels or 1,000 l plastic containers are used. Altogether, 19 different UV coatings were observed to be in use at the 8 surveyed plants. According to the material safety data sheets (MSDS) on file the most frequently observed UV coatings were composed of acrylate prepolymers and TPGDA (CAS No. 42978-66-5), but HDDA (CAS No. 13048-33-4) was often used in these coatings with TPGDA. The acrylate prepolymers were most often stated generally as oligomers, but some MSDS stated the prepolymer used was epoxy acrylate (5/19 coating formulations). Polyester acrylate was stated to be the prepolymer in 2/19 coating formulations.

TPGDA and HDDA are the most frequently used MufA in radiation curable coatings. (12) The percentage of acrylate monomers, HDDA and TPGDA, in the UV coatings, were most often reported to be between 10-30% in the MSDS reviewed. Less often, glycerol propoxy triacrylate (GPTA, CAS No. 52408-84-1), trimethylol propane triacrylate (TMPTA, CAS No. 15625-89-5), and ethyldiglycol acrylate (EDGA, CAS No. 7328-17-8) were reported. In addition, zinc sulfide, titanium dioxide, talc, silica, methyl ethanol amine, hydroxy alkyl phenone, butyl acetate, and pigments were reported in the MSDS. Monofunctional acrylates, e.g., 2-ethylhexyl acrylate (2-HEA, CAS No. 103-11-7), may be used in small quantities as reactive diluents to lower the viscosity of some types of radiation curable coatings, (2) but none of these were reported in the MSDS reviewed. In three work places hardeners were mixed into UV coatings in order to ensure uniform and maximum curing of the coating. Methyl ketone peroxide, polymer-

ic isocyanate, monomeric hexamethylene diisocyanate, or polyfunctional aziridine were used as hardeners in these work places.

### Job Category Description

Distinct job categories of occupational groups were identified as to having potential for occupational exposure to acrylates and UVR. Job titles were assigned according to a defined work task. UV lacquer line workers were divided into three different categories: controller, feeder, and receiver. UV filling line workers (fillers) were considered as a separate occupational group. Workers handling UVR cured products were assigned as finishers and their tasks included assembling, hand sanding, packing, etc. Results reported on worker exposures were categorized according to three occupational groups (UV line worker, filler, and finisher). We did not observe any differences in potential worker expo-

sure levels for UV line workers performing different tasks, and therefore, these workers were considered as a single group in our analysis.

### Exposure Measurements

**Dust**  
Altogether, 40 total dust samples were collected in the breathing zone of workers in the UVR surface coating industry (Fig. 2). The average 8 h time-weighted (8 h TWA) total dust concentration in workers' breathing zones was  $0.4 \text{ mg/m}^3$  (range  $0.02-1.0 \text{ mg/m}^3$ ) in the surveyed work places. The average total dust concentration in the breathing zones of the workers who worked on the UV lacquer line was  $0.3 \text{ mg/m}^3$  (range  $0.02-0.7 \text{ mg/m}^3$ ),  $0.5 \text{ mg/m}^3$  (range  $0.2-1.0 \text{ mg/m}^3$ ) on the UV filling line, and  $0.4 \text{ mg/m}^3$  (range  $0.1-0.7 \text{ mg/m}^3$ ) on finishing.

Twenty-six total dust samples collected in 4 of the work places surveyed were ana-

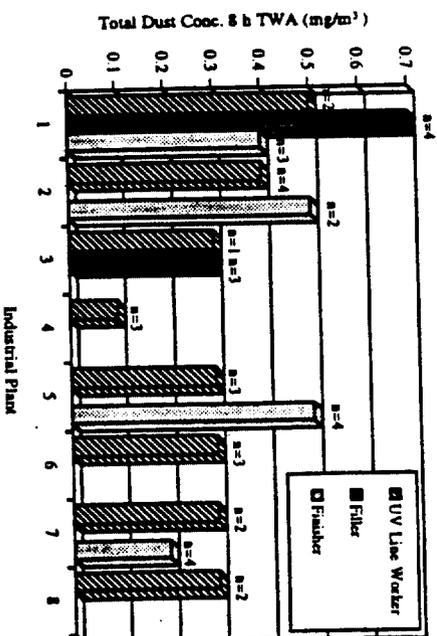


FIGURE 2. The average total dust concentrations (8 h TWA) in workers' breathing zones in the surveyed work places. The absence of data indicates the absence of a job category at a plant. For example, fillers are used only in parquet floor manufacturing. N equals the number of workers tested.

lyzed for acrylates and photoinitiators. Two samples contained trace levels of HDDA (detection level 2 µg/m<sup>3</sup>). A photoinitiator, BP, was detected in 9 samples. BP concentrations averaged 0.01 mg/m<sup>3</sup> (range 0.003-0.022 mg/m<sup>3</sup>).

Aerosol size distribution was obtained from breathing zone dust samples using personal cascade impactor. A total of 14 samples were collected in the breathing zones of the workers in the 7/8 surveyed work places (Table II). The average proportion of thoracic (< 10 µm) and respirable dust (< 5 µm) particle concentrations were 79% (range 62-99%) and 28% (range 12-62%), respectively, from the 8 h TWA total dust concentrations. The average particle size distribution on different stages of the cascade impactor from all collected samples is presented in Figure 3. No differences were observed between the different job categories (UV line workers, fillers, and finishers). No differences were observed when the total dust concentrations of the 13 parallel samples collected with the filter

method and the personal cascade impactor were compared. Both methods gave an average of 0.3 mg/m<sup>3</sup> as a 8 h TWA breathing zone concentration with a standard deviation of 0.2.

Stationary dust samples were collected in the vicinity of the UV line in 3 work places. A typical particle size distribution in the UVR curing environment is presented in Figure 4. Particle median diameters and lower and upper quartiles from the dust sample analyses are presented in Table III. Representative airborne particles shown in the scanning electron micrograph (Fig. 5.) of all the samples collected had a median diameter between 0.16-0.19 µm (avg. 0.17 µm) and were spherical in shape.

The use of hand sanding is limited to products identified as incompletely cured. We detected small amounts of TPGDA (0.018-0.04 w-%) in the only two dust samples, taken immediately after hand sanding of a UVR cured wood products, that were available to us during this survey.

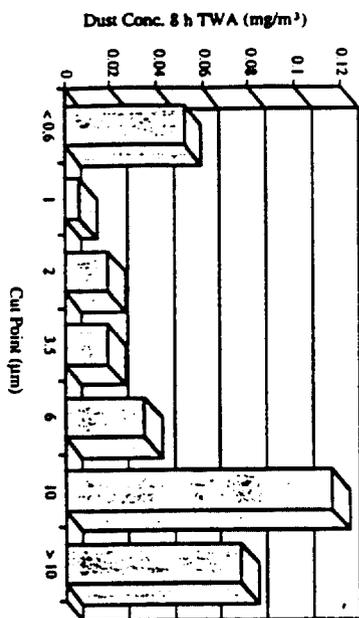


FIGURE 4. Particle size distribution in the measured breathing zone samples in the surveyed work places.

Table II. Average thoracic (<10 µm) and respirable (<5 µm) dust concentrations (mg/m<sup>3</sup>) in the workers' breathing zones in the surveyed UV surface coating manufacturing plants.

Worker / Plant ID	1	2	3	4	5	6	7	8
UV line worker: < 10 µm	0.386 (85%)*	0.384 (82%)*	0.065 (99%)*	0.112 (75%)*	0.257 (74%)*	0.197 (78%)*	ND	0.184 (68%)*
< 5 µm	0.154 (34%)*	0.141 (33%)*	0.040 (62%)*	0.028 (18%)*	0.124 (36%)*	0.072 (25%)*	ND	0.067 (26%)*
< 1 µm	0.077 (17%)*	0.106 (16%)*	0.037 (57%)*	0.021 (13%)*	0.028 (8%)*	0.071 (24%)*	ND	0.042 (16%)*
Filler:								
< 10 µm	0.483 (93%)*	NA	0.133 (84%)*	NA	NA	NA	NA	NA
< 5 µm	0.138 (27%)*	NA	0.018 (12%)*	NA	NA	NA	NA	NA
< 1 µm	0.063 (12%)*	NA	0.018 (12%)*	NA	NA	NA	NA	NA
Finisber:								
< 10 µm	ND	ND	NA	NA	0.326 (62%)*	NA	ND	NA
< 5 µm	ND	ND	NA	NA	0.091 (17%)*	NA	ND	NA
< 1 µm	ND	ND	NA	NA	0.044 (8%)*	NA	ND	NA

ND = not determined

NA = not determined due to absence of job category at that plant

\* = number in parenthesis indicates the per centage from the total dust concentration

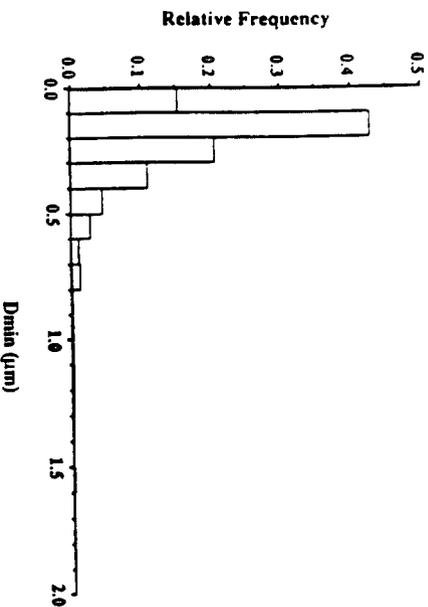


FIGURE 4. Particle size distribution ( $D_{min}$ ) of airborne particles collected on a polycarbonatic filter in the UV surface coating environment. A scanning electron microscopic analysis.

Table III. Result from the scanning electron microscopic measurements of the median particle diameter:  $D_{min}$  from 400 particles were measured.

Work place (Plant ID)	Median ( $\mu\text{m}$ )	Lower quartile ( $\mu\text{m}$ )	Upper quartile ( $\mu\text{m}$ )
UV filling line (1)	0.16	0.10	0.28
UV lacquer line (3)	0.16	0.13	0.25
UV lacquer line (7)	0.19	0.13	0.27

Also, photoinitiators, BP and benzil dimethylacetal were detected in these samples (0.4-1.6 w-% and 0.4-0.5 w-%, respectively).

#### Vapors and Aerosols

Eight charcoal tube samples were collected from the breathing zones of UV line workers in 4 different work places. Also, one stationary sample was collected directly above a surface of liquid UV lacquer in one work place. No acrylates, either mono- or multifunctional (e.g., HDDA, TPGDA), were detected in these samples using a standard method. (13) Small concentrations

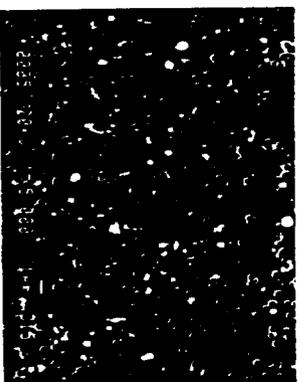


FIGURE 5. Scanning electron micrograph of the particles typical to the UV surface coating environment. White bar at the right bottom corner represents 1  $\mu\text{m}$ .

of different organic solvents were detected: toluene 0.64  $\text{mg}/\text{m}^3$  8 h TWA (0.03-2.03  $\text{mg}/\text{m}^3$ ), xylene 0.78  $\text{mg}/\text{m}^3$  8 h TWA (0.11-2.79  $\text{mg}/\text{m}^3$ ), butyl acetate 3.44  $\text{mg}/\text{m}^3$  8 h TWA (0.08-11.68  $\text{mg}/\text{m}^3$ ), and terpenes 2.68  $\text{mg}/\text{m}^3$  8 h TWA (0.02-6.34  $\text{mg}/\text{m}^3$ ). The average additive effect for solvent exposure was 0.03. The same organic solvents were detected in the stationary sample.

After a standard method for monofunctional acrylates failed to detect MuFA vapors or aerosols, 11 OVS tube samples were collected at a later date at 2/8 plants surveyed. Four samples were collected from the breathing zones of hand fillers, 5 from UV lacquer line workers, 1 from a hand sander, and 1 stationary sample in the vicinity of a roller coater application before a UV curing unit. Traces of TPGDA and HDDA were identified in all of these samples by peaks on the gas chromatography spectra coincident with spiked standards. However, the overall sensitivity of the sampling and analyzing methods precluded the quantification of some of the samples. Thus, the worker exposures at the UV lacquer lines were generally below the detection limit of 2  $\mu\text{g}/\text{m}^3$ , except for one worker, for whom HDDA was detected (15  $\mu\text{g}/\text{m}^3$ ). Also, TPGDA was detected in this sample (5  $\mu\text{g}/\text{m}^3$ ). The MSDS for the used formulation indicated that the UV lacquer contained ~3 times more HDDA than TPGDA. The greatest TPGDA concentrations were observed in the breathing zones of the hand fillers (average 11  $\mu\text{g}/\text{m}^3$ , range 8-14  $\mu\text{g}/\text{m}^3$ ). BP was identified in all of the samples (range <1-30  $\mu\text{g}/\text{m}^3$ ; LOD 1  $\mu\text{g}/\text{m}^3$ ). The highest BP concentrations (average 26  $\mu\text{g}/\text{m}^3$ ) were measured in the hand fillers' breathing zones. In the hand

sander's breathing zone TPGDA, HDDA, and BP were below the LOD.

#### Surface Contamination

Altogether, 27 surface wipe samples were collected in 4 of the surveyed work places. Only one sample contained TPGDA. In another two samples, taken from the top of the UV unit on the UV line, only traces of TPGDA were detected (<0.02  $\text{mg}/\text{sample}$ ). Photoinitiators were also found in these two samples; BP <0.005  $\text{mg}/\text{sample}$  and benzil dimethylacetal 0.009-0.015  $\text{mg}/\text{sample}$  (surface area 100  $\text{cm}^2$ ).

#### Ozone

Ozone concentrations were measured in 5/8 plants surveyed. In four plants the measurements were made in the ventilation exhaust pipes of UV lamp units during lamp ignition and the first hour of operation. Ozone concentrations varied in the ventilation exhaust pipes of UV lamp units from 0.05 to 3.0 ppm at lamp ignition, but ozone levels rapidly stabilized (0.01-0.8 ppm). Ozone was also measured in the work place ambient air in 4/8 plants (<50.01 ppm).

#### UV Radiation

The UV lamps observed in use were usually medium pressure mercury arc lamps, ranging in wavelength between 200-400 nm, and rated at 80 or 100 W/cm each. Gallium lamps, which also emit a large proportion of visual light for curing purposes in addition to the UVR (peak 400-450 nm), were used on one application line where white pigmented UV lacquer was used for surface coating. The UV lamps were enclosed in ventilated metal cases and the radiation was directed by elliptical reflectors (primary radiation) towards the

conveyor carrying surface coated wood material. Primarily, radiation to the work environment occurred through the entrance and exit openings for the conveyor in the UV units, but could also be observed through slots in the UV unit or through unshielded openings underneath the conveyor line and UV unit. The conveyor entrance and exit openings are equipped with outward built shielding funnels or hanging draperies. UVR from the openings is reflected primary radiation from the enclosure, from the conveyor line, and/or from the passing wood material (secondary radiation). Radiation which exits through openings and slots is reflected towards different machine parts and surfaces in the work environment (tertiary radiation).

The proportion of the UVR between different wavelength ranges at a distance of 0.5 m from different UV lamps in different UV regions, ranged for UV<sub>A</sub> from 41 to 65%, for UV<sub>B</sub> from 29 to 38%, and for UV<sub>C</sub> from 7 to 22%. Unshielded radiation from the UV lamps occasionally reached

the environment with intensities of 10-20 mW/cm<sup>2</sup>. UVR reflected from the entrance and exit openings at the line were usually in the range of 0.05-0.2 mW/cm<sup>2</sup>. From the transport line, 1-10% of this radiation was reflected to the environment.

#### Technical Measurements

##### UV Lamps

The UV lamps and housing units most commonly used, including reflectors and ancillary equipment (e.g., shutters, shielding), were supplied by Superfici, Monza, Italy. UV lamps were also supplied by Efsen Engineering, Vedbæk, Denmark and IST Stralentechnic GmbH, Wolfslughen, Germany. The UV units inspected varied in number between 1-6 per UV line and usually had between 1-5 lamps per unit. The greatest number of UV lamps (12) per UV unit was installed in a UV filling line. A common practice was to use fewer UV lamps in the curing process than the total number of lamps installed per unit (e.g., 2/4

total). The reflectors used in the UV units were constructed from aluminum and were elliptical in shape.

The performance of the UV units measured with the UVIMAP® showed that approximately half of the units needed some form of alteration (for example mounting, cleaning, or lamp change). An average of 58% of the UV lamp units were determined to be correctly focused at the time of the survey. When the uniformity of UVR intensity was measured in the different parts of the UV lamps (e.g., middle and sides) we observed that the intensity varied in different parts of the UV lamps (Fig. 6). Only in 1/9 of the UV lines was the radiation from all the measured UV lamps (4) correctly focused on the passing substrate. The temperature in the UV units varied between 40-100°C. Generally, UVR curing of wood surface coating is recommended to be carried out at the air temperature >50°C (14)

The observed operation times of the UV lamps in the surveyed UV lines varied greatly (range 7-7222 h). The efficiency also varied greatly, but we did not observe decreased UV lamp transfer efficiency with increased operation time (Fig. 7). A UV lamp operating over 7000 h was as effective as a new lamp. Also, a new lamp was found to be ineffective after only a few hours of use.

#### Environmental Conditions

Average air temperature in the surveyed plants varied from 19 to 23°C (avg. 22°C). Relative humidity varied from 28 to 57% (avg. 37%) and air velocity varied from 0.1 to 2.3 m/s (median 0.2 m/s).

#### Work Practices

Duration of potential for direct exposure to uncured UV coatings (acrylates) varied in accord with occupational group. We used individual questionnaires prepared for medical examinations as an aid for inter-

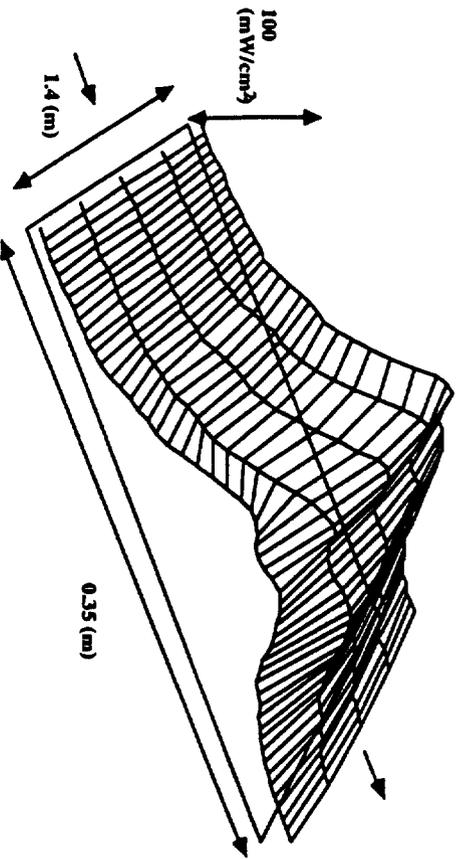


FIGURE 6. An example of UVR energy variation in different parts of the UV lamp.

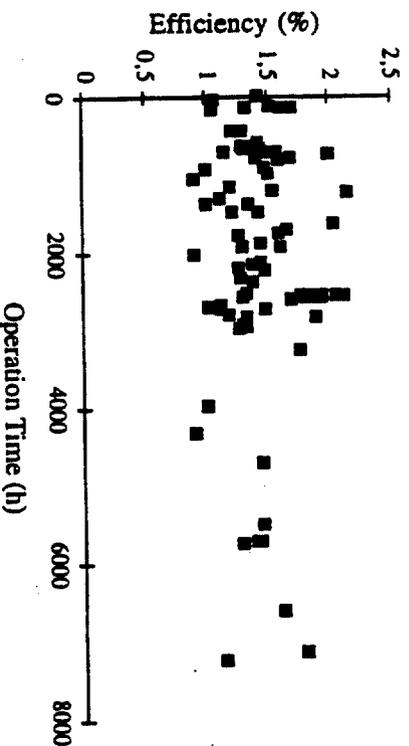


FIGURE 7. The variation of UV lamp transfer efficiency as a function of operation time of the UV lamp.

prising worker's occupational data. Thirty-seven out of 44 (84%) completed questionnaires for surveyed workers were available and 25 of the workers (68%) were surface coating workers at the UV line. Six of twenty five of the surface coating workers (24%) also worked part time (~40% of the work day) on a acid curing surface coating line. The workers average duration of employment in the wood surface coating industry was 11 years (range 0.5-45 years). The average time of employment on the UV line was considerably less, an average of 3 years (range 0.5-10 years).

Analysis of work practices performed during the work day was carried out by using a video recording made during the 1-day industrial hygiene survey. Preparations for the UV line operation required 2-5% of the work day, including the start and warm up procedures of the UV lamps to reach the optimal curing conditions, which took between 10-20 min. A common practice observed for the UV line (3-4 workers) required one individual to feed the products into the process, 1-2 working at the end of the line receiving the cured products, and 1 to control the curing process along the UV line. The feeders spent ~70% of the work time feeding products into the process. The receivers spent ~65% by receiving and stacking the cured products at the end of the UV line. The receivers checked cured surface by feeling the cured surface with the naked palm of their hands. The UV line controllers spent ~80% of their work time controlling the curing conditions on the UV line and were most likely to have direct skin contact with uncured UV coatings (~5% of the work time). The UV line controllers also cleaned the sanding machines on the UV line (1% of the work time). The hand fillers were most frequently in direct

skin contact with uncured UV coatings (~60% of the work time) of all the UV line workers.

Most of the plants surveyed provided some type of protective work clothing for their employees; 86% of the workers reported that they wore the company's work clothes (all or partly) during working hours. Three of the eight plants provided workers special work clothes either overalls or pants and jackets and 3 others provided only pants, while 2 plants did not provide any work clothes. The plants that provided the whole set of work clothes also provided industrial washing. In these plants separate closets were maintained for work and spare time clothes. One plant allowed the workers to choose between industrial washing or self-washing. None of the other plants surveyed provided industrial washing for their employees' work clothes. Despite the provision of work clothes and changing facilities in most of the work places, most of the workers were observed to arrive and leave work wearing their work clothes.

Operations during which gloves were worn by workers were feeding and receiving of the products on the UV line, direct contact with UV coating, and finishing work. The gloves most commonly used by UV line workers and finishers were cloth-leather gloves. These were worn in 7/8 of the surveyed work places. Latex rubber gloves were most commonly used (7/8 work places) when contact with uncured UV coating occurred. Also neoprene and/or disposable vinyl gloves were available in some work places. In all of the work places the gloves (latex rubber or neoprene), which were used when direct contact with UV coating was considered to occur, were used by several of the line workers. Gloves were located beside the UV line when not

used, and discarded and changed only when considered necessary; in other words, when they looked dirty enough. Ordinary hand cream was available for workers use in every work place.

We observed at one plant that protective gloves were not worn during cleaning of the roller coaters; workers carried out this work task bare handed, when skin contact with uncured UV coating was unavoidable, even though gloves were located beside the UV line and were easily accessible to workers.

Workers wore their own shoes at the work place. None of the work places offered safety shoes although the possibility of UV coating spills is frequent, especially when workers are carrying open coating containers and emptying them into the roller coater container. Only at one work place were the UV line controller and all others who entered the processing room required to wear disposable shoe covers.

Safety goggles or face shields were provided in 3/8 of the work places. Three of the eight plants provided respirators for the use of their employees, but none of the workers surveyed had worn one during UVR wood surface coating. Half masks with dust and chemical cartridges, or airline respirators were provided in 2/8 work places. Disposable dust masks were provided at one work place. The respirators were used occasionally during hand sanding of the UV cured products. However, during the survey we did not observe any worker to wear a respirator during any of the work tasks, not even during the cleaning procedure of the sanding machines with compressed air on the UV line.

#### Protective Measures at the UV Lines

The roller coater units were not enclosed, but in one work place local exhausts were installed onto the roller coaters. In another work place the local exhausts were located on the ceiling, ~5 meters above the roller coaters and, therefore, their efficiency for removing potential aerosols and vapors was considered null. In one work place the roller coater units were separated with gliding plexiglas shields. At another work place were local exhausts installed above the open UV coating containers located beside the roller coater.

The UV units and sanding units were always enclosed and exhausted to outside the plant. The primary purpose of local exhaust ventilation on UV units was to control ozone generated by the UV lamps and to ventilate UV lamps in order to prohibit overheating, and not due to acrylate exposure.

Most of the work places had placed cardboard underneath the UV coating containers, roller coaters, and UV units. This practice eased clean up procedures and protected floor and spreading of possible splashes. Special splash guards (metal plates) were used at some plants.

For the handling of used UV coating containers, 2/8 work places had a special curing closet equipped with a 400 W UV lamp in order to cure residue of coating in empty containers before disposing the containers to a communal land field. At another work place, containers were placed underneath the UV unit on the UV line. All other work places disposed UV coating containers directly without curing the coating residue to a land field. UV coating is not considered a hazardous waste and therefore used containers are discarded directly.

Half of the work places provided washing facilities beside the UV line. Eye shower stations were provided beside the UV line in 7/8 surveyed plants but only 1 work place had emergency shower on the UV line. Shower and changing facilities were located in the locker rooms in all of the surveyed plants. Separate lunch room facilities were provided at all plants. The results indicated that 78% of workers were satisfied with cleaning operations at the work place and considered their work environment to be satisfactory.

We were also interested in the type and duration of education the workers had received prior to starting work on a UV line. Only 35% of all the workers reported that they had received some form of information and education that considered handling, safety precautions, and risks of UV coatings. Forty-eight percent of workers on a UV line reported that they had received some form of information and education. Information and education was given primarily by the UV coating manufacturers and usually consisted of 1-4 h course. In 3/8 work places the company health service units also were engaged in providing information and education. One work place did not provide any form of information and/or education to their employees.

## DISCUSSION

The use of UV coatings in surface coating of wood products has increased, at least in part, due to the necessary reduction of solvents in the workplace and to the environment because of health concerns as well as desired economic benefits. Evaluation of new materials and/or processes for potential health effects before inclusion or substitution in the work place is difficult because of the unforeseen ways in which

use and work practices evolve. However, it is highly desirable to prospectively identify and evaluate potential health risks to new materials and work practices in the UVR wood surface coating industry as soon as possible in order to prevent or minimize unforeseen health risks that work to the disadvantage of both the worker and the industry. We have conducted an industrial hygiene survey on potential health risks and work place practices in cooperation with the Swedish industries to make such a prospective evaluation in the absence of any published known or suspected problems.

Overall, the results indicate that workers in the UVR wood surface coating industry in Sweden are exposed to small amounts of potentially harmful acrylate containing aerosols, vapors, and/or dusts and/or organic solvents. The most significant findings are: (1) an estimated 350/8,500 workers are potentially directly exposed to acrylates (uncured coatings, aerosols, and/or dusts) and UVR (2) respirable dust particles (28% of the total dust concentration was  $<5 \mu\text{m}$  dia) are present even though overall dust levels are low ( $\approx 0.4 \text{ mg/m}^3$ ), (3) aerosols and dusts containing sensitizing chemicals (TPGDA, HDDA, BP, etc.) were determined to be present in the work places surveyed, (4) protection devices for respirable dust were not consistently used (2/8 plants surveyed provided protective devices; however no device was ever observed in use), (5) potential for UV A, UV B, and UVC exposure to unprotected skin at biologically effective dose levels, (6) potential for direct skin exposure to acrylate contaminated surfaces and development of contact dermatitis, (7) lack and inconsistency of education programs for workers, and (8) indiscriminate disposal of acrylate

contaminated containers to the environment.

### Potential Risk for Exposure

During the past 10 years, the use of UVR curing of acrylate coatings in surface coating of wood has gradually increased and 48 plants now produce surface coated wood products using this technology in Sweden. Potential for exposure to uncured UV coating, acrylate containing dusts and/or aerosols and vapors may be related to occupation and primary product manufactured. In this survey, for example, the hand fillers had the longest contact (60% of work activity) with uncured UV coatings during the work day and their exposure to the TPGDA was greater than the UV line workers. With the development of an accurate method of measurement of MufFA this limited data set must be expanded and occupational categories reevaluated for potential exposure to MufFA. The overall total dust concentrations were well below Swedish 8 h occupational exposure limit value (OEL,  $3 \text{ mg/m}^3$ ) for nuisance dust. On average, worker exposure to different organic solvents observed in this survey (toluene, butyl acetate, xylene, and terpenes) were 100 fold or greater below the Swedish OELs.

### Particle Size Distribution

Particle size distribution measurements with a personal cascade impactor in the workers' breathing zones showed that a significant portion of the measured total dust exposure ( $0.4 \text{ mg/m}^3$ ) included particles in the thoracic and respirable region (79% and 28%, respectively). The electron microscopic analysis supports this observation. Mean diameter of analyzed particles averaged  $0.17 \mu\text{m}$  and the particles were

spherical in shape. The shape of the particles suggests the possibility of evaporation and condensation occurring from the UV coating during roller coating operation and from open coating containers feeding the roller coaters.

### Monitoring of Multifunctional Acrylates

The established methods for monitoring monofunctional acrylates (e.g., ethyl acrylate, methyl acrylate)(13, 15) were not useful for evaluation of worker exposure to MufFA that were observed to be used in the UV coatings. Inhalation exposure to this category of chemicals has not been a concern because of their low vapor pressure at room temperature. Because of the potential for worker exposure to acrylate aerosols and vapors we developed a monitoring method, (11) which was tested at 2/8 surveyed plants during 1 work day. The results show that this method is useful for analysis of aerosols and vapors containing MufFA.

### Surface Contamination

Possibility of direct skin contact and exposure to acrylates from contaminated work surfaces was evaluated in 3 work places. Only in 3/27 samples was contamination observed. These results indicate the possibility that contamination of skin and work clothes to acrylates from contaminated work place surfaces may exist. Good housekeeping, industrial hygiene, and work practices are considered very important in order to guard against acrylate contamination.

### Ozone

Ozone concentrations in the work environment were determined to be  $< 50.01 \text{ ppm}$ .

Table IV. The Intensity and erythema inducing properties of ultraviolet radiation in the UVR curing surface coating environment.

Radiation type	Maximal intensity (mW/cm <sup>2</sup> )	Erythema threshold time
Under the UV curing unit at the substrate passage level	100	< 1 sec
Direct radiation through bolts and slots	10	5-10 sec
Reflected radiation through entrance and exit slots	0.05-0.2	5-10 min
Secondary reflection to the work area	0.002	3-5 h

The local exhaust channels from the UV lamps were effective in removing ozone generated by the UV lamps. The risk for occupational exposure to ozone is considered minimal with use of local exhaust.

**Ultraviolet Radiation**

UVR from primary reflection through openings or slots from the UV unit at the distance of 0.5 m was estimated to range from 0.05-0.2 mW/cm<sup>2</sup>. Exposure to this type of radiation may result in erythema to pale skin within 3 min and at a distance of 1-2 m erythema may result in 10-20 min (Table IV). Exposure to unshielded lamps can result in skin symptoms within seconds. Long wave UVR (UVA) is slightly irritating while short wave UVR, UVB and especially UVC, is strongly irritating to skin and eyes. The sun gives a UVR intensity of 2-3 mW/cm<sup>2</sup> on the earth surface in a clear summer day in Sweden. Solar radiation contains 5-10% UVB. The UVR emitted from the UV lamps used in the surface coating of wood contains 30-40% UVB and 5-20% UVC. Therefore, the UVR from UV lamps used for curing wood surface coatings is considered to be 5-10 times more irritating for both skin and eyes than the radiation from the sun and that a

significant risk for UV damage may result if exposed to UVR from UV lamps.

**UV Lamps**

The performance of the UV lines in the surveyed plants was inconsistent and were not optimized for peak efficiency. Manufacturers of UV coatings did not provide published information on optimal curing conditions, e.g., required UVR (wave-length, energy, and time) for a given thickness of coating for products. Minimum UVR dosage (mJ/cm<sup>2</sup>) required for curing is dependent upon three major parameters: (1) line speed, (2) spectral distribution of incident radiation, and (3) UVR intensity (mW/cm<sup>2</sup>). The rate of curing is proportional to UVR intensity. Curing conditions are controlled by the geometry of the UV unit, including the reflector, the focusing of the UV lamp in the reflector, and the distance of the substrate from the lamp. UVR emitted from the lamp is focused by the reflector on a focal point at a fixed distance from the lamp. A substrate that passes under the UV unit at this distance receives the highest UVR intensity (peak intensity) if the UV unit is focused. Curing rate is dependent on the peak value of UVR intensity as well as the total UVR energy.

Intensity of the UVR on the coating significantly affects curing rate and the quality of the product. Therefore, correct mounting, focus, and cleanliness of the UV lamp and reflector is very important to achieving complete curing of the UV coating and economy (e.g., reduced power consumption and increased production).

Monitoring degradation of lamp output is another important parameter for directly controlling curing efficiency and, indirectly, controlling UVR and acrylate exposure. UV lamp output declines during its lifetime and may eventually result in poor curing performance. UV lamp manufacturers often state a guaranteed lamp life of 1000 h. However, the properties of UV lamps even from the same manufacturer may vary greatly and, therefore, the guaranteed lamp life cannot be considered as a guide to actual lamp replacement. In order to utilize the maximum effective lamp life and to prevent improper curing of UV coating and possible worker exposure to acrylates control and monitoring of UV lamps is necessary.

**Personal Protective Equipment**

General knowledge concerning health effects and risks associated with handling of uncured acrylate coatings was minimal. Basic worker education and instructions for working with UV coatings was insufficient. The use of personal protective equipment (e.g., gloves, respirators, safety shoes, aprons, disposable overalls) was not consistent and at the work places surveyed.

Existence of personal protective equipment did not ensure proper use. In order to minimize dermal and inhalation exposures to acrylates as well as other toxic and hazardous chemicals handled on-site, proper training and information on possible health

effects and risks are necessary. None of the gloves worn by the workers at any work place surveyed were sufficient to protect the workers from dermal exposure to acrylates. Acrylates penetrate worn rubber and vinyl gloves in a very short period. Only nitrile gloves are sufficient for protection from UVR curable acrylate formulations in excess of 8 h. (16, 17) PVP, viton-butyl, and surgical rubber gloves were penetrated after 30, 20, or 1 min, respectively. (18, 19) Nitrile gloves provide the protection required for workers handling UV coatings during a normal 8 h work day. Permeation of a nitrile glove occurs in less than 48 h, whether the glove is being worn or whether it is lying on the shelf after a short exposure time. Therefore, gloves, once exposed, are not reusable and must be discarded at the end of the work shift. Premium latex gloves can be used for short periods when the risk of direct contact with uncured UV coating is minimal and discarded immediately after use. The time factors cited above are based on the initial contact of the glove with the coating material. If other chemicals are used that are not compatible with glove materials (e.g., nitrile gloves and organic solvents), the permeability characteristics of the coating material may change as well as the protection factor of the gloves.

Actual respirator use was not observed during the survey at any workplace. On average, total dust concentrations were low (0.4 mg/m<sup>3</sup>), however, ~30% of the particles were respirable. Certain job activities (e.g., cleaning of sanding machines with pressurized air, or hand sanding) may dramatically increase dust concentrations. Therefore, during these work activities, respirators must be used. Airline respirators offer the most protection from respirable

dust particles. Half mask respirators with dust cartridges are acceptable, but, in general, do not filter smallest respirable particles and require regular replacement of cartridges depending upon dust concentration. When not properly used and taken care, respirators can create a false sense of protection.

#### Disposal of Used UV Coating Containers

Very little is known about the effects of uncured UV coating in the environment. Containers should not be discarded without first curing the UV coating residues. The best method for handling containers with acrylates, that we observed, was curing the residue in a special closet equipped with a UV lamp powerful enough to cure the coating residue lining the container. Another practice observed was placing the containers underneath the UV unit on the UV line for curing. This practice is questionable because the efficiency of the UVR weakens as a function of distance and satisfactory curing of the coating residue may not be obtained.

#### CONCLUSIONS

The use of UVR for curing of acrylate coatings in wood surface coating industry involves a potential exposure risk for workers involved. The significance of the health effects of the exposures described in our study of 8 independent manufacturers using this process is unknown. Overall, uncured or partially cured UV coatings as a group must be considered as potentially harmful to eyes and skin. Exposure to acrylate aerosols and vapors, cured or partially cured coating dusts, and UVR are factors involving special risk that must be considered more extensively and in more detail. Our initial assessment indicates that

work practices (e.g., routines for changing and washing of clothes, lack of use of personal protective equipment) portends a significant risk for skin exposure. We have found that work place air contains acrylates as a vapor or as aerosols alone or as a part of sanding dust created in the UVR curing process and in the process of finishing. Poorly shielded UV units at some installations also resulted in a potentially increased exposure to UVR. Ozone is produced during the ignition and use of a UV lamp, but concentration levels were not observed to exceed the general background concentration of ozone in the work place air.

Our knowledge of potential health risks associated to specific exposures observed in this type of industrial process is limited. There is no basis at present for estimating risk to workers involved. The use of UVR to cure surface coated products is increasing. Future research on exposure assessment, risk assessment, and managing risk by process, emission, and exposure control technology is required and urgent. We believe that it is advisable to treat the UV coatings with great care and consideration. Recommendations given to users by the coating manufacturers in Sweden indicate the necessity to use special protective gloves, disposable overalls, separate areas for work cloths and private clothes, etc. Control of the UVR curing process in respect to complete curing and shielding of UV units are also important measures to reduce potential risk of exposure. Finally, regular industrial hygiene assessment focusing on aerosol and vapor exposure to airways and skin, UVR and efficiency of curing, and monitoring of work practices to reduce potential to exposure are required.

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# A Method for Monitoring Worker Exposure to Airborne Multifunctional Acrylates

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Increased use of multifunctional acrylates in surface coatings cured with ultraviolet radiation may result in increased worker exposure to airborne contaminants, but present methods are inadequate to assess the exposure levels. We have developed a method for sampling and analyzing aerosols and vapors containing multifunctional acrylates and benzophenone in order to investigate worker exposure levels. Aerosols were sorbed on glass fiber filter and vapors on Tenax sorbent, desorbed with acetone, and analyzed by a gas chromatograph using a flame ionization detector and a 5% phenyl methyl silicone capillary column. The method was applied to the multifunctional acrylates 1,6-hexanediol diacrylate and tripropylene glycol diacrylate, as well as to the photoinitiator benzophenone. The parameters investigated were recovery (85%), precision (<5%), storage stability (20 days at 4°C), and detection limits. Concentrations as low as 2 µg/m<sup>3</sup> for multifunctional acrylates and 1 µg/m<sup>3</sup> for benzophenone can be measured. The concentrations of multifunctional acrylates ranged from <2 µg/m<sup>3</sup> to 15 µg/m<sup>3</sup> and those of benzophenone from <1 µg/m<sup>3</sup> to 30 µg/m<sup>3</sup> in the workers' breathing zones. Our results indicate that it is possible to measure worker exposure to low concentra-

tions of multifunctional acrylates with this method, which is based on a two-phase sampling system and gas chromatographic analysis.

## INTRODUCTION

Occupational exposure to potential hazardous agents has increased in association with the manufacturing and use of acrylated surface coatings cured with ultraviolet radiation (UV). This increase is mainly due to the government mandated requirements to reduce solvent emissions from solvent based curing systems for coated surfaces in European countries. The acrylic resin systems used in the industry are normally composed of the following three basic components: an acrylated prepolymer (urethane acrylate, polyester acrylate, etc.), a multifunctional acrylate monomer (e.g., tripropylene glycol diacrylate, TPGDA; 1,6-hexanediol diacrylate, HDDA), and a photoinitiator system (e.g., benzophenone, BP). (1) Resins can also contain other additives (e.g., fillers, plasticizers, stabilizers, catalysts, pigments).

Potential health effects of exposure to acrylate esters include skin irritation and allergic contact dermatitis. (2-8) Many inhibitors are also considered to be possible sensitizers. (9, 10) In general, our knowledge of the potential health effects of acry-

lates used in the UV curing of acrylate coatings for surface coatings is poor. With increased use and the potential for increased exposure, sensitive methods for monitoring worker exposure to acrylates is particularly important.

Few methods have been published on the sampling and analysis of multifunctional acrylates (MuFA).<sup>(11, 12)</sup> There are significant technical problems associated with these procedures.<sup>(12)</sup> The recommended workplace environmental exposure level (WEEL) for HDDA and other MuFA is 1 mg/m<sup>3</sup>. In the United States the Occupational Safety and Health Administration (OSHA) has not set allowable air concentrations for any MuFA. We were not able to obtain a complete sampling method for any MuFA, including HDDA and TPGDA.

In an earlier field study, we attempted to sample and analyze for MuFA using a standard method (charcoal tube) for monofunctional acrylates<sup>(13, 14)</sup> in the UV cured surface coating industry and were unsuccessful.<sup>(15)</sup> The analysis of sanding dust and total dust measurements indicated the presence of trace levels of MuFA, but we were uncertain of these results. In response, we reevaluated our approach to sampling methods and also to the determination of exposure levels. We chose to test a two-phase sampling system, which we believed could be suitable for collecting both aerosols and vapors of MuFA. In this report we present a sampling and analytical method for monitoring worker exposure to MuFA (aerosols or vapors), as tested for HDDA, TPGDA, and the photoinitiator BP.

## MATERIALS AND METHODS

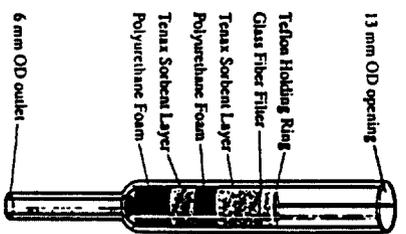
### Sampling Techniques

#### *Collection of Aerosols and Vapors Under Experimental Condition*

Stationary aerosol samples were collected during the roller coating application of UV curable lacquers under laboratory conditions. Aerosol samples were collected on a 37 mm glass fiber filter (Whatman, GFF) or a 37 mm cellulose ester filter (Millipore, 0.8 µm) using a Reciprotor pump operating at a nominal flow rate of 20 U/min. Stationary aerosol samples were also collected for electron microscopic analysis with a 25 mm Nuclepore® gold coated polycarbonate filter (0.4 µm) placed in a nonconductive holder and a personal sampling pump (DuPont P 2500, E. I. du Pont de Nemours and Co. Inc., Wilmington, DE, USA) operating at a nominal flow rate of 2 U/min. The sampling time was ~20 min. The collection devices were placed directly in front of the roller coating machine, into which commercial UV curable acrylate lacquer (Becker Acroma AB, Sweden, containing acrylate prepolymer 50-60%, TPGDA 10-20%, HDDA 1-5%, and BP 1-5%) was continuously fed.

Airborne vapors were collected with both an OSHA Versatile Sampler tube (OVS; Cat. No. 226-56, SKC Inc., Eighty Four, PA, USA) containing a glass fiber filter, tenax sorbent (14070 mg), and polyurethane foam or a SKC Polyurethane Sampler tube (PUF; Cat. No. 226-92, SKC Inc., Eighty Four, PA, USA) containing purified polyurethane (Fig. 1) and a personal sampling pump (DuPont P 2500) operating at a nominal flow rate of 1 U/min or 3 U/min, respectively. The sampling tubes were placed 10 cm above the roller coating machine, and the sampling was carried out simultaneously with the aerosol sampling.

OSHA Versatile Sampler  
SKC Cat. No. 226-56



PUF Sampler Tube  
SKC Cat. No. 226-92

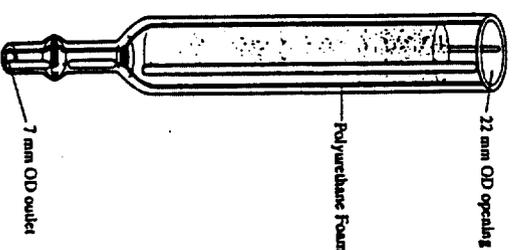


FIGURE 1. Illustration of the OSHA Versatile Sampler (OVS) tube and the PUF Sampler tube.

Air (1 U/min) was drawn through the sampler using a personal sampling pump (SKC Inc., Eighty Four, PA, USA) for 280 min. Sample collection was conducted under laboratory conditions: 20°C room temperature and 40% relative humidity.

#### *Collection of Aerosols and Vapors with an OVS Tube above Acrylate Lacquer Surface*

The evaporation of MuFA and photoinitiators from a commercial UV curable acrylate lacquer, containing polyester acrylate >30%, TPGDA >30%, and hydroxyalkylphenone 1-5% according to the material safety data sheet, was tested using an OVS tube as shown in Figure 2.

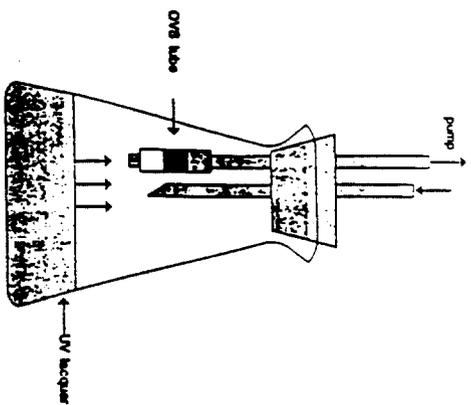
#### *Collection of Aerosols and Vapors with OVS Tubes Under Work Place Conditions*

The monitoring of worker exposure to airborne MuFA was conducted during a complete 8 h work day on a UV surface coating line at two work places. Samples were collected with OVS tubes and personal sampling pumps (DuPont P 2500) operating at a nominal flow rate of 1 U/min in the workers' breathing zones.

#### *OSHA Versatile Sampler Tube Method*

##### *Recovery and Precision*

Recovery and precision test samples were prepared by spiking the OVS tubes with a known concentration of HDDA, TPGDA, and BP. The solution contained



**FIGURE 2.** Illustration of the collection procedure used for the analysis of the multifunctional acrylates MFAAs from the UV curable acrylate lacquer surface.

1.02 g HDDA, 1.03 g TPGDA, and 1.00 g BP in 10 ml of acetone (p.a. >99.5% quality; Merck, Darmstadt, Germany). Five microliters of this solution was injected onto the glass fiber filter of an OVS tube. Air flow (1 l/min) through the sampler continued for 25 min. The spiked samples were stored capped in the dark at 4°C and analyzed within 24 h.

#### Storage Stability

Samples were prepared by spiking the OVS tubes in the same way as for the samples for the recovery test. They were stored capped in the dark at 4°C and analyzed at 0, 10, 20, or 30 days. The first set contained 10 samples and the rest had 3 samples each per time point.

#### Analysis of the Samples

##### Chemicals and Calibration

Diacylate standards were prepared from chemicals purchased from Aldrich Chemical Co. Inc., Milwaukee, WI, USA (HDDA, Aldrich No. 24618-6 and TPGDA, Aldrich No. 24683-2). Only technical grade chemicals (purity >90%) were commercially available. BP was obtained from Fluka Chemical Co., Buchs, Switzerland (Fluka No. 12309, purity >99.5%). The diacylate standards were not further purified because of technical difficulties associated with distillation.

Stock solutions were made by weighing 1.0 g of each compound and dissolving in 10 ml of acetone (p.a. >99.5% quality; Merck). They were stable for 2 months when stored in closed volumetric flasks in the dark at 4°C. The loss of HDDA and TPGDA was <3%. Working standards were prepared by diluting a stock solution (0.1-10 µl/5 ml acetone) using a microliter syringe (Hamilton, Bonaduz, Switzerland).

##### Sample Analysis

The glass fiber filters were extracted with 5 ml of methanol (LiChrosolv Gradient Grade; Merck) and the cellulose ester filters with 5 ml of carbon disulfide (p.a. quality; Merck). The OVS tubes were extracted with acetone (p.a. >99.5% quality; Merck). Three successive 5 ml fractions were collected into 5 ml volumetric flasks, which were sealed immediately. A PUF sample was extracted seven times with 10 ml of a diethyl ether/hexane (5/95 v/v, p.a. >95% quality; May & Baker Ltd., Dagenham, UK/J.T. Baker Chemicals, Deventer, Holland) solution.

The analysis of the samples was conducted using a HP7671A autosampler to inject a 2 ml sample into a gas chromatog-

raph (HP5880A; Hewlett-Packard, Waldbronn, Germany) equipped with a flame ionization detector (FID). Both split and splitless injection modes were used. The splitless injection mode was used for workplace samples because of the observed increased sensitivity. The analytical column was a cross linked 5% phenyl methyl silicone capillary column (HP-5, 50 m, ID 0.32 mm, film 0.52 µm; Hewlett-Packard) that enabled simultaneous analysis of both organic solvents and diacylates. The injector and detector temperatures were both 300°C. The temperature program used for the split mode was 45°C for 5 min, 10°C/min up to 250°C, and 250°C for 15 min. For the splitless mode we used the following temperature program: 45°C for 1 min, 10°C/min up to 250°C, and 250°C for 15 min. The injector and detector temperatures were the same as those used in the split mode. Unknown peaks in the chromatograms were identified by another gas chromatograph (HP5890, Hewlett-Packard) equipped with a HP7673A autosampler (Hewlett-Packard) and a HP5971A quadrupole mass selective detector (MSD; Hewlett-Packard). MSD parameters were: Electron impact mode, 70 eV ion source, mass scan 34-400 amu. The temperature program and column were the same as described above.

For the determination of particle size and shape the filters were gold coated and analyzed using a JEOL scanning electron microscope (JSM-840 A, Japan) connected to an image analyzer (KONTRON, SEM-IPS, Germany). The smallest particle diameter ( $D_{min}$ ) was measured from 400 particles. The median diameter and lower and upper quartiles were calculated.

## RESULTS AND DISCUSSION

The need for an effective and sensitive monitoring method for the MuFA shown in Table I led us to investigate the possibility of using OVS and PUF sampling tubes because of their potential to capture both aerosols and vapors and their use of large air volumes.

#### Collection of Aerosols and Vapors Under Experimental Condition

The average aerosol concentration measured with the filter method under experimental conditions was 0.05 mg/m<sup>3</sup> (range 0.048-0.054 mg/m<sup>3</sup>, n=4) for the glass fiber filters and 0.06 mg/m<sup>3</sup> (range 0.054-0.064 mg/m<sup>3</sup>, n=3) for the cellulose ester filters. Only trace levels of HDDA, TPGDA, and BP were detected (<0.001 mg/m<sup>3</sup>) in any of the samples.

The particle median diameters and lower and upper quartiles from the aerosol sample analyses are presented in Figure 3. Representative aerosols, shown in the scanning electron micrograph (Fig. 4), of all the samples collected had a median diameter of 0.16 µm and they were spherical in shape.

The analysis of the collected OVS and PUF tube samples revealed that the PUF tube was slightly more effective for airborne MuFA than the OVS tube under experimental conditions (Table II). However, we chose to develop and test the OVS tube instead of the PUF, mainly because it was easier to handle during both the collection and preparation of a sample and because it more efficiently recovered the acrylates. PUF required 7 extractions with 10 ml versus 3 extractions with 5 ml for the OVS tube, to achieve maximum quantitative recovery.

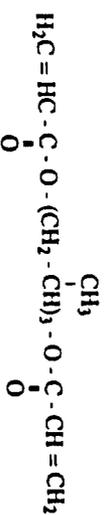
Our analysis of samples collected under experimental laboratory conditions con-

Table I. The chemical properties and structures of multifunctional acrylates and benzophenone used in UV curable acrylate lacquers.

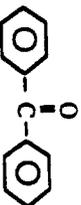
Compound	Mol. wt.	Density	Vapor pressure (mmHg)	Boiling point (°C)	Solubility
HDDA CAS: 13048-33-4	226	1.01	< 0.01	> 315.5	insoluble in water, soluble in many organic solvents
TPGDA CAS: 42978-66-5	300	1.03	< 0.01	* 315	—
BP CAS: 111-61-9	184	1.11	< 0.01	305.4	—



1,6-Hexanediol diacrylate (HDDA)



Tripropylene glycol diacrylate (TPGDA)



Benzophenone (BP)

vinced us that aerosols containing MuFA were produced during the application of UV curable acrylate coating onto the roller coater. Aerosols that are produced in the UV curing process may be due to movement of the rollers in the coating machine, as well as to the condensation of coating from open coating containers. Because of the possibility that MuFA might exhibit a dual character (vapor and aerosol) when evaporated in air, we tested a collection device (OVS tube) that would be able to collect both aerosols and vapors (Fig. 2). The concentration measured in the vicinity of the UV curable acrylate coating surface was 0.46 mg/m<sup>3</sup> for TPGDA and 0.28

mg/m<sup>3</sup> for BP. These results confirmed that MuFA evaporate from UV curable acrylate coatings.

#### OSHA Versatile Sampler Tube Method

##### Recovery and Precision

The results of the recovery and precision tests from spiked samples are shown in Table III. A representative gas chromatographic separation of diacrylates and BP is illustrated in Figures 5, and 6. The average recovery was 85% for HDDA and TPGDA and 86% for BP when three successive extractions (3 × 5 ml) were used. The first fraction contained over 95% of the total amount of each chemical extracted (~85%),

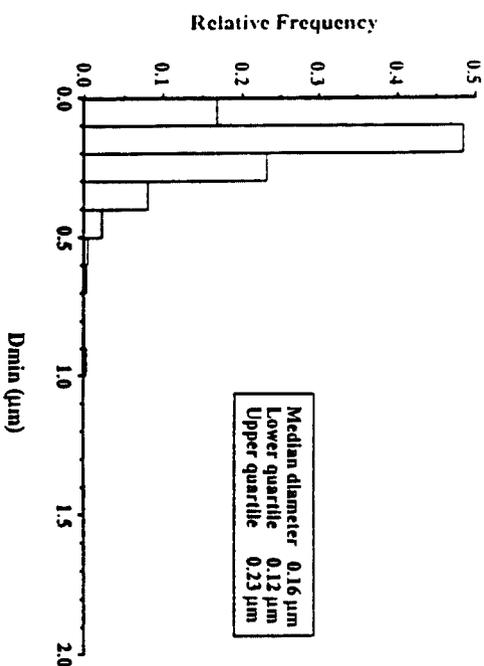


FIGURE 3. Particle size distribution ( $D_{min}$ ) of airborne aerosols collected on a polycarbonate filter in the application of UV curable acrylate lacquer onto a roller coater. A scanning electron microscopic analysis.

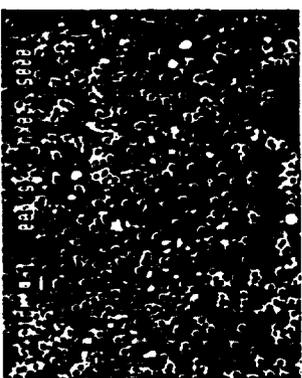


FIGURE 4. Scanning electron micrograph of the collected aerosols. White bar at the right bottom corner represents 1 µm.

the second fraction 1-5%, and the third fraction <1%. Thus, some bias was observed in the recovery of HDDA, TPGDA, and BP from OVS tubes. This bias was possibly due to an incomplete extraction procedure. Therefore, a recovery corre-

ction (1.17) should be applied to the final results when this extraction procedure is used.

The coefficient of variation (CV%), an estimate of precision, within one run (one sample run 10 times) was 2.6% for HDDA, 2.6% for TPGDA, and 2.7% for BP (Table III). The "overall uncertainty" calculated according to the proposal in the European CEN standard(16) was about 23%. This value complies with the standard proposal (maximum "overall uncertainty" 30%).

##### Storage Stability

Storing samples at 4°C resulted in retention of 298.3% for the first 10 days (Table IV). A significant loss (~9%) was observed between days 10 and 20. This loss may have been a result of analytical error or error in the test sample preparation rather than a real loss of analytes. However, the sampling system can be considered stable (loss <10% for 20 days) according to NIOSH criteria. (17)

Table II. Concentrations of HDDA, TPGDA, and BP in the OVS and the PUF samples collected under experimental conditions.

Sampler (Air volume)	HDDA		TPGDA		BP	
	µg/sample	µg/m <sup>3</sup>	µg/sample	µg/m <sup>3</sup>	µg/sample	µg/m <sup>3</sup>
OVS A (319 l)	48	150	33	103	95	297
PUF B (1169 l)	200	171	152	130	395	337

A OVS = OSIIA Versatile Sampler  
 B PUF = Polyurethane Sampler Tube

Table III. Results of the recovery tests of the spiked samples. Theoretical amount 0.500 mg/sample for HDDA, TPGDA, and BP.

Sample No.	HDDA (mg/sample)	TPGDA (mg/sample)	BP (mg/sample)
1	0.401	0.401	0.404
2	0.418	0.425	0.417
3	0.409	0.412	0.417
4	0.427	0.432	0.428
5	0.436	0.436	0.434
6	0.407	0.436	0.434
7	0.442	0.447	0.439
8	0.450	0.457	0.451
9	0.426	0.431	0.424
10	0.447	0.452	0.446
Average	0.426	0.430	0.426
Recovery	85.3 %	86.0 %	85.3 %
SD A	0.0174	0.0190	0.0164
RSD = CV B	4.1 %	4.4 %	3.8 %

A SD = Standard Deviation

B RSD = Relative Standard Deviation (%), CV = Coefficient of variation

**Detection Limit**

The estimated limit of detection (LOD) is 1 µg/sample for HDDA and TPGDA and 0.5 µg/sample for BP. These values correspond to concentrations of 2 µg/m<sup>3</sup> for MuFA and 1 µg/m<sup>3</sup> for BP on the basis of an 8 h sampling time at a flow rate of 1 l/min.

Concentrations of TPGDA and HDDA in work place air are not expected to be great due to the very low vapor pressures of these compounds. Thus, the capacity of the sampling system was not considered to be a critical factor and was not investigated.

Table IV. Storage stability of the spiked samples before and after storage at 4°C. Theoretical amount 0.500 mg/sample for HDDA, TPGDA, and BP.

Storage time (days)	HDDA (mg/sample)	TPGDA (mg/sample)	BP (mg/sample)	n
0	0.498 (100%)	0.503 (100%)	0.498 (100%)	10
10	0.482 (96.3%)	0.501 (99.5%)	0.496 (99.5%)	3
20	0.452 (90.6%)	0.452 (90.0%)	0.453 (90.8%)	3
30	0.441 (88.5%)	0.455 (90.5%)	0.440 (88.3%)	3

Results are corrected due to incomplete recovery/desorption (85%, k=1.17).

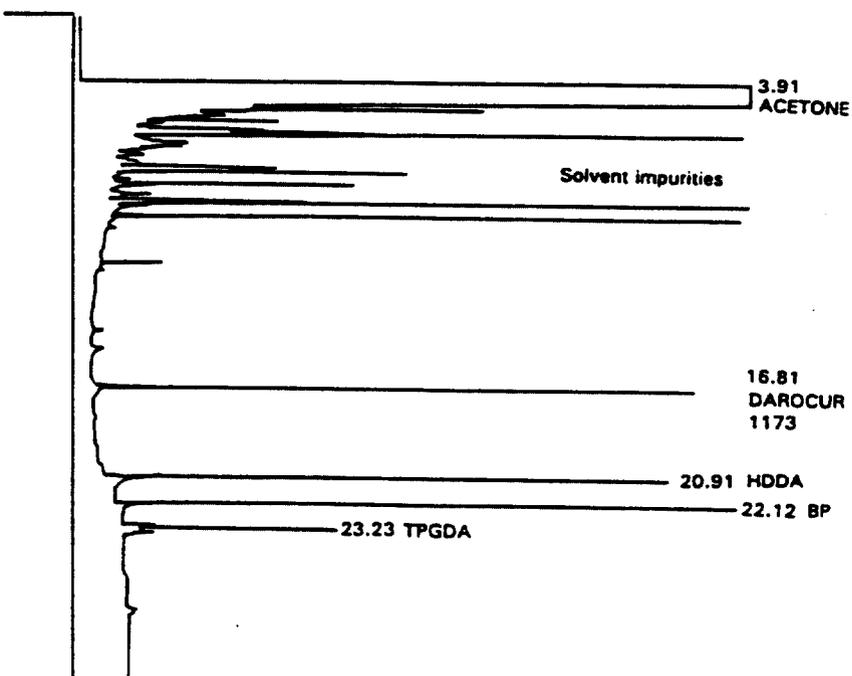


FIGURE 5. A gas chromatogram showing the separation of TPGDA, HDDA, BP, and Darocur 1173 (photoinitiator) in a standard sample by a gas chromatograph. Concentration 10 µg/5 ml sample for each component.

3.91  
ACETONE

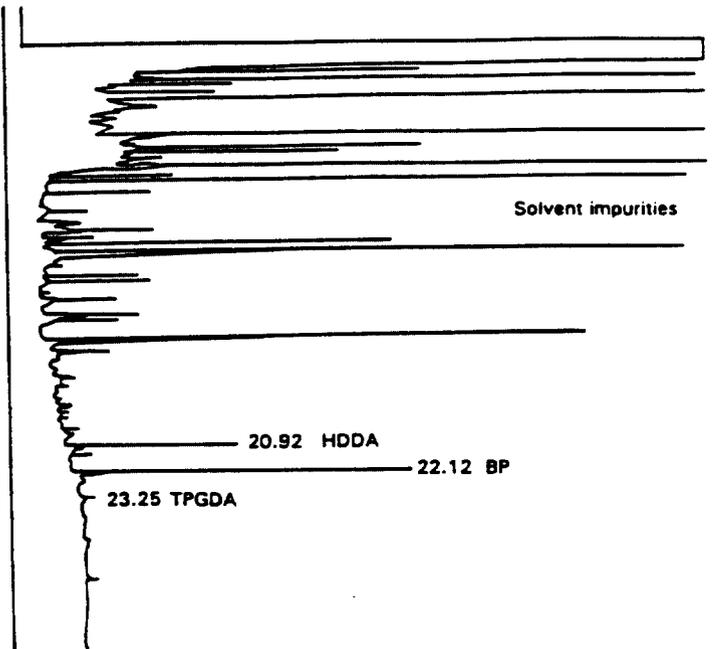


FIGURE 6. A gas chromatogram showing the separation of HDDA, TPGDA, and BP in a work place sample.

### OVS Tube Method Validation in the Work Place

The use of a filter method (glass fiber filter or cellulose ester filter) for measuring worker exposure to MuFA in the manufacture of UV cured acrylate coating for wood was insufficient to detect low concentrations of MuFA.

We used the OVS tube sampling method successfully to monitor worker exposure to MuFA in the manufacture of wood products coated with UV curable acrylate

TPGDA was also detected in this sample (5  $\mu\text{g}/\text{m}^3$ ). The material safety data sheet for the used formulation indicated that the UV lacquer formulation contained ~3 times more HDDA than TPGDA. The greatest TPGDA concentrations were observed in the breathing zones of the hand fillers (average 11  $\mu\text{g}/\text{m}^3$ , range 8-14  $\mu\text{g}/\text{m}^3$ ). BP was identified in all of the samples (range <1-30  $\mu\text{g}/\text{m}^3$ ; detection limit 0.5  $\mu\text{g}/\text{m}^3$ ). The highest BP concentrations (average 26  $\mu\text{g}/\text{m}^3$ ) were measured in the hand fillers' breathing zones. In the hand sander's breathing zone TPGDA, HDDA, and BP were below the detection limits of this method.

### CONCLUSIONS

Because of the potential for worker exposure to uncured UV acrylate coating aerosols and vapors and inadequate monitoring methods, we initially developed a monitoring method under experimental conditions. We tested the method at two work places manufacturing wood products coated with UV curable acrylate lacquer or filler.

The method developed to measure MuFA had good recovery (85%) and precision (<5%). The samples were stable for 20 days. Low concentrations of MuFA in a worker's breathing zone can be measured with this two-phase collection device (OVS tube) and a capillary gas chromatograph. We have demonstrated that the OVS tube sampling method for MuFA under work place conditions is successful. The concentrations of TPGDA and HDDA ranged from <2  $\mu\text{g}/\text{m}^3$  to 13  $\mu\text{g}/\text{m}^3$  and those of BP from <1  $\mu\text{g}/\text{m}^3$  to 26  $\mu\text{g}/\text{m}^3$ . Thus, we can state that this sampling method is a convenient and accurate method for measuring worker exposure to MuFA.

Inhalation exposure to MuFA has not previously been a concern because of the low vapor pressure of these compounds at room temperature. The use of UV curable acrylate coatings is increasing, and worker exposure to aerosols and vapors of MuFA is possible and may involve a potential health risk for workers, since particles are in the respirable range and may occur in sufficient concentrations to sensitize individuals in high exposure jobs. (19) Additional research and large scale exposure assessments of worker exposures to MuFA is required, and urgent, if the potential health risk is to be understood and allowable worker exposure concentrations to MuFA are to be determined.

### ACKNOWLEDGEMENTS

We thank the Swedish lacquer manufacturers Becker Acroma AB and Nobel Coatings AB for their assistance and the wood surface coating manufacturing plants and their workers for their assistance and participation in testing this method. We also thank Ilpo Ahonen for the mass spectrometric analysis, Marja-Liisa Aijälä for the gas chromatographic analysis, and Birgitta Kaprali for the preparation of the illustrations.

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# Upper Airway Symptoms and Function in Wood Surface Coating Industry Workers

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Respiratory and ocular effects from exposure to airborne contaminants in workers employed in the manufacture of wood products using ultraviolet radiation curing (UV) or acid curing (AC) of surface coating were investigated. Surface coating line or finishing workers exclusively employed in one or both processes were compared to a matched control group. Symptoms of exposure were investigated by questionnaire and medical examination. Nasal, pharyngeal, and ocular symptoms of discomfort, but not lower airway, were common among all exposed groups. These symptoms were most frequent in UV line workers and finishers of UV surface coated wood products. Mucociliary clearance was significantly decreased in UV line workers. Significantly impaired olfaction was observed in UV line and AC line workers and finishers of UVA/C surface coated wood products. Low levels of organic solvents and coating dusts (composed in part of wood dust, chemical composition unknown) were measured in the workers' breathing zones. Although remarkable improvements have been made in both AC and UV surface coating, additional control measures to eliminate airborne contaminants and improved work practices are required.

**Key words:** occupational exposure, nasal irritants, respiratory physiology, dust, acrylates, mucociliary clearance, olfaction, surface coating, wood industry

## INTRODUCTION

In recent years the use of acid curing coatings containing organic solvents in the furniture industry has diminished, due to more restrictive environmental regulations for solvent emissions. New types of coatings have been introduced for the surface coating of wood. Especially, coatings that are cured (i.e., polymerized) by ultraviolet radiation (UV coatings) have increased significantly during the last ten years in Sweden [Nylander-French et al., in press a]. The UV coatings are composed of three basic components: an acrylated prepolymer (urethane acrylate, polyester acrylate, etc.), a multifunctional acrylate monomer (e.g., tripropylene glycol diacrylate, TPGDA; 1,6-hexanediol diacrylate, HDDA; etc.), and a photoinitiator system (e.g., benzophenone, etc.) [Allen et al., 1991]. The number of workers employed in this industry is increasing as the industry expands; the volume growth of radiation curable acrylate use is estimated to be 15-20% per annum until the year 2000 [del Rosso Arrighetti and Moretti, 1992; Dufour, 1991; Hussain, 1991].

UV coatings are applied to wood surfaces and cured in automated production lines requiring only 3-5 workers per line. Workers on the application lines are exposed to uncured acry-

latic coatings, cured and/or partially cured acrylate coating dust, acrylate vapors, wood dust, and ultraviolet radiation (UVR) from all UV wavelength regions UVA, UVB, and UVC [Nylander-French et al., in press a]. UVR that is reflected into the work environment from the UV lamps may affect both the eyes and the skin [International Agency for Research on Cancer, 1992]. After application and curing of UV coating, wood products are further processed by hand sanding and/or drilling, assembling, and packing. Thus, differences in chemical and UVR exposure and exposure patterns exist between workers employed in different work tasks.

Our knowledge of the overall potential health risks associated with specific exposures and patterns observed in this industry is inadequate. The biological effects of multifunctional acrylates has been insufficiently investigated. No data on absorption, metabolism, and/or distribution, and dermal, oral, inhalation, or ocular toxicity of multifunctional acrylates has been published.

The goal of this study was to investigate potential health effects to the upper airways and eyes of workers employed in the UVR curable coatings of wood products. For comparison, workers employed in the acid curing of surface coatings for wood as well as unexposed workers were examined.

## MATERIALS AND METHODS

### Study Population

Workers exposed to uncured UV acrylate coatings and UVR and/or cured acrylate coatings were identified [Nylander-French et al., in press a]. This was a cross-over field study and all available individuals were examined. Five groups of subjects based on work tasks were formed from the study population and examined. All investigations were performed in the work places during the working hours; examination began approximately 2 h after the start of work. Summary statistics of the study population are presented in Table 1. Group (I) consisted of 26 UVR surface coating line workers (*UV line*), exposed to uncured and/or cured acrylate coatings and UVR. Group (II) consisted of 24 acid curing surface coating line workers (*AC line*), exposed to organic solvents, formaldehyde, and uncured and/or cured acid curing amino resins. Group (III) consisted of 33 workers employed in the finishing processes of UVR cured acrylate surface coated products (*UV finisher*), exposed to cured and/or partially cured acrylate coating dust and wood dust. Group (IV) consisted of 35 workers employed in the finishing processes of both UVR cured acrylate and acid curing surface coated products (*UV/AC finisher*), exposed to cured and/or partially cured acrylate and acid curing coating dust and wood dust. Group (V) was a control group of 30 office workers (*control*) from the same village, where the majority of surveyed plants were located, and who had no history of occupational exposure to acrylates, solvents, and/or wood dust.

### Worker Exposure Assessment

Distinct occupational groups were identified and job titles were assigned according to a defined work tasks [Nylander-French et al., in press a]. UV lacquer line workers were divided into three different job categories: controller, feeder and receiver. UV filling line workers were considered as a separate group, as were finishers, who worked at a station for

Table 1. Summary statistics of the study population according to the type of exposure.

Study Group	I. <i>UV line</i> (N=26)	II. <i>AC line</i> (N=24)	III. <i>UV finisher</i> (N=33)	IV. <i>UV/AC finisher</i> (N=35)	V. <i>Control</i> (N=30)
Sex (female/male)	9/17	4/20	18/15	8/27	19/11
Age	34.8	45.7	41.8	39.2	41.5
Employment / Exposure (years) <sup>1</sup>	2.8	10.7	6.7	8.0	20.6
Employment in wood industry (years)	9.6	17.4	13.0	14.3	0
Smokers (%)	50	46	45	23	27
Alopy (%)	19	17	18	23	17

<sup>1</sup> Employment equals the time employed at the present job. Workers were assigned to the corresponding exposure groups according to their present work tasks, thus, employment corresponds to exposure time at the current work environment.

the finishing of cured products. Exposure assessment was determined during a 1-day industrial hygiene measuring survey, which is described in detail elsewhere [Nylander-French et al., in press a]. Briefly, sample collections were conducted with personal sampling equipment placed in the breathing zone of the worker during a complete 8 h work day. The workers wore the sample affixed to the right side of their work apparel label, or if two samples were collected on the same worker, samplers were attached to opposite apparel labels.

Airborne total dust samples were collected with a 37 mm glass fiber filter (Whatman, GF/F) or a 37 mm cellulose ester filter (Millipore, pore size 0.8 µm) and a personal sampling pump (flow rate of 2 l/min) and analyzed gravimetrically [Swedish National Board of Occupational Safety and Health, 1979]. Particle size selective sampling was conducted using a personal cascade impactor and a personal sampling pump (flow rate of 2 l/min). The aerosol size distribution was determined through gravimetric analysis for the sample collected on each 34 mm Mylar or 34 mm PVC filter. Airborne acrylate vapors and aerosols were collected with an OSHA Versatile Sampler (OVS) tube and a personal sampling pump (flow rate of 1 l/min) and analyzed by a gas chromatograph [Nylander-French et al., in press b]. Airborne vapors were collected with a SKC charcoal tube and a personal sampling pump (flow rate of 50 or 200 ml/min) and analyzed by a gas chromatograph [Swedish National Board of Occupational Safety and Health, 1979]. Worker exposure to formaldehyde and organic solvents had been determined in an earlier study [Hultengren et al., 1992]. In this study, solvents were collected with the charcoal tube method [Swedish National Board of Occupational Safety and Health, 1979] and formaldehyde with the fritted midjet bubbler method [Swedish National Board of Occupational Safety and Health, 1977]. Sampling was performed in the workers' breathing zones on 15 min occasions selected at random during the 8 h work day.

Duration of potential direct exposure to acrylates varied in accord with occupational group. Preparations for the UV line operation required 2-5% of the work day (10-20 min), which included the start and warm up procedures of the UV lamps to reach the optimal curing conditions. This procedure was conducted by all the line workers. The UV line controllers spent

~80% of their work time controlling the curing conditions on the UV line and were most likely to have direct skin contact with uncured acrylic lacquer (~5% of the work time). They also cleaned the sanding machines on the UV line (1% of the work time). The feeders spent ~70% of the work time feeding products into the process. The receivers spent ~65% by receiving and stacking the cured products at the end of the UV line. The hand fillers were most frequently in direct skin contact with uncured acrylic coatings (~60% of the work time) of all the UV line workers.

We did not observe any differences in potential worker exposure levels for UV line workers performing different tasks (controller, feeder, and receiver), therefore, these workers were considered as a single exposure group in our analysis. The average 8 h time-weighted (8 h TWA) total dust concentration in workers' breathing zones was 0.4 mg/m<sup>3</sup> (range 0.02-1.0 mg/m<sup>3</sup>) in all work places surveyed. The average total dust concentration in the breathing zones of the workers who worked on the UV lacquer lines was 0.3 mg/m<sup>3</sup> (range 0.02-0.7 mg/m<sup>3</sup>), 0.5 mg/m<sup>3</sup> (range 0.2-1.0 mg/m<sup>3</sup>) on the UV filling lines, and 0.4 mg/m<sup>3</sup> (range 0.1-0.7 mg/m<sup>3</sup>) during finishing work. Total dust concentrations in the breathing zones of acid curing line workers had been measured in an earlier study (Alexandersson et al., 1987) and were observed to be <0.5 mg/m<sup>3</sup>.

Particle size distribution was determined in the workers' breathing zones and no differences were observed in the samples collected on workers conducting different work tasks (UV line workers, fillers, and finishers). The average proportion of thoracic (<10 µm) and respirable dust (<5 µm) particle concentrations were 79% (range 62-99%) and 28% (range 12-62%), respectively, from the 8 h TWA total dust concentrations.

Worker exposure to TPGDA and HDDA at the UV lacquer line was generally below the limit of detection (LOD 2 µg/m<sup>3</sup>) except for one worker (out of 9), for whom HDDA was detected (15 µg/m<sup>3</sup> 8 h TWA). Also, TPGDA was detected in this sample (5 µg/m<sup>3</sup> 8 h TWA). The greatest TPGDA concentrations were observed in the breathing zones of the hand fillers (average 11 µg/m<sup>3</sup> 8 h TWA, range 8-14 µg/m<sup>3</sup> 8 h TWA). Benzophenone was identified in all of the samples (range <1-30 µg/m<sup>3</sup> 8 h TWA; LOD 1 µg/m<sup>3</sup>). The highest benzophenone concentrations (average 26 µg/m<sup>3</sup> 8 h TWA) were measured in the hand fillers' breathing zones. In the hand sander's breathing zone TPGDA, HDDA, and benzophenone were below the limit of detection.

Small concentrations of different organic solvents were detected in the workers' breathing zones working at the UV lines: toluene, xylene, butyl acetate, and terpenes. In acid curing lines workers were exposed to toluene, xylene, *n*-butyl acetate, ethanol, *n*-butanol, isobutanol, ethyl acetate, propylene glycol methylether (1-methoxy-2-propanol), propylene glycol methyl ether acetate (1-methoxy-2-propyl acetate), and formaldehyde. Worker exposure to organic solvents (calculated for additive exposures [American Conference of Governmental Industrial Hygienists, published yearly]) in the UV lines was ~26% of that in acid curing lines.

#### Medical Examination

The medical examinations were performed during November and December to minimize the effects of seasonal allergies. The study was conducted in accordance of the approval from

the ethical committee at Huddinge University Hospital. The medical examination focused primarily on the nose and the throat.

Questionnaires were distributed to all participants before the medical examination, completed by the participant and reviewed in detail by the medical examiner with the participant during the examination. The questionnaire solicited information concerning previous and present work tasks, employment time, sex, age, smoking history, airway, eye and skin symptoms, and allergy and hyperreactivity.

Nasal airway flow (NPEF) was measured with a peak expiratory flow meter (The Right Peak Flow Meter, Airmed, England) connected to an anesthetic face mask by an adapter. Nasal peak expiratory flow rate (l/min) was recorded after clearing the nose, by forced expiration through the nose into the mask with closed lips, while in a standing position. The average value of the two best of three maximal expirations was used for statistical analysis.

In a single blind fashion, nasal mucociliary clearance was measured with one-quarter of a saccharin tablet (Hermescetas<sup>®</sup>) applied on the inferior turbinate, 1 cm behind the anterior border of the turbinate. The test person was told not to inhale or exhale through his/her nose. Time was recorded until the subject could describe the correct associated taste. A time of transport (taste sensation) exceeding 20 min was regarded as pathological.

Olfaction was determined by measuring the olfactory threshold according to Cain and colleagues [1988]. The odorant stimulus was an aqueous dilution of 1-butanol where successive dilutions (step 1-13) differed by a factor of three. The highest concentration was 4% (step 1) and the lowest 0.4×10<sup>-6</sup>% (step 13). The test solution was presented in 250 ml squeezable plastic bottles containing 60 ml of the solution. The subject was presented two bottles at a time, one with 1-butanol and one containing deionized water. The examiner squeezed the bottle under the nostril of the subject who then had to decide odor smelled stronger. The lowest concentration that was repeatedly correctly detected was marked as that individual's threshold level.

Spirometry was performed with a Vitalograph<sup>™</sup> (Buckingham, England). At least three efforts were recorded for each subject, and the greatest one was used for all calculations. Vital capacity (VC) and forced vital capacity in one second (FVC<sub>1.0</sub>) were recorded. All the individual values were compared with expected normal values (based on age, sex, height, weight and smoking habits) according to Quanjer [1983].

#### Statistics

For analysis of the questionnaire and mucociliary clearance odds ratios (OR) with 95% confidence limits (95% CI) were calculated. When OR was not applicable, Fisher's exact test was used. Nasal peak expiratory flow (NPEF) was analyzed with Student's paired one-tail t-test and olfaction with Mann-Whitney's U test and Fisher's exact test. The differences were classified as significant if the p-value was ≤0.05.

#### RESULTS

Altogether, 148 individuals participated this cross-over field study and the participant drop-out was estimated to be between 2-3%. There were no significant differences in tobacco use, age, or frequency of atopy between the study groups, but there were more females in group III

(UV Finisher) and in the unexposed control group compared to all others.

From the questionnaire, we observed that nasal, pharyngeal, and ocular symptoms of discomfort, but not complaints about the lower airways, were more common among all the exposed workers than the unexposed control group (Table II). In the four groups of exposed workers nasal complaints were the most frequent in group III (UV Finisher) compared to the control group. Pharyngeal symptoms were over represented in groups I (UV line), III (UV Finisher), and IV (UV/VAC Finisher), whereas ocular symptoms were significantly more common in groups I (UV line), II (AC line), and III (UV Finisher), when compared to the control group. When nasal, pharyngeal, and ocular symptoms were summed in each group and compared, groups I (UV line) and III (UV Finisher) were observed to have more symptoms of discomfort compared to groups II (AC line) and IV (UV/VAC Finisher). Daily symptoms of discomfort were significantly more often observed in group I (UV line), while more infrequent symptoms (a few times per month) were observed more often in all four exposed groups.

Specific nasal symptoms associated with nasal complaints, such as nasal obstruction, runny nose, nose bleeding, dryness, and an impaired sense of smell, were not statistically over represented in any of the exposed groups compared to the control group, except for sneezing. Sneezing was more frequent in groups III (UV Finisher; OR 14.5, 95% CL 1.74-120.9) and IV (UV/VAC Finisher; OR 11.4, 95% CL 1.34-96.19) compared to the control group. Specific pharyngeal symptoms, such as sore throat, dryness, and lump, were not statistically more frequent in the exposed groups compared to the control group. For ocular symptoms, however, itching was significantly more frequent in groups I (UV line;  $p<0.01$ ; Fisher's exact test), II (AC line;  $p<0.05$ ), and III (UV Finisher;  $p<0.5$ ) and ocular secretion was significantly more frequent in groups I ( $p<0.05$ ) and III ( $p<0.05$ ) compared to the control group.

A history of sinusitis was more frequent in group III (UV Finisher), but pharyngeal infections, bronchitis, and pneumonia were not more often reported in exposed groups than in the control group (Table III).

The frequency of hyperreactivity and/or work related allergic symptoms was estimated based on the answers in the questionnaire (discomfort when exposed to odors, cigarette smoke, exhaust, fresh paper, cold air, etc.). Summary statistics of the hyperreactivity in

Table II. Summary statistics of systemic discomfort experienced by surface coating industry workers when compared to matched unexposed controls.

Symptom	I. UV line		II. AC line		III. UV finisher		IV. UV/VAC finisher		Total	
	OR	95% CL	OR	95% CL	OR	95% CL	OR	95% CL	OR	95% CL
Nasal discomfort	2.1	0.63-7.07	1.7	0.47-5.78	4.8	1.56-14.81	3.0	0.98-9.17	2.8	1.08-7.47
Pharyngeal discomfort	10.7	1.22-93.92	2.6	0.22-30.97	12.6	1.50-105.81	8.6	1.01-73.32	8.6	1.12-66.12
Lower airway discomfort	1.2	0.26-5.28	4.0	0.41-38.70	3.9	0.43-36.12	2.8	0.30-27.16	3.0	0.37-23.94
Ocular discomfort	15.4	1.79-131.94	9.7	1.07-87.00	10.9	1.29-92.03	2.7	0.27-27.62	8.6	1.12-66.12

different study groups is presented in Table IV. Nasal hyperreactivity was significantly more often observed in groups III (UV Finisher) and IV (UV/VAC Finisher) compared to the control group, and was almost significant in group I (UV line). Pharyngeal hyperreactivity was significantly more frequent in all exposed groups ( $p<0.01$  for groups I, III, and IV;  $p<0.05$  for group II; Fisher's exact test), while ocular hyperreactivity was most frequent but not statistically significant in group I (UV line). For lower airway hyperreactivity, there was no difference between the exposed groups and the control group.

On examination a large number of exposed workers had signs of nasal inflammation, such as redness of the nasal mucosa (Table V). There was also a great number of workers in all exposed groups with dryness of the nasal lining, crusting, and blood. A few cases with nasal polyps and septal perforation were also observed. In the control group these signs were more infrequent.

Nasal peak expiratory flow (NPEF) showed no significant differences, except for a lower mean value in group III (UV Finisher) compared to the control group (Table VI).

The olfaction test disclosed the lowest threshold values in the control group (Table VI). In all exposed groups, the sense of smell was significantly deteriorated compared to the control group except in group III (UV Finisher). The duration of employment in the particular work tasks in the wood surface coating industry did not affect significantly the olfaction. When we compared the effect of age (groups divided into two by the average age of the group) between the exposed groups and the control group, olfaction was significantly decreased ( $p<0.05$ , Fisher's exact test) in the older workers in group I (UV line), II (AC line), and IV (UV/VAC Finisher) but in the younger workers only in group IV (UV/VAC Finishers). A tendency

Table III. Summary statistics of airway infections experienced by surface coating industry workers when compared to matched unexposed controls.

Infection	I. UV line		II. AC line		III. UV finisher		IV. UV/VAC finisher		Total	
	OR	95% CL	OR	95% CL	OR	95% CL	OR	95% CL	OR	95% CL
Sinusitis	2.4	0.21-28.31	1.3	0.07-21.27	9.3	1.08-79.40	6.0	0.68-53.01	4.9	0.62-38.25
Pharyngitis	2.4	0.21-28.31	0.0	-	2.1	0.18-24.15	1.9	0.16-21.75	1.6	0.19-14.18
Bronchitis	2.4	0.21-28.31	0.0	-	5.2	0.57-47.16	1.8	0.15-20.40	2.4	0.29-19.86
Pneumonia	0.6	0.05-6.56	1.3	0.17-9.77	1.9	0.33-11.39	0.9	0.11-6.42	1.2	0.24-5.65

Table IV. Summary statistics of nasal, lower airway, and ocular hyperreactivity in surface coating industry workers when compared to matched unexposed controls.

Hyper-reactivity	I. UV line		II. AC line		III. UV finisher		IV. UV/VAC finisher		Total	
	OR	95% CL	OR	95% CL	OR	95% CL	OR	95% CL	OR	95% CL
Nasal	2.8	0.90-8.40	2.0	0.62-6.18	2.9	1.01-8.42	6.0	2.04-17.65	3.3	1.34-7.91
Lower airway	1.6	0.33-8.10	1.8	0.36-8.96	2.9	0.69-12.08	1.9	0.42-8.19	2.1	0.57-7.42
Ocular	3.4	0.91-12.97	1.3	0.68-10.56	1.4	0.37-5.71	1.9	0.52-7.18	2.2	0.71-6.87

Table V. Results of nasal and pharyngeal examination of the study groups.

Study Group	I. UV line (N=26)	II. AC line (N=24)	III. UV finisher (N=33)	IV. UV/AC finisher (N=35)	V. Control (N=30)
Redness of the nasal mucosa	20	21	18	18	5
Swollen nasal mucosa	2	5	10	5	2
Increased nasal secretion	4	2	9	2	1
Dryness of the nasal mucosa	9	15	12	11	2
Nasal crusting blood	6	14	15	8	0
Pharyngeal irritation	9	8	9	2	1

of impaired olfaction was observed in all groups with increasing age. We did not observe smoking to affect the sense of smell in any of the study groups.

The number of subjects with impaired nasal mucociliary clearance was greater in all four exposed groups compared to the control group (Table VI). The difference was, however, statistically significant only for group I (UV line). The mean value of clearance time among subjects within normal transport time (below 20 min) did not differ statistically between the exposed groups and the control group. Nasal complaints were not more frequent among subjects with impaired mucociliary clearance compared to those with a value in the normal range. Mucociliary clearance was not significantly different in exposed groups and in the control group when adjusted for duration of employment in the present work task or smoking. However, mucociliary clearance was significantly impaired ( $p < 0.05$ ; Fisher's exact test) in young UV line workers ( $< 39$  years of age) when compared to young controls ( $< 42$  years of age).

Table VI. Results from the nasal peak expiratory flow (NPEF) test (l/min), olfaction test, and nasal clearance test in the exposure and the control groups. The number of subjects with impaired nasal clearance in different exposure groups is compared statistically (odds ratios and 95% confidence limits) to the control group.

Exposure Group	NPEF Mean $\pm$ STD	Olfaction test Mean $\pm$ STD	OR	Clearance 95% CL	(%)*
I. UV line (N=26)	324 $\pm$ 18.3	4.9 $\pm$ 1.3**	9.2	1.02-82.21	23
II. AC line (N=24)	315 $\pm$ 22.9	4.7 $\pm$ 1.8**	6.4	0.67-62.31	17
III. UV finisher (N=33)	252 $\pm$ 17.9	5.6 $\pm$ 1.9	2.9	0.28-29.51	9
IV. UV/AC finisher (N=35)	318 $\pm$ 16.6	4.8 $\pm$ 1.7***	3.7	0.39-35.47	11
V. Control (N=30)	294 $\pm$ 15.7	6.4 $\pm$ 1.9			3

\* Percent of subjects in the study group with impaired mucociliary clearance.

\*\*  $p < 0.01$

\*\*\*  $p < 0.001$

Table VII. Results of the pulmonary function tests in the exposed groups. In the statistical analysis comparisons were made between the observed and expected values.

Exposure Group	I. UV line (N=26)	II. AC line (N=24)	III. UV finisher (N=33)	IV. UV/AC finisher (N=35)	V. Control (N=30)
Vital capacity					
Observed	4.604	4.348	4.045	4.553	4.180
Expected	4.480	4.470	3.946	4.538	3.928
FEV <sub>1</sub> % <sup>1</sup>					
Observed	83.3	80.0	80.8	82.6	81.6
Expected	81.5	80.6	80.5	80.6	80.5

<sup>1</sup> FEV<sub>1</sub>% = (100  $\times$  forced expiratory volume in 1 s)/forced vital capacity.

Spirometry measurements indicated that vital capacity and FEV<sub>1.0</sub> were close to expected values in all exposed groups as well as in the control group (Table VII).

## DISCUSSION

Release of organic solvents to the ambient environment and concomitant worker exposure is reduced (~26%) in the UV radiation curing of acrylic coatings compared to the acid curing coatings as intended by the introduction of new organic solvent free coatings. In order to compare the potential differences in health effects between these two manufacturing processes, we compared surface coating line and finishing workers exclusively using one or the other process or either process to a matched control population without a history of exposure in either work environment. For every exposed worker we attempted to obtain one control individual matched for sex and age among workers employed at acid curing surface coating lines, finishers employed in UV surface coating, finishers in acid curing surface coating, and office workers. There were too few finishers in acid curing surface coating manufacturing to obtain a satisfactory control match. Therefore, finishers with mixed exposures were also included, but are presented separately, in this study. Airway and ocular symptoms of potential exposure were examined by questionnaire and medical examination, during which the tests of airway function were administered.

A similar pattern of symptoms and dysfunction were observed within the given job categories of all exposed workers when compared to unexposed matched controls. Thus, symptoms observed may be independent of work place chemical exposures (acid curing versus UV radiation curing). A common exposure between the two different work groups is the coating dusts (composed, at least, in part of wood dust) present in concentrations below the limit value of 3 mg/m<sup>3</sup> [Nylander-French et al., in press a]. The actual chemical composition of these work place dusts is unknown, but a significant portion (~30% of the total dust concentration) is in the respirable range [Nylander-French et al., in press a].

Nasal, pharyngeal, and ocular discomfort were cited most often by surface coating line and finishing workers using either or both processes, and this pattern of prevalence is consistent with our analysis of site specific complaints and their medical evaluation (Tables III and IV). Although complaints concerning lower airway discomfort were not significant and exposure to dust low, ~30% of dust are respirable [Nylander-French et al., in press], the absence of

significant complaint from lower airways is opposite to that observed in formaldehyde-wood dust exposed workers [Holmström and Wilhelmsson, 1988]. A nonspecific nasal hyperreactivity was observed in exposed groups which may explain a greater frequency of complaints. This nonspecific nasal hyperreactivity was also reported in formaldehyde-wood dust exposed workers [Wilhelmsson and Holmström, 1992] and was not more frequently observed in atopics. Theoretically, dust and toxins may induce an inflammatory reaction in the nasal mucosa followed by hyperreactivity due to eosinophilic migration as reported by Fabri and colleagues [1987] in toluene diisocyanate induced late asthmatic reactions.

Nasal irritation was frequently observed in all exposed groups on examination (Table V). Redness and dryness of the nasal mucosa, especially, were frequently seen. Similar findings as well as inflammation, were also reported in wood furniture workers [Hadfield, 1969]. Crusting was mainly observed in the anterior part of the middle turbinate and anteriorly in the septal wall. Crusting may contribute to the sense of nasal obstruction as reported by many of the workers investigated in this study.

Physiological measurements of nasal passage function (peak expiratory flow, olfaction, and mucociliary clearance), shown in Table VI, indicate that mucociliary clearance was impaired in all exposed groups, but was significantly impaired only in the UV line workers. Olfaction was significantly impaired in UV line and AC line workers and UV/AC finishers. These workers may be exposed to low levels of uncured acrylate aerosols and vapors [Nylander-French et al., in press a] that deposit and act at contact site in the anterior nasal passage. This effect may be distinct from mechanical irritation by fine dust that we suspect may be the major cause of the observed symptoms and complaints.

Changes in mucociliary clearance may be a result of either altered rheological properties of the mucous covering the ciliated epithelial cells or altered function of the ciliated cells due to a lowered cilia beat frequency or loss of cilia. These changes may be caused by exposure to dusts and/or toxins, including carcinogenic chemicals [Andersen and Møhlave, 1983; Bogdanffy et al., 1987; Boysen et al., 1986; Morgan et al., 1986a; Morgan et al., 1986b; Wilhelmsson and Lundh, 1984; Wilson et al., 1985]. The effect of impaired mucociliary activity is obvious to the worker, who may notice it as nasal obstruction or discharge. Ciliary stasis may result in increased dust or chemical toxicity to the mucosa.

Impaired olfaction may be a result of a toxic effect of a chemical or of a blocked nose. In this study, using NPEF, we did not observe significant signs of nasal obstruction in any exposed groups compared to the control group. However, there are some difficulties in interpreting these results since nasal flow is related to the height of the subject tested. Females were overrepresented in groups III and V, and differences in male and female average height may be related to the lower values in these groups. NPEF is not the best method for detection for a swollen lining in the roof of the nose. Animal studies conducted on monofunctional acrylates (e.g., methyl acrylate, ethyl acrylate, methyl methacrylate) have indicated an irritative and degenerative effects (e.g., loss of olfaction epithelium and replacement of the olfaction epithelium to respiratory epithelium) of these vapors in the nasal mucosa [Chan et al., 1988; Klimisch and Reinmighaus, 1984; Miller et al., 1985; Reinmighaus et al., 1991; Tansy et al., 1980].

No signs of airway restriction or airway obstruction were observed using the Vitalograph.

However, a hidden pulmonary obstruction cannot be excluded, since we did not perform a test for reversibility of pulmonary resistance in this study.

Health effects of wood dust are well recorded and known [Nylander and Dement, 1993]. Wood dust is an irritant and a sensitizer to the airways and after long periods of exposure, correlated with an increased incidence of sinonasal cancer [Nylander and Dement, 1993]. Other health related effects, e.g., nasal clearance and inflammation, are insufficiently studied. However, nasal obstruction and hypersecretion, as well as an increased incidence of common colds has been reported in wood furniture workers [Ruppe, 1973; Wilhelmsson and Dretnier, 1984]. These symptoms seem to correlate to wood dust concentration [Andersen et al., 1977]. Inflammation, skin irritation, and allergic contact dermatitis from exposure to acrylates are, potentially, the most likely health effects to workers [Björkner et al., 1980; Emme, 1977; Malen, 1979; Malen et al., 1979; Nelhercoit, 1981; Nelhercoit et al., 1983; Whittington, 1981]. However, no studies have been published, to our knowledge, on health complaints and/or pathophysiology in upper airways in workers exposed to multifunctional acrylates. Both multifunctional acrylates and methacrylates are potent sensitizers in humans and guinea pigs; triacrylates appearing to be more potent sensitizers than diacrylates. Many inhibitors that are added to acrylate formulations to prevent spontaneous polymerization of the acrylate monomer and prepolymer are also considered to be possible skin sensitizers [Rietschel, 1986; van der Walle et al., 1982]. However, the potential sensitization due to inhibitors can usually be considered negligible because of their low concentration (10-250 ppm) in acrylate formulations. The toxicity, including carcinogenicity [Andrews and Clay, 1986; DePass et al., 1985] and mutagenicity [Cameron et al., 1991] of some multifunctional acrylates are known and the results indicate to us that additional studies are needed to understand the biological effects of acrylate exposure. Epidemiological investigations of worker exposure and potential health effects (e.g., cancer) from long term exposure to acrylates have been limited. This may be due to the long latent period of cancer induction and the relatively brief period of industrial use of this technology. An excess of colon cancer was observed in men employed in the manufacturing and polymerization of acrylate monomers in acrylic sheet manufacturing [Walker et al., 1991]. Although, the cohorts studied had other work exposures to industrial chemicals, it was concluded that exposure to monofunctional acrylates (ethyl acrylate and/or methyl acrylate) at concentrations used in the manufacturing process was a tenable explanation for the excess cancer mortality.

Evidence of toxicity and potential carcinogenicity of acrylates used in UV curable coatings, like tripropylene glycol diacrylate (TPGDA) or hexanediol diacrylate (HDDA) are limited or unknown. Recent studies suggest that TPGDA may have the potential to induce or promote epithelial tumors when topically applied to the skin of mice [Nylander-French and French, submitted a] and that it is toxic to normal human skin and lung cells in vitro [Nylander-French and French, submitted b]. Together, these studies indicate that exposure to certain acrylates, at least, may pose a risk to workers and that this risk must be carefully evaluated and managed.

## CONCLUSION

In summary, a similar pattern of upper airway and ocular symptoms of discomfort was observed in all wood surface coating industry workers. The prevalence of symptoms were significantly different from the control group. However, symptoms were most frequent in UV line workers and finishers of UV surface coated wood products. Mucociliary clearance was significantly decreased in UV line workers. Significantly impaired olfaction was observed in UV line and AC line workers and finishers of UV/AC surface coated wood products. Organic solvent exposure levels were low for exposed workers (less than one tenth of the Swedish occupational exposure limit values). The measured dust concentrations were similar in both UV curing and AC curing work environments. Dust, along with formaldehyde (not determined for the UV curing process environment) and other airborne aerosols and vapors, may be responsible for the observed symptoms. More information is required on worker exposure levels to these hazardous agents and dust composition in the respective work environments in order to determine the etiology of the observed symptoms. Even though remarkable improvements have been made in both the acid curing and radiation curing of surface coatings, additional control measures to eliminate airborne contaminants and improved work practices are required.

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# Dermatological Risk to Workers in the Ultraviolet Radiation Wood Surface Coating Industry

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Surface coating of wood with ultraviolet radiation (UV) curable acrylate coatings is rapidly increasing due to an attempt to reduce the industrial use of organic solvents. Workers from 38 (out of 48) industrial plants using this technology were identified. Altogether, 659 individuals participated in this investigation. Workers who had past or present eczematous skin disease (184) were included in the dermatologic examination. Of those, 144 were investigated clinically and patch tested with TRUE Test™ standard panels and a test panel containing ingredients that are included in UV coatings. Forty individuals were telephone interviewed. Present eczema or history of eczema excluding seborrheic

dermatitis was observed in 28% of the UV line workers and finishers and in 18% of the unexposed matched controls. The total eczema frequency in the UV line workers was the greatest of the exposure groups and significantly different from the control group. One individual had a positive reaction to acrylates and had previously experienced eczema, when working on the UV line. The UV radiation emitted from the UV curing units is highly irritating. This study indicates problems of irritant dermatitis but low frequency of contact allergy in workers employed in UV surface coating industry. Protective measures are required to reduce worker exposure to UV coatings and UV radiation.

## INTRODUCTION

New types of coatings have been introduced to the wood surface coating during the last ten years in Sweden. Occupational exposure to new, potentially hazardous, agents has increased in association with the use of acrylate surface coatings cured with ultraviolet radiation (UV coating).<sup>1,5</sup> The UV coatings are composed of three basic components: an acrylated prepolymer (urethane acrylate, polyester acrylate, etc.), a multifunctional acrylate monomer (e.g., tripropylene glycol diacrylate, TPGDA; 1,6-hexanediol diacrylate, HDDA; etc.), and a photoinitiator system (e.g., benzophenone, etc.).<sup>1</sup> The UV coatings can also contain other additives (e.g., fillers, plasticizers, stabilizers, catalyzers, pigments,

etc.). The number of workers employed in the UV surface coating industry is increasing as the industry expands; the volume growth of the use of acrylate surface coatings is estimated to be 15-20% per annum until the year 2000.<sup>3,4,8</sup>

Our knowledge of the overall potential health effects associated with exposure to acrylate surface coatings is inadequate. The biological effects of multifunctional acrylates has been insufficiently investigated. No data on absorption, metabolism, and/or distribution, and oral, inhalation, or ocular toxicity of multifunctional acrylates has been published. Skin irritation and allergic contact dermatitis from exposure to acrylates are the most likely health effects

to workers.<sup>2, 5, 11-14, 21</sup> Both multifunctional acrylates and methacrylates are potent sensitizers in humans and guinea pigs; triacrylates appearing to be more potent sensitizers than diacrylates. Many inhibitors that are added to acrylate resins to prevent spontaneous polymerization are also considered to be possible skin sensitizers.<sup>19, 20</sup> However, the potential sensitization due to inhibitors can usually be considered negligible because of their low concentration (10-250 ppm) in acrylate resins. Previously, we conducted an industrial hygiene survey in this industry to determine potential worker exposure to hazardous agents and work practices.<sup>15</sup> Based on the survey results we decided to evaluate clinically the incidence of skin disease and sensitization due to acrylate surface coatings in workers employed in this industry.

## MATERIALS AND METHODS

### Study Population

Identification of industrial sites and the characterization of the work force have been previously described.<sup>15</sup> In this study, we identified all workers exposed to uncured acrylate coatings and UV radiation and/or cured acrylate coatings in Sweden.

Table I. Summary statistics of the study population according to the type of exposure.

Study Group	I.	II.	III.	IV.	V.	VI.	VII.
(N=173)	(N=146)	(N=57)	(N=54)	(N=97)	(N=71)	(N=61)	(N=61)
Sex (females/males)	24/122	3/54	29/23	26/71	7/64	13/48	61/112
Age (average)	37.7	41.6	39.9	39.3	39.3	39.2	42.2
Employment in UVR surface coating (years)	3.6	0	5.0	4.1	3.8	2.3	0
Employment in wood industry (years)	11.4	16.0	9.8	15.0	12.4	13.8	14.8
Smokers (%)	52	30	41	28	32	20	18
Alopy (%)	22	13	26	17	17	34	23

Seven groups of subjects were formed from the study population of 659 workers and investigated. All investigations were performed in the work places during the working hours. Summary statistics of the study population are presented in Table I. Group (I) consisted of 146 UV surface coating line workers (*UV line*), exposed to uncured and/or cured acrylate coatings and UV radiation (UVR). Group (II) consisted of 57 workers employed in acid curing surface coating line and/or spray painting with acid curing lacquers and paints (*AC worker*), exposed to organic solvents, formaldehyde, and uncured and/or cured acid curing amino resins. Group (III) consisted of 54 workers employed in the finishing processes of UVR cured acrylate surface coated products (*UV finisher*), exposed to cured and/or partially cured acrylate coating dust and wood dust. Group (IV) consisted of 97 workers employed in the finishing processes of both UVR cured acrylate and acid curing surface coated products (*UV/AC finisher*), exposed to cured and/or partially cured acrylate and acid curing coating dust and wood dust. Group (V) consisted of 71 workers employed in the surface coating line using both UV acrylate and acid curing

coatings (*UV/AC line*). Group (VI) consisted of 61 workers previously employed in the UV line, but had moved to other work tasks, that did not include exposure to UV acrylate coatings (*prior UV*). Group (VII) was a control group of 173 office workers (*control*) from the same companies and a village, where many of the surveyed plants were located.

### Assessment of Symptoms and Clinical Diagnosis

Questionnaires were distributed to all workers in the study population and evaluated before examination and patch testing by an occupational dermatologist. The questionnaire included questions concerning previous and present work tasks, employment time, sex, age, smoking history, airways and eye symptoms, alopy, previous and current skin problems with special regard to problems with exposure to different working materials, use of personal protective equipment, and relationship between work tasks and skin symptoms. Clinical examination of the skin of hands, arms, face, and upper body was conducted in all workers that expressed skin problems (eczema) as a child or an adult. Patch testing was performed using a standard test panel (Table II; TRUE Test™, Pharmacia AB, Uppsala, Sweden) and a special test series with chemicals included in UV coatings (Table III) obtained from Chemotechnique Diagnostics AB, Malmö, Sweden (acrylates and inhibitors) and from Ciba-Geigy AB, Göteborg, Sweden (initiators). White petrolatum was used as the vehicle for the chemicals in the special acrylate test series. The patch tests were placed on the upper back. Patch tests were evaluated according to international standards (Table

IV),<sup>6 72 h</sup> after application and 24 h after the removal of the patches.

## RESULTS

Altogether, 659 workers, 496 men (75.3%) and 163 women (24.7%), participated in the study. The age distribution between the study groups was even (avg. 40 ± 11 years) as was the employment time in the wood industry (avg. 13 ± 10 years) in the exposure groups. Most of the workers at the UV lines had been employed 55 years (83%) in their present work. More than half of these workers (66%) had been working at the UV lines 1-5 years.

From the questionnaire, a total of 132 workers (19%) were determined to be alopic. The highest frequency of alopy (34%) was observed in group VI (*prior UV*) and the lowest (13%) in group II (*AC line*). Group I (*UV line*) had a similar frequency of alopy than the control group (22% and 23%, respectively).

Dermatologic symptoms such as erythema, itching, and dryness experienced by individuals in different exposure groups are presented in Table V. Workers in groups I (*UV line*) and III (*UV finisher*) experienced skin irritation, itching, and dryness from UV acrylate coating and coating dust. Workers in group II (*AC line*) reported itching and dryness, but not erythema from acid curing coatings and coating dust. Itching and dryness were also reported from individuals in all exposure groups when exposed to wood dust.

Dermatologic symptoms from UV lamps were reported by few individuals. In those individuals, the most frequently reported skin symptoms were erythema, edema, and pain after an accidental exposure to UVR during lamp service. Erythema and smarting was also reported by hand fillers, who

Table II. Standard test series (TRUETest™). Vehicles used for test material: hydroxypropylcellulose, carboxymethylcellulose, or polyvinylpyrrolidone.

1. Nickel Sulfate	13. p-Tert-Buylphenol Formaldehyde Resin
2. Wool Alcohols	14. Parabene Mix
3. Neomycin Sulfate	15. Carba Mix
4. Potassium Dichromate	16. Black Rubber Mix
5. Caine Mix	17. Cl+Me Isobiazolinone
6. Fragrance Mix	18. Quaternium-15
7. Colophony	19. Microgynobenzothiazole
8. Epoxy Resin	20. p-Phenylene diamine (PDD)
9. Quinoline Mix	21. Formaldehyde
10. Balsam of Peru	22. Mercapto Mix
11. Ethylenediamine Dihydrochloride	23. Thimerosal
12. Cobalt Chloride	24. Thiuram Mix

Table III. Acrylate patch test series. Vehicle used for test material was white petrolatum. The weight percent of the chemical in the vehicle is expressed in parenthesis.

25. Epoxy Acrylate (0.5%)	30. Triethylol Propane Triacrylate (TMPTA; 0.1%)
26. Oligo Triacrylate (OTA) = Glycerol Propoxy Triacrylate (0.1%)	31. Tripropylene Glycol Diacrylate (TPCDA; 0.1%)
27. Methylhydroquinone (1%)	32. 2-Hydroxy-2-methyl-1-phenylpropane (Dacour 1173; 2%)
28. 1,6-Hexamethiol Diacrylate (HDDA; 0.1%)	33. 2,2-Dimethoxy-1,2-diphenylethane-1-one (Ugacure 651; 2%)
29. Triethylene Glycol Dimethacrylate (TEGDMA; 2%)	34. 1-Hydroxy-cyclohexyl-phenyl-ketone (Ugacure 184; 2%)

Table IV. Patch test scoring.

-	Negative reaction
7/IR	Questionable or Irritative reaction, which do not fulfill criteria for positive reaction
+	Weak positive reaction, redness, infiltration, and discrete papules
++	Distinct positive reaction, redness, infiltration, papules, and sometimes vesicles
+++	Strong positive reaction, intensive redness, infiltration, and joining vesicles

Table V. Experienced intolerance for some working materials in the exposure groups. Results for erythema (E), Irritation (I), dryness (D) are expressed as percentage (%) of discomfort in the exposed groups.<sup>1</sup>

Study Group	UV acrylate coating		Acid curing coating		Coating dust		Wood dust									
	E	D (%)	E	D (%)	E	D (%)	E	D (%)								
I and VI <sup>2</sup>	7	4	9	135	2	2	10	93	5	1	3	89	0	3	16	122
III and IV <sup>2</sup>	3	5	14	63	0	4	19	57	4	4	25	72	1	4	25	76
V	4	9	9	56	3	5	28	60	2	2	25	56	2	4	15	53
II	0	0	9	111	2	11	30	44	0	5	38	40	0	3	24	33

<sup>1</sup> The control group was not considered relevant concerning these questions in the questionnaire, because their work history did not include working with these agents.

<sup>2</sup> Groups I and VI and III and IV were pooled together because worker exposure and/or work tasks (present or previous) were considered similar.

worked in the front of poorly shielded UV units. ers used skin protective creams during work and ~40% used hand cream after work.

The use of gloves in performing work tasks did not differ between the different exposure groups. Approximately half of the workers reported that they use gloves some or most of the time (32% and 19%, respectively) and 20% reported to use gloves all the time. Operations during which gloves were worn by workers in the UV surface coating were feeding and receiving of the products on the UV line, direct contact with coating, and finishing work. The gloves most commonly used were cloth-leather gloves. Rubber gloves were most commonly used when contact with uncured acrylate coating occurred. Approximately 30% of the exposed workers used skin protective creams during work and ~40% used hand cream after work. Altogether, 144 individuals with history of past or present dermatitis were investigated clinically and with the patch tests. Forty individuals with history of eczema were interviewed by telephone. The reason for the most of the telephone interviews was the distant location of an industrial plant and very few individuals (usually one or two) with skin disease(s) were involved at each plant. The distribution of investigated individuals with skin disease in the different study groups are presented in Table VI. The clinical investigation disclosed an expected panorama of non-eczematous skin

Table VI. Number of individuals investigated with a history of eczematous skin disease according to the study group.

Study Group	I. (N=146)	II. (N=57)	III. (N=54)	IV. (N=97)	V. (N=71)	VI. (N=61)	VII. (N=173)
Clinical investigation	44	12	18	13	11	14	32
Telephone interview	9	3	1	12	8	1	6

disease among individuals investigated. Different types of eczema were diagnosed in 168 individuals investigated (Table VII). Irritant contact dermatitis was significantly more common among workers in groups I (*UV line*; odds ratio 3.77, 95% confidence limit 1.86-7.63) and II (*AC line*; odds ratio 2.85, 95% confidence limit 1.16-7.02) than in the control group. Distribution of eczematous disease and relation to work tasks in all exposure groups was significantly different from the control group ( $p < 0.05$ , Fisher's exact test; Table VIII). However, in group I (*UV line*) the total eczema frequency was the greatest of the exposure groups and significantly different (odds ratio 2.07, 95% confidence limit 1.24-3.43) from the control group. Twenty of the exposed workers (13%) judged the work with the UV curable acrylic coatings to be the main cause of the disease. No cases of skin cancer were observed in this study.

Altogether, 49 positive patch test reactions were observed in 31 individuals (Table VIII). The test reactions and their

intensity are presented in Table IX. Only one individual was allergic to acrylates. This individual left the UV line work due to eczematous skin disease, which healed after leaving the work environment. No other patch test reaction had direct association to the work with UV coatings. The three reactions to colophony were associated with previous or present contact with wood. The strongest reaction was observed in an office worker who had had hand eczema when working in wood finishing. Four of the nickel reactions were potentially work related since they occurred in individuals with hand eczema who assembled furniture with nickel plated components. Two reactions, one to formaldehyde and one to *p*-phenol-formaldehyde resin were related to exposure to acid curing coatings or to hard board dust. Most of the remaining positive patch test reactions were related to past or present work activities (e.g., chromium - construction work) or leisure activities. The relevance for the caine-mix, carba-mix and thimerosal reactions is unknown. Except for methyl hydroquinone, which gave

Table VII. The number of individuals working in the wood surface coating industry and the type of eczema observed according to exposure groups (I-VI) and the matched control group (VII). Those with a positive patch test are indicated by parentheses.

Eczema / Study Group	I.	II.	III.	IV.	V.	VI.	VII.
Atopic dermatitis	6 (1)	2	6 (1)	9 (2)	2	2	13 (2)
Contact dermatitis	32† (3)	10† (3)	7 (1)	6 (2)	10 (1)	7 (3)	12 (5)
Nummular eczema	1	-	1	1	2	1	1
Schoenleichen dermatitis	9 (1)	4	2 (1)	5	-	1	3
Tyloid eczema	-	-	-	1	-	-	-
Vesiculosis	1	-	1	-	-	1	3 (2)
Other eczema	-	-	-	1	3	-	2
Total	49*†	16	17	23	17	12*	34*

\* One individual with psoriasis had a positive patch test reaction.

† Significantly different from the control group.

Table VIII. Eczematous disease among exposure groups in the wood surface coating industry and the unexposed control group. Results are expressed as the number of workers, whose disease was not work related compared to those, whose disease was determined to be work related to the previous or present occupation.

Eczema / Study Group	I.	II.	III.	IV.	V.	VI.	VII.
Hand eczema							
- past work	1/7	-	1/2	0/1	0/1	1/0	4/0
- present work	1/3	1/2	-	1/1	2/0	1/1	3/0
Other eczema localization							
- past work	6/6	1/3	3/1	4/1	4/1	1/0	8/0
- present work	3/4	1/1	3/1	2/3	1/1	0/3	7/0
Hands and other eczema localization							
- past work	2/3	2/1	0/1	3/1	2/4	0/3	3/0
- present work	1/3	-	1/2	0/1	1/0	0/1	6/0
Total	14/26	5/7	8/7	10/8	10/7	3/8	31/0
Total % in the group	27.4	21.1	27.8	18.6	23.9	18.0	17.9

18 irritant reactions of erythematous type, the acrylic test series presented few irritant reactions.

## DISCUSSION

Forty-eight industrial plants producing UV radiation cured acrylic surface coated wood products were identified. Approximately 8,500 workers are employed in these industrial plants and ~350 employees work on the UV lines and ~700 in finishing processes of UV cured wood products. For this study, 38 of these industrial plants chose to participate. With few exceptions, we identified all UV line workers and workers previously employed on a UV line in the participating plants. Thus, the investigated group of UV line workers represents a majority of the Swedish UV line workers with this particular occupational exposure. For every exposed worker we attempted to obtain one control individual matched for

sex and age among workers employed at acid curing surface coating lines, finishers employed in UV surface coated products, and office workers. However, there were too few acid curing line workers as well as UV finishers to obtain a satisfactory match. Therefore, finishers with mixed exposures were also included, but are presented separately, in this study.

Skin exposure to UV coatings varies, but for all plants a significant exposure to UV line workers occurred when cleaning UV line equipment.<sup>15</sup> We were therefore surprised, that only one individual was positive for an allergic reaction to a acrylic patch test series. The special acrylic patch test series included most commonly used acrylics and other proprietary ingredients used in the UV curable coatings for wood. During the investigation, we observed, that some coatings contained acrylics (i.e.,

Table IX. Number of positive test reactions for different allergens.

Chemical	+	++	+++
1. Nickel Sulfate	2	7	3
4. Potassium Dichromate	1	2	
5. Gaine Mix	1		
6. Fragrance Mix	1	3	1
7. Colophony	1	1	1
10. Balsam of Peru	1	1	1
11. Ethylenediamine Dihydrochloride	1	1	
12. Cobalt Chloride		2	1
13. p-tert-Butylphenol Formaldehyde Resin			
15. Carba Mix		1	
18. Quaternium-15		4	
20. p-Phenylenediamine (PDD)		1	
21. Formaldehyde		2	
22. Mercapto Mix	1		
23. Thimerosal	1	2	
25. Epoxy Acrylate (0.5%)	1		
26. Oligo Triacrylate (OTA; 0.1%)	1		
28. 1,6-Hexanediol Diacrylate (HDDA; 0.1%)	1		
29. Triethylene Glycol Dimethacrylate (TEGDMA; 2%)	1		
30. Trimethylol Propane Triacrylate (TMPTA; 0.1%)	1		
31. Tripropylene Glycol Diacrylate (TPGDA; 0.1%)	1		

glycerol propoxy triacrylate and ethylidiglycol acrylate) and/or hardeners (i.e., methyl ketone peroxide, polymeric isocyanate, monomeric isocyanate, polyfunctional aziridine) that were not included in this patch test series. However, the use of these chemicals was restricted to a few plants.

This investigation of dermatologic symptoms indicates irritant problems with the skin connected to acrylate and/or UV radiation exposures at UV lines. The workers on the UV lines reported three different clinical types of irritant dermatitis: (1) localized well demarcated distinct "burns" on back of hands and arms at the site of accidental contact with uncured UV coatings;

(2) dry, fissuring, skin with fine scaling observed in all types of irritant work; and (3) diffuse itchy erythematous swelling of the hands and arms followed by scaling, which lasted during work on the UV line and disappeared within a few days, but reappeared with repeated exposure; these symptoms were reported mainly with UV coatings no longer in common use.

UV radiation emitted from the UV lamps was measured to have high intensity and a highly irritant spectral distribution and a direct exposure to this type of radiation could result in burns within seconds.<sup>15</sup> Most of the UV units provided protection from direct UV radiation. However, work stations

with emission of UV radiation from entrance and exit slits were observed and this sometimes resulted in minor erythema and pigmentation on exposed workers. Reports of accidental overexposure and burns while servicing UV lines with lamps on and without adequate protection for the skin.

The UV coatings contain chemicals which may be toxic to the skin and may cause skin irritation, sensitization, and allergic contact dermatitis.<sup>2, 5, 9-14, 18-21</sup> In this study the irritant and sensitizing potential of acrylates was also observed. Clinical investigation of upper airways and eyes in the UV line workers and finishers of UV surface coated wood products disclosed nasal, pharyngeal, and ocular symptoms of discomfort and impaired mucociliary clearance and olfaction.<sup>7</sup> These findings indicate that workers employed in these particular occupations are exposed to irritant and/or sensitizing agents. Further investigations are required to determine the etiology of these observed symptoms.

Dermal toxicity of the acrylates used in the UV coatings has been insufficiently investigated. Recent studies suggest that tripropylene glycol diacrylate, a commonly used multifunctional acrylate in UV coat-

ings, may have the potential to induce and/or promote epithelial tumors when topically applied to the skin of mice.<sup>16</sup> Synergistic effects between UV radiation and acrylate toxicity have been observed in human cells in vitro.<sup>17</sup> No cases of skin cancer were observed in workers employed in the UV surface coating industry in this study. However, the average length of employment in the UV line is 3 years<sup>15</sup> and further observations will be required. Further investigations on occupational UV radiation and acrylate exposure are required to estimate the risk to chronic exposures. Protective measures, worker education, and improved work practices are required to minimize and manage a possible risk to workers employed in this industry.

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V

# Chemical Effects in TG·AC (v-Ha-ras) Mice: Tripropylene Glycol Diacrylate, but not Ethyl Acrylate, Induces Skin Tumors in a Twenty Week Short Term Carcinogenesis Study

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## Summary

The toxicity and potential carcinogenicity of the esters of acrylic acid are poorly understood. We used the TG·AC transgenic mouse to conduct comparative short term bioassays to determine the potential carcinogenicity of tripropylene glycol diacrylate (TPGDA) alone and in a reference ultraviolet radiation curable lacquer (Lacquer A). Ethyl acrylate (EA) was used as a reference acrylate. Insertion of the  $\zeta$ -globin promoter v-Ha-ras transgene into the FVB mouse genome (TG·AC) introduced a defined genetic lesion, which is critical, but insufficient by itself, to induce tumorigenesis. EA is a monofunctional acrylate that induces forestomach tumors when administered by intubation. The toxicity of TPGDA, a difunctional acrylate, and Lacquer A (TPGDA) was unknown. The dose of TPGDA that killed 50% of the mice was estimated to be 120  $\mu$ moles/mouse (4 mmoles or 1.2 g/kg bw). Based on dose related increases in skin hyperplasia in range finding studies, dosages of EA (0, 60, 300, or 600  $\mu$ moles/mouse), TPGDA, or Lacquer A (applied equimolar for TPGDA concentration; 0, 1, 5, or 10  $\mu$ moles/mouse) were selected. Starting at 12 weeks of age, the doses were administered topically (200  $\mu$ l of acetone vehicle) 3x/week for 20 weeks

to the shaved dorsal skin of female TG·AC mice (10/group). TPGDA and reference Lacquer A (equimolar for TPGDA) at 5 or 10  $\mu$ moles/mouse, but not EA (60, 300, or 600  $\mu$ moles/mouse) or TPGDA or Lacquer A at 1  $\mu$ mole/mouse induced a dose related increase in papillomas between 6 and 12 weeks of treatment that reached a maximum number of papillomas per mouse between 19 and 20 weeks of treatment. We conclude from these results that TPGDA is significantly more toxic than EA and is a potential rodent carcinogen at the site of contact when applied to the skin.

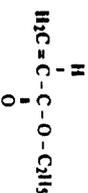
## Introduction

Esters of acrylic acid are used in the manufacture of lacquers, paints, inks, etc. [1]. Recently, the use of UV radiation cured acrylate based surface coatings has increased as fundamental changes in the industry have occurred [2]. There are approximately 200 acrylates listed on the United States Environmental Protection Agency's Toxic Substances Control Act Inventory that are of potential industrial use [3] and most are of unknown potential biological activity or toxicity. Only a few of these important acrylates have been studied for their effects in biological systems.

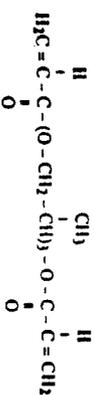
Ethyl acrylate (EA), a monofunctional acrylate, is a carcinogen when administered orally by intubation, causing papillomas and squamous cell carcinomas in the forestomachs of rats and mice [4] and is considered a potential human carcinogen [5]. However, under the experimental conditions employed, EA was not found to be carcinogenic to rats or mice by dermal application [6] or inhalation [7]. 2-Ethylhexyl acrylate [8, 9] and neopentyl glycol diacrylate [8] induced benign and malignant skin tumors in C3H/HeJ mice when administered topically. Tetraethylene glycol diacrylate or triethylene glycol diacrylate, but not trimethylol propane triacrylate, 1,6-hexanediol diacrylate, tripropylene glycol diacrylate (TPGDA), tetraethylene glycol dimethacrylate, or trimethylol propane trimethacrylate, induced a low incidence of either benign or malignant skin tumors in male C3H/HeJ mice (age not stated) when treated 2x/week for 80 weeks (2.5 mg/mouse for acrylates or 25 mg/mouse for methacrylates in mineral oil) [10].

In general, simple monofunctional acrylates, like EA, are considered to be more toxic, but less potent contact sensitizers, than multifunctional acrylates, like TPGDA (see Fig. 1 for chemical structures). The acrylates have been classified based on a comparison of their structure activity relationships and potential biological activity [3]. Acrylates are electrophiles and have an inherent potential for carcinogenicity. As potential direct alkylating agents, their toxicity is expected to be limited by their ability to interact with biological systems. The major route of detoxification of acrylates and methacrylates is their conjugation with glutathione [11-13], via Michael's addition reaction or catalysis with glutathione-S-transferase [14]. Conjugation of

acrylates with glutathione is expected to be proportional to the number of functional acrylate groups. The available data suggests that acrylates most likely act at the site of contact, conjugate available glutathione, and are hydrolyzed via carboxylesterases [11, 12, 15-18].



Ethyl Acrylate (EA)



Tripropylene Glycol Diacrylate (TPGDA)

Fig. 1. Chemical structure of ethyl acrylate and tripropylene glycol diacrylate. Electrophilic structural alerts of the acrylate functional groups are in bold.

Short term dermal carcinogenesis studies for identification of hazardous chemicals that may act directly with the skin or where systemic exposure occurs via skin are a potential valuable complement to long term toxicity and carcinogenicity studies. In the TG•AC transgenic mouse, insertion of a  $\zeta$ -globin promoted v-Ha-ras transgene into the genome introduces a defined genetic lesion, which is critical, but insufficient by itself, to induce tumorigenesis [19, 20]. Treatment of TG•AC mice with the tumor promoter, 12-O-tetradecanoyl-13-phorbol acetate (TPA) results in a dose-dependent and rapid induction of epidermal papillomas [19]. Both complete carcinogens and tumor promoting agents applied topically

are active in this mouse line [20]. We describe here a short term carcinogenesis study comparing EA to TPGDA, and a reference ultraviolet radiation cured lacquer (Lacquer A; containing TPGDA monomer as the active ingredient) for the purpose of determining (1) if acrylate toxicity (i.e. carcinogenicity) is a function of the number of acrylate functional groups and (2) the potential dermal toxicity of these important acrylates.

## Materials and methods

### Chemicals

Ethyl acrylate (EA; CAS No. 140-88-5; 100.12 g/mole) was received from Aldrich Chemical Co. (Madison, WI, USA) as a colorless liquid with a pungent odor at 99% purity. Tripropylene glycol diacrylate (TPGDA; CAS No. 42978-66-5; 300.25 g/mole), Lacquer A (reference lacquer containing only the base components for ultraviolet radiation curable wood surface coating lacquer), and a commercially available ultraviolet radiation curable wood surface coating lacquer (Lacquer B) were obtained from Nobel Coatings (Malmö, Sweden). TPGDA was received as a pale-yellow tinted liquid with a mild odor at 80% pure monomer with >18% TPGDA oligomer. Lacquer A was a pale-yellow tinted liquid with an acrid odor and according to the manufacturer contained 56.4% TPGDA monomer (remaining components were proprietary ingredients). Lacquer B was a dark opaque and viscous liquid with a acrid odor and according to the manufacturer contained 24.8% TPGDA monomer (remaining components were proprietary ingredients). Due to the tendency of the acrylates to polymerize when exposed to light, hydroquinone (<200 ppm) was added as an inhibitor to each test article by the

manufacturer. Samples were stored in the dark at 4-6°C. Analyses of the stability of the test compounds were not carried out. All studies were conducted during the manufacturer's warranty for chemical stability. Acetone (CAS No. 67-64-1, Aldrich Chemical Co. Madison, WI, USA) was used as a vehicle control. For a positive control, 12-O-tetradecanoyl-phorbol-13-acetate (TPA; CAS No. 16561-29-8) was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

### Mice

Female homozygous TG•AC transgenic mice ( $\zeta$ -globin promoter fused with the v-Ha-ras oncogene on the FVB strain) were obtained from Taconic Farms (Germantown, NY, USA). Upon receipt all animals were examined and acclimated for two weeks. Beginning at 12 weeks of age each mouse received three weekly (Monday, Wednesday, Friday) topical applications of the appropriate chemical or vehicle (200  $\mu$ l/mouse, ~8 cm<sup>2</sup>). Mice were housed 3-5 per cage. Animal rooms were maintained at 71  $\pm$  7 °F and 50  $\pm$  20 % relative humidity with 10 fresh air changes per hour and 12 hours of fluorescent light daily (animals were protected from receiving direct radiation). City water and Purina PicoChow No. 5058 in pellet form (Barnes Supply Co., Durham, NC, USA) were available *ad libitum*.

### Selection of test chemical dosage

A series of 2 week dose finding studies were carried out using the same mouse strain, sex, age, and experimental conditions. EA (60, 300, or 600  $\mu$ moles, TPGDA, (1.0, 5.0, 60, 300, or 600  $\mu$ moles), Lacquer A (0.5, 1.0, or 5.0  $\mu$ moles), Lacquer B (0.5, 1.0, or 5.0  $\mu$ moles) or TPA (2 nmole) were administered to groups of

3-4 female mice once daily on 7 separate days (Monday, Wednesday, Friday, etc.) over a 15 day period. Chemicals were applied in acetone vehicle in a 200  $\mu$ l dosing solution (total volume) to the shaved back of each mouse from the mid-back (interscapular region) to near the base of the tail ( $\approx 8$  cm<sup>2</sup>) using a calibrated micropipette. To evaluate the dose dependency for increasing skin thickness (epidermal keratinocyte hyperplasia), three concentrations of EA, TPGDA, and Lacquer A in acetone were selected for the short term experiment.

#### Treatment

Based on the body weights, mice were randomly assigned to treatment or control groups: 10 female TG-AC mice per dose group were selected for the 20 week study. These animals were divided into 3 dose groups per test substance except for the positive and negative control groups which both had only one dose group. One positive and one vehicle control group served as control groups for all the test substances (EA, TPGDA, and Lacquer A) for data comparison. Prior to the first dose application, all animals were inspected and their backs closely shaved on the site of application, while avoiding the production of abrasions or cuts. On each day before the treatment all animals were inspected for hair growth and shaved again, if needed. The first anagen phase of hair growth in mice is usually completed by 10-12 weeks of age.

The test substances, dissolved in acetone in 200  $\mu$ l total volume were applied as described above. Mice were treated with a single dose of 200  $\mu$ l total volume per animal three times a week (Monday, Wednesday, and Friday) for 20 weeks.

#### General observations

Individual body weights were recorded at the beginning of the study, twice weekly through the study, and at study termination. All animals were observed in their cages twice daily for signs of morbidity or mortality. Weekly, clinical observations were performed and the test sites were examined for signs of irritation and local skin lesions as well as the onset and progression of tumor growth.

#### Post-mortem procedures

**Dose Finding Study:** At study termination, gross necropsies were performed on all mice. A section of skin from the test site was collected in Bouin's fixative and the inguinal lymph nodes were collected in 10% neutral buffered formalin. The skin test site and the inguinal lymph nodes were processed and subjected to histopathological analysis. In addition, the test site skins were coded and graded in a blind fashion for epidermal thickness.

Relative skin thickness was determined by preparing each mouse's dorsal skin by excision and fixation for 24 h in 10% neutral buffered formalin, processed, and embedded in paraffin. Approximately 6  $\mu$ m sections were bichromically stained with hematoxylin and eosin for histological examination. Morphometric analysis was performed with an ocular micrometer. The number of nucleated epidermal cell layers was counted at five randomly selected locations per slide and averaged. The thickness of the nucleated cell layers of the epidermis was also measured in a similar manner, and the means  $\pm$  standard errors of the mean were calculated.

**Twenty Week Study:** Complete necropsies were carried out on mice that died or were sacrificed when moribund. A section

Table 1. Survival from acute toxicity to tripropylene glycol diacrylate applied to the skin of TG-AC mice.

Study	0	12	60	120	300	600
	Jumoles / Mouse / Application					
# 1	4/4	4/4	4/4	2/4*	0/4*	0/4*
# 2	4/4	ND	4/4	2/4**	ND	ND
Total	8/8	4/4	8/8	4/8	0/4*	0/4*

ND = not done

\* 2/4 or 4/4 died by day 2 before 2nd dose application

\*\* 2/4 died by day 3 before before 2nd dose application

of skin from the test site was collected in Bouin's fixative and all gross lesions were placed in 10% buffered formalin.

#### Results

##### Acute Dose Finding Study

The dose required to kill 50% of the mice that received a single topical application of TPGDA was estimated (Table 1) at 120  $\mu$ moles per mouse (4  $\mu$ moles or 1.2 g/kgbw). All mice receiving lower doses received 7 doses total without fatality. The minimum lethal dose of EA was greater than 600  $\mu$ moles per mouse ( $>20$   $\mu$ moles or  $>2$  g/kg bw) under the conditions of this study (data not shown). Pozzani *et al.* [21] reported the LD<sub>50</sub> value in rabbits (strain not cited) to be  $\approx 1.8$  g or 20  $\mu$ moles EA/kg bw.

Normal mouse skin is 1 or 2 epithelial cell layers in thickness. In response to 2  $\mu$ moles TPA per mouse topically applied to the dorsal epidermis a thickening of the epidermis occurred (Fig. 2). No treatment related differences were observed in histopathological examination of the inguinal lymph nodes (data not shown). EA at 600  $\mu$ moles per mouse induced a similar level of relative skin thickness or hyperker-

atosis when compared to 5  $\mu$ moles TPGDA per mouse (both significantly different from the control at P $<0.029$ ; Fig. 2). A no effect level for increasing relative skin thickness was observed at 300  $\mu$ moles EA per mouse or 1  $\mu$ mole TPGDA alone or in Lacquer A or Lacquer B (Fig. 2). Therefore, we selected doses of 60, 300, or 600  $\mu$ moles EA per mouse or 1, 5, or 10  $\mu$ moles TPGDA alone or in Lacquer A for the short term carcinogenesis studies by skin paint. Because differences in acute toxicity were not observed between the two lacquer formulations, further studies with Lacquer B were not conducted.

##### Short Term (20 Week) Carcinogenicity Study

After repeated application of TPA, TPGDA, or TPGDA in Lacquer formulation A, three times a week for 20 weeks, focal areas of hyperkeratosis developed into papillomas. TPGDA showed a marginal dose related increase in papillomas per mouse over time when compared to the vehicle control (acetone) or EA (Fig. 3). Similar effects were observed with Lacquer A (adjusted equimolar to TPGDA alone based on percent TPGDA) at 5 or 10

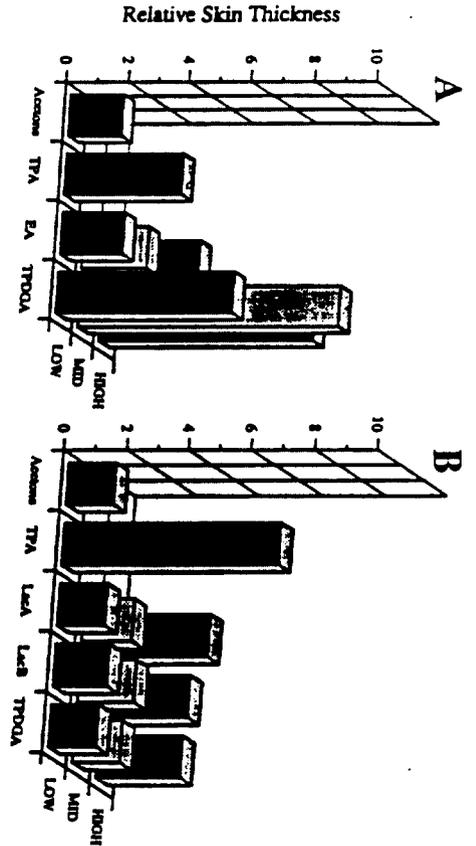


Fig. 2. Relative skin thickness after chemical treatment in the acute dose finding studies (3x/week for 7 applications total). Relative skin thickness was determined by counting the number of cell layers of the epidermis at five random sites in four different mouse skins from the same treatment group. A. Doses ( $\mu$ moles/mouse). TPA: 0.002. Ethyl acrylate (EA): LOW, 60; MID, 300; HIGH, 600. Tripropylene glycol diacrylate (TPGDA): LOW, 12; MID, 60; HIGH, 120. B. Doses ( $\mu$ moles/mouse). TPA: 0.002. Lacquer A (Lac A), Lacquer B (Lac B), or TPDGA: LOW, 0.5; MID, 1.0; HIGH, 5.0. Acetone vehicle control and all doses were delivered in 200  $\mu$ l acetone.

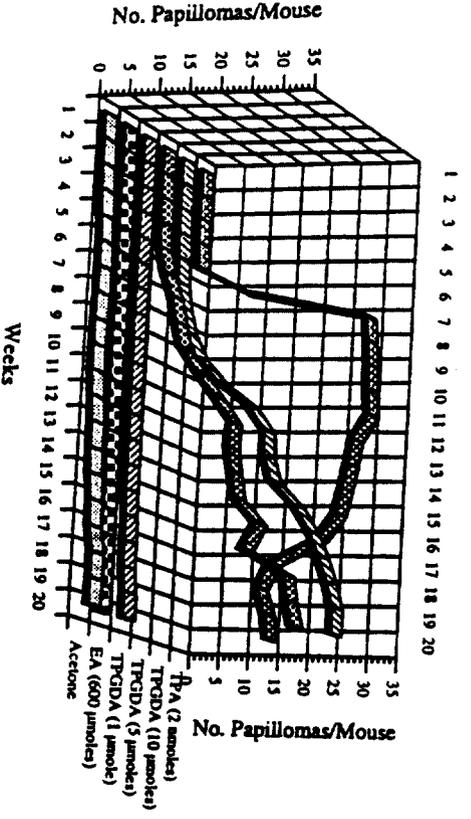


Fig. 3. Mean number of papillomas per mouse per week during a 20 week treatment period with ethyl acrylate (EA) and tripropylene glycol diacrylate (TPGDA).

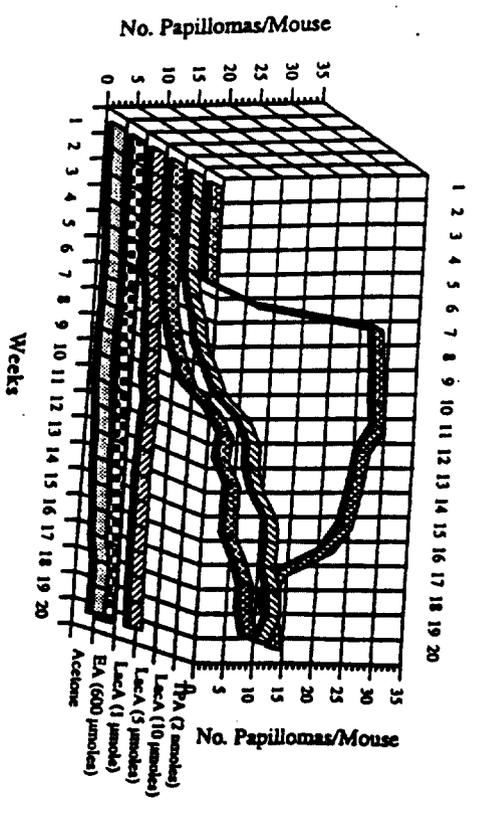


Fig. 4. Mean number of papillomas per mouse per week during a 20 week treatment period with Lacquer A (Lac A).

Table II. Effect of topical application of acrylates to the skin of TG-A-C mice: Incidence, survival, induction of papillomas, and tumor latency.

Chemical	Incidence <sup>1</sup>	Mean Survival Time (days $\pm$ SD)	Maximum Papillomas/Mouse (Mean $\pm$ SD)	Latency (days $\pm$ SD)
TPA (2 $\mu$ moles)	5/10	169.1 $\pm$ 59.4	31.7 $\pm$ 0.9	34.6 $\pm$ 2.8
Acetone (200 $\mu$ l)	10/10	200.1 $\pm$ 46.7	5.0 $\pm$ 6.8	120.4 $\pm$ 92.3
EA (60 $\mu$ moles)	2/10	217.1 $\pm$ 39.1	7.5 $\pm$ 3.5	107.5 $\pm$ 84.1
(300 $\mu$ moles)	5/10	227.2 $\pm$ 23.8	4.4 $\pm$ 5.9	103.6 $\pm$ 56.1
(600 $\mu$ moles)	2/9	186.2 $\pm$ 75.8	2.0 $\pm$ 1.4	123.5 $\pm$ 46.0
TPGDA (1 $\mu$ mole)	9/9	217.9 $\pm$ 30.0	5.4 $\pm$ 5.7	136.9 $\pm$ 41.5
(5 $\mu$ moles)	9/9	166.9 $\pm$ 62.6	29.2 $\pm$ 5.7	43.9 $\pm$ 16.8
(10 $\mu$ moles)	10/10	219.3 $\pm$ 30.6	30.4 $\pm$ 5.1	47.8 $\pm$ 12.1
Lac A (1 $\mu$ mole)	10/10	194.4 $\pm$ 60.5	5.8 $\pm$ 5.2	43.3 $\pm$ 20.0
(5 $\mu$ moles)	10/10	209.0 $\pm$ 31.8	18.3 $\pm$ 11.4	50.9 $\pm$ 10.0
(10 $\mu$ moles)	10/10	200.5 $\pm$ 38.7	23.1 $\pm$ 10.1	43.2 $\pm$ 11.3

<sup>1</sup> Incidence equals the number of tumor bearing mice over the total number of mice per group surviving more than 10 weeks of treatment

µmoles (Fig. 4). No effects were observed at 1 µmole TPGDA, 1 µmole Lacquer A (adjusted to equimolar TPGDA), or at even the highest dose of 600 µmoles EA (Fig. 4; lower dose EA data not shown).

In the TPA treated (positive control) group, early mortality, possibly due to tumor burden and other treatment effects, resulted in a dramatic decrease in the maximum number of papillomas per mouse (Fig. 3 or 4) in surviving mice. The rate of tumor occurrence was less in the acrylate treated groups (Fig. 3 and 4) compared to the TPA treated group.

Tumor incidence varied between the different treatment and control groups, but did not appear to be dose related (Table II). No significant differences were observed in mean survival time between any treatment group and the negative control (Table II). Mean latency (no. days  $\pm$  std dev until each mouse developed the first papilloma) was reduced between the negative control and TPA, 5 or 10 µmoles TPGDA, or 1, 5 or 10 µmoles Lacquer A (Table II). The mean number of papillomas per mouse was significantly different ( $P < 0.009$ ) between the negative control and the TPA positive control, 5 or 10 µmoles TPGDA or Lacquer A (Table II).

Body weights were depressed in the EA high dose group (Fig. 5A), but a dose response relationship was not observed in the TPGDA or TPGDA in lacquer formulation treatment groups (Fig. 5B and 5C).

## Discussion

In acute exposure studies, the difunctional TPGDA or TPGDA in lacquer formulation at 5 µmoles/mouse induced a level of relative skin thickness (hyperplasia) similar to 600 µmoles/mouse of the monofunctional EA ( $\sim 100$  fold difference in dose).

TPGDA applied at the same dose rate for 20 weeks (alone or in lacquer formulation) induced a maximum number of papillomas per mouse similar to the TPA positive control (2 nmoles/mouse), but a longer period of time was required. EA applied at these dose rates did not induce any chemical related tumors, even though 600 µmoles/mouse was sufficient to induce hyperplasia similar to TPGDA (5 µmoles/mouse) in the acute studies and resulted in hyperkeratosis in the 20 week study. These results suggest that properties inherent of TPGDA other than the number of functional acrylate groups may play a role in tumor induction when applied to the skin of these mice.

Only by oral administration (intubation, using corn oil as a vehicle) and repeated application to sustain hyperplasia has EA been carcinogenic to the site of application (forestomach) in rodents [4]. In a previous lifetime study, dermal application of EA (25 µl, neat) was negative for induction of skin tumors in C3H/HeJ mice [6]. The requirement for sustained cellular proliferation in the EA induction of forestomach tumors in rats has been well documented and may be dose rate and concentration dependent [22]. Few other acrylates have been investigated for their ability to induce skin tumors [8, 9].

In the acute studies (Fig. 2), apparent thresholds were observed for induction of an increase in relative skin thickness. For TPGDA the threshold appears between 1 and 5 µmoles/mouse. A significant increase in dose was required for EA to achieve a similar increase in relative skin thickness (between 300 and 600 µmoles/mouse). No differences existed in the induction of relative skin thickness between TPGDA alone, or TPGDA in Lacquer A or Lacquer B (when applied at equimolar

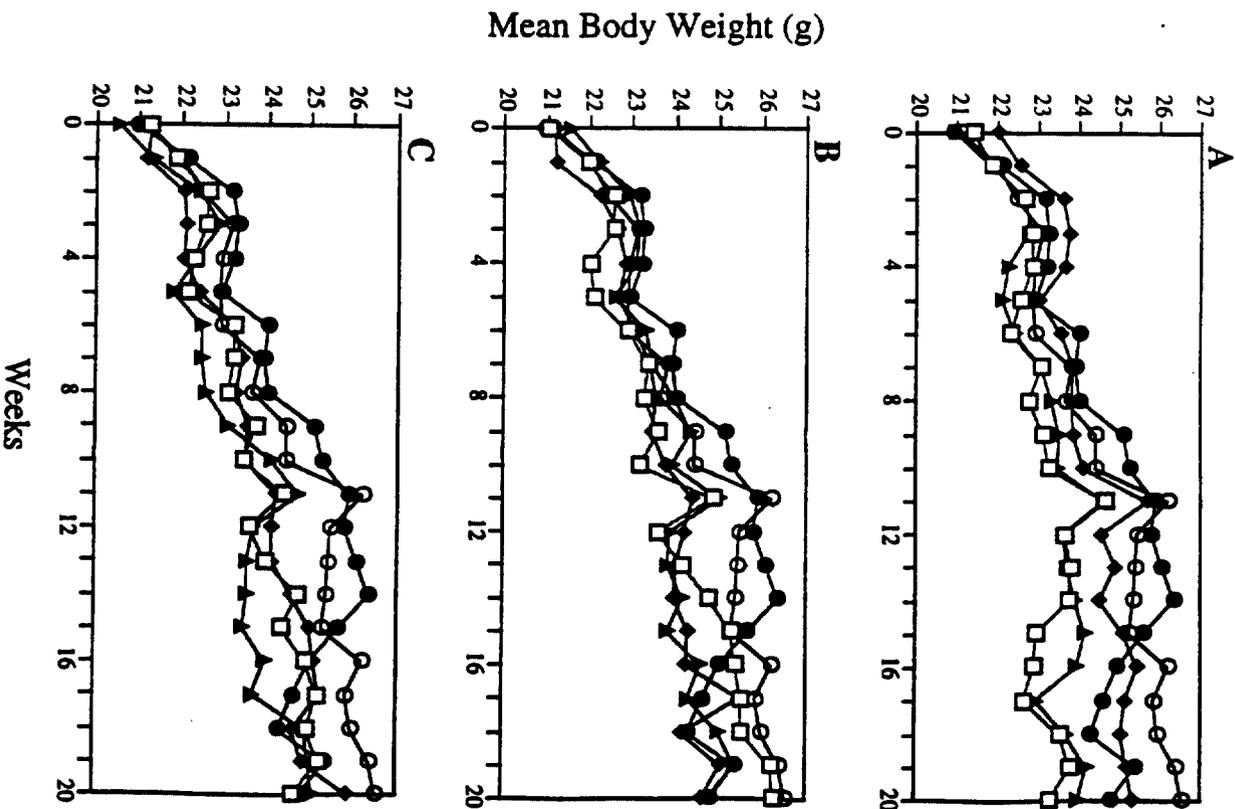


Fig. 5. Mean individual mouse body weights per week per dose group during a 20 week treatment period. A. EA, TPA, and acetone controls. B. TPGDA, TPA, and acetone controls. C. Lacquer A (Lac A), TPA, and acetone controls. Acetone (○); TPA (●); LOW (◆); MID (▲); HIGH (□) for EA (◇); TPGDA (■), and Lac A (△).

doses, based on their respective concentration). Toxicity, thus, may be attributed to the TPGDA, which is apparently not significantly affected by the other lacquer components.

Mean number of papillomas induced by TPGDA was similar (between TPGDA alone and Lacquer A; Fig. 3 and 4), but the mean number of papillomas per mouse were not significantly different ( $P < 0.05$ ). This apparent difference may be attributed to either an altered vapor pressure in acetone and/or to spontaneous polymerization of the TPGDA monomer when painted in a complete radiation curable lacquer, which may reduce the effective dose applied. Latency (Table II) and the kinetics of papilloma appearance (Fig. 3 and 4) are similar between TPGDA and TPGDA in Lacquer A. At similar dose levels of TPGDA (in mineral oil) Andrews and Clary [10] did not observe an increase in skin tumors in male C3H/HeJ mice after 80 weeks of treatment (2x/week). Differences in mouse strain, vehicle, and dose rate of application may have contributed to the differences observed between these two studies.

Ethyl acrylate failed to induce papillomas at even the highest dose, which did induce an increase in relative skin thickness in acute studies. No differences were observed between any dose of EA or the lowest dose of TPGDA or TPGDA in Lacquer A when compared to the vehicle (acetone) control. The vapor pressure difference between TPGDA and EA is significant [23-25] and the high vapor pressure of EA could have resulted in a relative decrease in dermal exposure to EA in these studies and affected the outcome. However, depression of the body weights in the EA high dose group indicated that a systemic toxicity via topical application was induced, but was

not sufficient to have induced a caloric restriction and depress potential tumorigenicity. Also, the clinical appearance of hyperkeratosis at the test site was similar between EA, TPGDA, and Lacquer A throughout the study.

Application of acetone has not induced papillomas in previous studies [20, 26], in which female mice had been group housed since weaning. In the studies reported here, mice, which had been housed together since weaning, were randomized and assigned by weight to treatment groups immediately before the first treatment. Restructuring of the mouse hierarchy within a cage most likely resulted in bite wounds (only some of which can be documented in the clinical observations), which resulted in late appearing papillomas (Table II). Only a one or two mice from each cage of the acetone vehicle and EA treatment groups were affected (Table II).

The sharp threshold and the maximum induction of papillomas (relative to TPA positive control) by the mid and high dose groups suggest that a threshold exists, which can be rapidly saturated. Glutathione and glutathione-S-transferase and hydrolysis of the conjugated product is believed to be the main source of protection from acrylate toxicity [11, 12, 15-18] and conjugation may be expected to be proportional to the number of functional groups.

The differences observed in these studies suggest that other inherent characteristics of TPGDA may be responsible for the increased toxicity relative to EA and may be both qualitatively and quantitatively different from the known tumor promoter TPA. Toxicity and the potential of acrylates to promote skin tumors may depend upon the number of functional groups, molecular structure, and molecular weight differences

that affects their ability to be absorbed through and interact with the skin. Additional studies will be required to determine the significance of the role of TPGDA induced cellular proliferation in induction of papillomas. The role of chemically induced cellular proliferation in tumorigenesis and the implications for risk assessment has received considerable attention [27].

The TGAC mouse, which carries an inducible activated Harvey *ras* oncogene in all cells and tissues, does not require an initiating carcinogen and is very useful in identifying and determining skin tumor promoters and carcinogens [19, 20, 26]. These mice have a very low incidence of skin tumors, unless induced by a chemical or physical agent and may be a valuable mouse line for identification of environmental and occupational chemicals and conducting dosimetric risk analysis.

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# Absence of Systemic In Vivo Genotoxicity after Dermal Exposure to Ethyl Acrylate and Tripropylene Glycol Diacrylate in TG•AC (v-Ha-ras) Mice

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Acrylates may be polymerized to stable surface coatings (paints, lacquers, inks, etc.) by alkylation via Michael's type addition reaction. Thus, acrylates have an inherent potential as electrophiles to be mutagenic; limited in their biological activity by their physicochemical properties. To evaluate the systemic genotoxicity of ethyl acrylate (EA) as a reference acrylate, tripropylene glycol diacrylate (TPGDA), and a reference ultraviolet radiation curable lacquer (Lacquer A; containing TPGDA as the active ingredient) when applied dermally to TG•AC mice, peripheral blood was sampled periodically during the 20 week treatment period. Leukocytes were evaluated for DNA damage (single strand breaks, alkali labile sites) at weeks 4, 8, 12, 16, and 20 using the Single Cell Gel assay, while polychromatic erythrocytes (PCE) were evaluated for the presence of micronuclei at week 20. The percentage of polychromatic erythrocytes (%PCE) at week 20 was determined to evaluate the effect of treatment on the rate of erythropoiesis. The percentage of migrated DNA in leukocytes was not significantly increased at any sample time for EA at 60, 300, or 600  $\mu$ mole/mouse or TPGDA at 1, 5 or 10  $\mu$ mole/mouse when administered alone or in Lacquer A. The frequency of micronucleated PCE was not significantly elevated after 20 weeks of treatment. The lack of genotoxicity in leukocytes or bone marrow suggests that these two acrylates are not genotoxic systemically. A significant, dose dependent increase in the %PCE relative to the vehicle control was present only in mice treated with TPGDA. A dose dependent, but nonsignificant increase in %PCE was observed in mice treated with Lacquer A. This observed rate of increase in erythropoiesis may reflect a homeostatic mechanism of response to the treatment induced tumor burden and/or hematopoietic response to epidermal keratinocyte cytokines induced by tissue injury.

**Key words:** monofunctional acrylates, multifunctional acrylates, skin, mutagenicity, transgenic mouse

## INTRODUCTION

Esters of acrylic acid are used in the manufacture of lacquers, paints, inks, etc. [IARC, 1986]. Recently, the use of UV radiation curable acrylate based surface coatings has increased as fundamental changes in the industry have occurred [Nylander-French et al., in press]. Approximately 200 acrylates are listed on the United States Environmental Protection Agency's Toxic Substances Control Act Inventory [Lawson and Jurs, 1990] and most are of unknown biological activity or toxicity. Few of these important acrylates have been studied for their effects in biological systems.

Simple monofunctional acrylates, like ethyl acrylate (EA), are considered to be more toxic, but less potent contact sensitizers, than multifunctional acrylates, like tripropylene glycol diacrylate (TPGDA; see Figure 1 for chemical structures). Acrylates may be polymerized by alkylation via Michael's type addition reaction. Thus, acrylates have an inherent potential as electrophiles to be mutagenic; limited in

their biological activity only by their physicochemical properties and access to biological systems. The acrylates have been classified and assigned to groups based on a comparison of their structure activity relationships and potential biological activity [Lawson and Jurs, 1990]. The major route of detoxification of acrylates and methacrylates is their conjugation with glutathione [deBehlitz et al., 1987; Frederick et al., 1992; Ghanayem et al., 1987] via Michael's addition reaction or glutathione-S-transferase [Boylard and Chasseaud, 1968]. Conjugation of acrylates by glutathione is expected to be proportional to the number of functional acrylate groups. The available data suggests that acrylates most likely act at the site of contact, conjugate available glutathione, and are hydrolyzed via carboxylesterases [Corkill et al., 1976; deBehlitz et al., 1987; Delbressine et al., 1981; Ghanayem et al., 1987]. Based on their chemical structure and molecular reactivity, acrylates have the potential to interact with biomolecules, in-

cluding nucleic acids and nucleoproteins and to induce mutations.

EA is a carcinogen when administered orally by intubation, causing papillomas and squamous cell carcinomas in the forestomachs of rats and mice [NTP, 1986] and is considered a potential human carcinogen [IARC, 1986]. However, under the experimental conditions employed, EA was not found to be carcinogenic to rats or mice by dermal application [DePass et al., 1984; Nylander-French and French, submitted] or inhalation [Miller et al., 1985]. 2-Ethylhexyl acrylate [DePass et al., 1985; Wenzel-Harung et al., 1989] and neopentyl glycol diacrylate [DePass et al., 1985] induced benign and malignant skin tumors in C3H/HeJ mice when administered topically. Tetraethylene glycol diacrylate or triethylene glycol diacrylate, but not trimethylene propane triacrylate, 1,6-hexanediol diacrylate, tripropylene glycol diacrylate (TPGDA), tetraethylene glycol dimethacrylate, or trimethylol propane trimethacrylate, induced a low incidence of either benign or malignant skin tumors in male C3H/HeJ mice (age not stated) when treated 2x/week for 80 weeks (2.5 mg/mouse for acrylates or 25 mg/mouse for methacrylates in mineral oil) [Andrews and Clary, 1986]. More recently, we have demonstrated that TPGDA (either alone or equimolar in Lacquer A), but not EA, induces skin papillomas when topically applied to the skin of the TG-Ac transgenic mice over a 20 week exposure period [Nylander-French and French, submitted].

In general, evidence for the potential genotoxicity of the acrylates (mono- or multifunctional) appears to be limited. Acrylates have been reported as negative for mutagenicity in the *Salmonella typhimurium* reverse mutation assay in either

the presence or absence of metabolic activation [Cameron et al., 1991; Gordon et al., 1991; Ishida et al., 1981; Waagemackers and Bensch, 1984]. The multifunctional acrylates, trimethylolpropane triacrylate and trimethylolpropane trimethacrylate, were weakly mutagenic in *Salmonella* (strain TA1535) [Cameron TP et al., 1991].

In contrast, the in vitro mouse lymphoma assays (L5178 TK +/- assay) of acrylates are generally positive in the presence or absence of metabolic activation systems, preferentially inducing small colonies, indicative of a possible clastogenic mechanism [Cameron et al., 1991; Dearfield et al., 1989; Moore et al., 1988; Moore et al., 1989]. The possible inherent clastogenic activity of acrylates is not strongly supported by other in vivo assays. At concentrations one-half of the minimum lethal dose, EA and methyl acrylate (ip); two consecutive daily doses) induced a 3 to 4 fold increase in micronuclei relative to mitomycin C (positive control) in BALB/c mice [Przybojewska et al., 1984]. However, in a similar study using the same exposure conditions, EA failed to increase the incidence of micronuclei in C57BL/6 Aplk or BALB/c mice [Ashby et al., 1989]. Finally, EA was toxic in vitro and in vivo (ip), but failed to induce an increase in sister chromatid exchange rate, chromosomal aberrations, or micronuclei in C57BL/6 mice in vivo [Kligerman et al., 1991]. Only after in vitro acrylate exposure to splenocytes was chromatinid-type aberrations observed. Possible differences in assay conditions (dosing regimens, chemical purity, substrain differences, etc.) and/or inability to deliver sufficiently toxic dose in vivo were cited as possible explanations in the differences observed. No studies were found in the literature on the genotoxicity of multi-

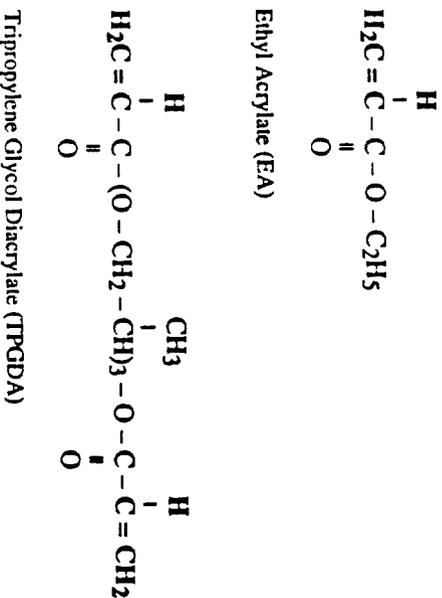


Fig. 1. Chemical structures of ethyl acrylate and tripropylene glycol diacrylate. Electrophilic structural alert of the acrylate functional groups are in bold.

functional acrylates. Furthermore, no studies were found in the literature on either mono- or multifunctional acrylates that used the expected routes of human exposure, i.e., dermal or inhalation. On the basis of this database, the *in vivo* genotoxicity of mono- or multifunctional acrylates remains equivocal.

To evaluate the systemic toxicity of EA or TPGDA when applied dermally, DNA damage assays were integrated into 20 week tumorigenesis studies using TG•AC transgenic mice [Nylander-French and French, submitted]. The genotoxicity endpoints used in these studies included an analysis of the frequency of micronucleated polychromatic erythrocytes (MN-PCE) in peripheral blood and the extent of DNA damage in nucleated cells of the blood, as measured by the alkaline Single Cell Gel microgel electrophoresis (SCG) assay. The analysis of micronucleated erythrocytes is the approach most commonly used for evaluating the ability of suspect genotoxicants to induce either structural or numerical damage in proliferating *in vivo* cell populations [Heddle et al., 1983; MacGregor et al., 1987; Tice and Iwert, 1985]. The assay is conducted in mice by evaluating the frequency of MN in immature erythrocytes (PCE) scored in bone marrow or in peripheral blood preparations [Heddle et al., 1983; MacGregor et al., 1980; Schlegel and MacGregor, 1982]. Peripheral blood sampling is especially useful because it allows for repeat sampling on the same animal and a temporal evaluation of DNA damage induced in bone marrow [Luke et al., 1988; Luke et al., 1988; MacGregor et al., 1980; Schlegel and MacGregor, 1982]. In addition, an analysis of the percentage of PCE (%PCE) in peripheral blood allows an

evaluation of the effect of chemical treatment on the rate of erythropoiesis.

Techniques which permit the sensitive detection of DNA damage are needed to evaluate the genotoxic potential of biologically reactive substances. Since the effects of many reactive substances are tissue and cell type specific, it is important to utilize techniques which can directly detect DNA damage in individual cells. Cytogenetic techniques, while providing information at the level of the individual cell, are limited to proliferating cell populations. Furthermore, these techniques require the processing of DNA damage into microscopically visible lesions. Biochemical techniques, such as alkaline elution and nucleoid sedimentation, circumvent these difficulties in that DNA damage can be evaluated directly in any cell population. However, the resulting data do not provide any information about the distribution of damage or repair among individual cells. These inherent limitations can be overcome by using an electrophoretic technique capable of detecting DNA single strand breaks and alkalilabile sites in individual cells [Singh et al., 1988]. Eukaryotic cells are embedded in an agarose gel on a microscope slide, lysed by detergents and high salt at pH 10, and then electrophoresed for a short time under alkaline conditions. Cells with increased DNA damage display increased migration of the DNA from the nucleus towards the anode. The importance of this technique lies in its ability to detect intercellular differences in DNA damage in virtually any eukaryotic cell population and in its requirement for extremely small numbers of cells (1,000 to 10,000 cells). In this study, this methodology was used to evaluate systemic toxicity as measured by the level of DNA damage in blood leukocytes fol-

lowing topical exposure of EA or TPGDA to TG•AC mice.

## MATERIALS AND METHODS

### Chemicals

Ethyl acrylate (EA; CAS No. 140-88-5; 100.12 g/mole) was received from Aldrich Chemical Co. (Madison, WI, USA) as a colorless liquid with a pungent odor at 99% purity. Tripropylene glycol diacrylate (TPGDA; CAS No. 42978-66-5; 300.25 g/mole), and Lacquer A (reference lacquer containing only the base components for ultraviolet radiation curable wood surface coating lacquer) were obtained from Nobel Industrial Coatings (Malmö, Sweden). TPGDA was received as a pale-yellow tinted liquid with a mild odor at 80% pure monomer. Lacquer A was a pale-yellow tinted liquid with an acrid odor and according to the manufacturer contained 56.4% TPGDA monomer (remaining components were proprietary ingredients). Due to the tendency of the acrylates to polymerize when exposed to light, hydroquinone (<200 ppm) was added as an inhibitor to each test article by the manufacturer. Samples were stored in the dark at 4-6°C. Analyses of the stability of the test compounds were not carried out. All studies were conducted during the manufacturer's warranty for chemical stability. Acetone (CAS No. 67-64-1, Aldrich Chemical Co.) was used as a vehicle control. For a positive control, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA; CAS No. 16561-29-8) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Dimethylsulfoxide (DMSO) was obtained from Fisher Scientific Co. (Fairlawn, NJ, USA); agarose and Triton X-100 from Bethesda Research Laboratories (Gaithersburg, MD, USA); acridine orange, EDTA, ethidium bromide, fetal bovine serum, sodi-

um hydroxide, sodium sarcosinate, and Tris were obtained from Sigma Chemical Co.; Hanks Balanced Salt Solution (HBSS) was obtained from Irvine Scientific (Irvine, CA, USA).

### Mice

Female homozygous TG•AC transgenic mice (5-globin promoter fused with the Ha-ras oncogene on the FVB strain) were obtained from Taconic Farms (German town, NY, USA). Upon receipt all animals were examined and acclimated for two weeks. Mice were housed 3-5 per cage. Animal rooms were maintained at 71 ± 7°F and 50 ± 20% relative humidity with 10 fresh air changes per hour and 12 hours of fluorescent light daily (animals were protected from receiving direct radiation). City water and Purina PicoChow No. 5058 in pellet form (Barnes Supply Co., Durham, NC, USA) were available *ad libitum*.

### Treatment

Experimental details for exposure conditions and dose selection have been described [Nylander-French and French, submitted]. Briefly, at 12 weeks of age, female TG•AC mice, identified by a unique number by tail tattoo, were randomly assigned by body weight to treatment or control group. Each chemical was investigated at three doses for comparison to positive (TPA, 2 nmoles/mouse) and vehicle (acetone) controls, which served as concurrent controls. Prior to the first dose, each mouse was inspected and shaved from mid-scapular region to the base of the tail. Dose selection was based on the results of a series of 2 week dose finding studies using the same strain, age, sex, and exposure conditions to evaluate toxicity and determine the relative skin thickness (indirect measurement of epidermal keratinocyte hyperpla-

sia). Each mouse received three weekly (Monday, Wednesday, Friday) topical applications of the appropriate chemical and/or vehicle (200  $\mu$ l/mouse,  $\approx$ 8  $\text{cm}^2$ ). At 4, 8, 12, 16, and 20 weeks of treatment, 1-2 mm of the terminal portion of the tail was snipped and 3 drops of blood collected; 1 drop for SCG assay and 2 drops to prepare blood smears for MN analysis.

#### Micronucleus Analysis

Peripheral blood smears were air dried, fixed using absolute methanol, and then stained with acridine orange [Tice et al., 1990]. Coded slides were scored in numerical order at 1,000 $\times$  magnification using epi-illuminated fluorescence microscopy (450-490 nm excitation, 520 nm emission). To assess if chemical treatment affected the rate of bone marrow erythropoiesis (signified by a change in the proportion of PCEs within the total erythrocyte population), the number of PCE among a total of 1,000 erythrocytes was determined at 1,000 $\times$  magnification. For the determination of the frequency of micronucleated cells, 2,000 PCEs were evaluated at 1,000 $\times$  magnification.

#### Single Cell Gel Analysis

Each blood sample was placed in a prelabelled vial containing 1 ml of ice cold Hank's Balanced Salt Solution (HBSS) with 20 mM EDTA and 10% DMSO [Tice et al., 1992]. The cell suspension was pelleted by centrifugation, mixed with 0.5% low melting point agarose at 37°C and layered onto microscope slides [Singh et al., 1988], one per mouse. All slides were placed in a lysing solution consisting of 1% sodium sarcosinate, 2.5 M NaCl, 100 mM Na<sub>2</sub>EDTA, 10 mM Tris (pH 10) with 10% DMSO and 1% Triton X-100, added fresh at 4°C until electrophoresed. After remaining in the lysing solution for at least 1 h, the

SCG slides were treated with alkali (1 mM Na<sub>2</sub>EDTA and 300 mM NaOH; pH > 13) for 60 min to express alkali labile damage, followed by electrophoresis for 20 min at 25 V/300 mA. Following electrophoresis, the alkali was neutralized with 0.4 M Tris (pH 7) and stained with ethidium bromide (0.5  $\mu$ g/ml). Slides were scored in numerical order from randomly numbered mice to eliminate biased analysis. Observations were made at 250 $\times$  magnification using a fluorescent microscope equipped with an excitation filter of 515-560 nm and a barrier filter of 590 nm. Twenty five cells per mouse were evaluated for DNA damage (single strand breaks and alkali labile sites) using the Comet Image Analysis System (Kinetic Imaging Ltd., Liverpool, UK).

The parameters used to evaluate DNA damage was the percentage of migrated DNA. The variability in the number of mice scored per dose group at different sample times reflects both treatment associated mortality and the loss of some slides due to degeneration of the agarose.

#### Statistical Analysis

Data analysis was conducted using a micronucleus assay data management and statistical analysis software system [Integrated Laboratory Systems, 1990]. In this program, a one-tailed trend test using pooled data that incorporates a variance inflation factor to account for excess interanimal variability. The trend test is used to determine if a treatment related increase in MN-PCE frequency occurred at an alpha level of 0.05. An analysis of variance (ANOVA) test based on individual mouse data was used to determine if a treatment related difference in the percentage of PCE occurred at an alpha level of 0.05. Pairwise comparisons between dose groups and the control group were conducted using the appropriate

one-tailed (for MN) Pearson Chi-Square test (pooled data) or two-tailed Student's *t* test (for %PCE) to determine if a significant effect had occurred at any single dose.

The effect of dose on the percentage of migrated DNA (based on individual mouse data) at each sample time was analyzed using a one-tailed trend test with the alpha level set at 0.05. Also, each dose group was compared against the concurrent control using an uncorrected two-tailed Student's *t* test to identify any individual dose at which a significant increase in the percentage of migrated DNA occurred.

## RESULTS

For SCG analysis, peripheral blood smears collected at 4, 8, 12, 16, and 20 weeks of treatment were evaluated for an increase above vehicle control level in the percentage of migrated DNA. The MN and %PCE analysis was limited to the 20 week treatment blood sample.

EA at 60, 300, or 600  $\mu$ moles/mouse did not induce a significant increase in the percentage of migrated DNA in leukocytes, either by a trend test or by a pairwise comparison of each treatment dose against the concurrent vehicle control at any sample time (Table I). Similarly, at 20 weeks, the frequency of MN-PCE was not significantly elevated and the %PCE was not significantly altered (Table II). The percentage of migrated DNA and the frequency of

MN-PCE was not increased in the mice treated with 2 nmoles TPA (positive control for tumorigenesis studies) (Table I and II). A marginally nonsignificant increase in %PCE was observed in these mice ( $P=0.054$ ) at 20 weeks treatment.

The percentage of migrated DNA in leukocytes of the mice treated with TPQDA at 1, 5, or 10  $\mu$ moles/mouse was not significantly increased, either by a trend test or by a pairwise comparison of each treatment dose against the concurrent vehicle control, at any sample time (Table III). Similarly, at 20 weeks, the frequency of MN-PCE was not significantly elevated (Table IV). However, a highly significant, dose dependent increase in the %PCE was observed in TPQDA treated mice (Table IV). The lowest effective dose inducing a significant change was 5  $\mu$ moles/mouse.

The percentage of migrated DNA in leukocytes of the mice treated with Lacquer A, equimolar for TPQDA, at 1, 5, or 10  $\mu$ moles/mouse was not significantly increased, either by a trend test or by a pairwise comparison of each treatment dose against the concurrent vehicle control, at any sample time (Table V). Similarly, at 20 weeks, the frequency of MN-PCE was not significantly elevated and, while increased in an apparent dose dependent manner, the %PCE was not significantly altered (Table VI).

Table I. Percent migrated DNA<sup>†</sup> in blood leukocytes of female TG+AC mice treated with ethyl acrylate.

Dose µmol/mouse	Week 4		Week 8		Week 12		Week 16		Week 20					
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM				
TPA, 0.002	10.5	4.0	7	15.0	3.0	10	20.0	2.6	8	28.8	5.5	7	25.7	1
0	11.6	5.4	6	13.0	2.7	9	19.5	1.5	7	19.4	2.6	7	14.6	0.9
60	17.8	4.6	5	13.4	2.5	9	20.6	2.3	10	14.5	1.3	7	33.5	4.1
300	11.5	4.3	6	13.6	3.3	9	19.8	1.2	7	14.8	1.3	4	28.1	3.3
600	16.9	4.2	7	13.0	2.2	9	18.8	2.0	5	15.5	3.7	3	17.4	2.1
p-value <sup>‡</sup>	0.326			0.514			0.679			0.919			0.233	

<sup>†</sup> Mean percent migrated DNA and standard error of the mean (SEM) among N mice. Data based on 25 cells/mouse.

<sup>‡</sup> One-tailed trend test p-value.

Table II. Micronucleated polychromatic erythrocytes (MN-FCE) and the percentage of polychromatic erythrocytes (%PCE) in female TG+AC mice treated with ethyl acrylate.

Dose µmol/mouse	Mean	MN-FCE <sup>a</sup>		Mean	%PCE <sup>b</sup>	
		SEM	N		SEM	N
TPA, 0.002	2.1	0.7	7	10.7	3.9	7
0	1.4	0.4	9	3.5	0.5	9
60	2.2	0.3	9	4.6	1.5	9
300	1.4	0.3	10	3.4	0.3	10
600	1.4	0.3	7	3.4	0.3	7
p-value <sup>‡</sup>	0.873			0.650		

<sup>a</sup> Group mean frequency of MN-FCE per 1,000 FCE and standard error of the mean (SEM) among N mice. Data based on 2,000 FCE scored per mouse.

<sup>b</sup> Group mean percentage of FCE and standard error (SEM) of the mean among N mice. Data based on 1,000 erythrocytes scored per mouse.

<sup>‡</sup> One-tailed trend test p-value.

Table III. Percent migrated DNA<sup>†</sup> in blood leukocytes of female TG+AC mice treated with tripropylene glycol diacrylate.

Dose µmol/mouse	Week 4		Week 8		Week 12		Week 16		Week 20					
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM				
TPA, 0.002	10.5	4.0	7	15.0	3.0	10	20.0	2.6	8	28.8	5.5	7	25.7	1
0	11.6	5.4	6	13.0	2.7	9	19.5	1.5	7	19.4	2.6	7	14.6	0.9
1	17.7	3.9	7	13.3	4.2	8	20.3	1.9	7	31.8	3.9	9	16.0	2.5
5	13.1	3.8	7	12.2	4.1	7	19.4	2.9	7	27.2	6.7	5	21.9	4.7
10	16.1	2.8	6	15.9	2.4	7	19.9	2.7	8	30.2	4.7	6	18.9	1.4
p-value <sup>‡</sup>	0.404			0.290			0.495			0.203			0.147	

<sup>†</sup> Mean percent migrated DNA and standard error of the mean (SEM) among N mice. Data based on 25 cells/mouse.

<sup>‡</sup> One-tailed trend test p-value.

Table IV. Micronucleated polychromatic erythrocytes (MN-FCE) and the percentage of polychromatic erythrocytes (%PCE) in female TG+AC mice treated with tripropylene glycol diacrylate.

Dose µmol/mouse	Mean	MN-FCE <sup>a</sup>		Mean	%PCE <sup>b</sup>	
		SEM	N		SEM	N
TPA, 0.002	2.1	0.7	7	10.7	3.9	7
0	1.4	0.4	9	3.5	0.5	9
1	1.8	0.4	10	3.4	0.3	10
5	1.9	0.6	5	7.5*	0.8	5
10	2.0	0.3	9	9.7*	1.3	9
p-value <sup>‡</sup>	0.142			<0.001		

<sup>a</sup> Group mean frequency of MN-FCE per 1,000 FCE and standard error of the mean (SEM) among N mice. Data based on 2,000 FCE scored per mouse.

<sup>b</sup> Group mean percentage of FCE and standard error of the mean among N mice. Data based on 1,000 erythrocytes scored per mouse.

<sup>‡</sup> One-tailed trend test p-value.

\* Significantly different from the concurrent control at P < 0.05.

Table V. Percent migrated DNA<sup>†</sup> in blood leukocytes of female TG•AC mice treated with Lacquer A (equimolar for tripropylene glycol diacrylate).

Dose µmol/mouse	Week 4		Week 8		Week 12		Week 16		Week 20				
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM			
TPA, 0.002	10.5	4.0	15.0	3.0	10	20.0	2.6	8	28.8	5.5	7	25.7	1
0	11.6	5.4	13.0	2.7	9	19.5	1.5	7	19.4	2.6	7	14.6	0.9
1	14.0	4.1	13.7	3.2	7	16.0	1.5	6	26.6	4.5	6	19.1	3.6
5	13.4	3.9	6	9.3	4.0	8	18.2	2.5	7	28.2	4.4	8	13.6
10	22.1	5.3	7	7.4	1.7	7	17.7	1.6	9	20.9	2.3	7	19.3
p-value <sup>d</sup>	0.058		0.945		0.572		0.406		0.403				

<sup>†</sup> Mean percent migrated DNA and standard error of the mean (SEM) among N mice. Data based on 25 cells/mouse.

<sup>#</sup> One-tailed trend test p-value.

Table VI. Micronucleated polychromatic erythrocytes (MN-PCE) and the percentage of polychromatic erythrocytes (%PCE) in female TG•AC mice treated with Lacquer A (equimolar for tripropylene glycol diacrylate).

Dose µmol/mouse	MN-PCE <sup>a</sup>			%PCE <sup>b</sup>		
	Mean	SEM	N	Mean	SEM	N
TPA, 0.002	2.1	0.7	7	10.7	3.9	7
0	1.4	0.4	9	3.5	0.5	9
1	2.1	0.4	8	3.5	0.4	8
5	2.1	0.7	9	4.9	0.9	9
10	1.6	0.3	7	8.3	3.5	7
p-value <sup>d</sup>	0.604			0.251		

<sup>a</sup> Group mean frequency of MN-PCE per 1,000 PCE and standard error of the mean (SEM) among N mice. Data based on 2,000 PCE scored per mouse.

<sup>b</sup> Group mean percentage of PCE and standard error of the mean among N mice. Data based on 1,000 erythrocytes scored per mouse.

<sup>d</sup> One-tailed trend test p-value.

DISCUSSION

The TG•AC mouse, which carries an inducible activated Harvey ras oncogene in all cells and tissues, does not require an initiating carcinogen and is very useful in identifying and determining skin tumor promoters and carcinogens [Hansen and Tennant, 1994; Leder et al., 1990; Spalding et al., 1993]. In this mouse, topical application with EA for 20 weeks failed to induce papillomas even at doses which induced significant skin hyperplasia in 2 week studies and hyperkeratosis at 20 weeks [Nylander-French and French, submitted]. However, TPGDA at 5 or 10 µmoles/mouse induced a highly significant increase in papillomas. The maximal response (~30 papillomas/mouse) to TPGDA was similar to the response of 2 nmole/mouse TPA (positive control). Lacquer A (equimolar for TPGDA) also significantly increased the incidence of papillomas, although the maximal response (~15 papillomas/mouse) occurred at 10 µmoles/mouse and, thus, was only one-half that for TPGDA alone [Nylander-French and French, submitted].

Systemic genotoxicity assays were readily integrated into the 20 week short term carcinogenesis protocol in order to evaluate the effects of EA and TPGDA. Assays using peripheral blood were used because of availability and collection is minimally invasive and allows multiple collections due to the small sample volumes required [Luke et al., 1988; Luke et al., 1988; Tice et al., 1992; Vijaylaxmi et al., 1992]. In addition, a comparative analysis of SCG and MN data provide for an evaluation of damage induced in circulating leukocytes versus a proliferating bone marrow cell population. In this study, the dermal application of EA, a monofunctional acrylate, or TPGDA,

a multifunctional acrylate, failed to induce a significant increase in circulating leukocytes, as assessed by the SCG assay. The absence of systemically induced damage in peripheral blood leukocytes suggests that these two acrylates are not systemically genotoxic. However, the contact happens and potent genotoxicity may only be observed in the presence of contact [NTP, 1986; DeGruy et al., 1991; Chanayem et al., 1991; Nylander-French et al., 1991]. Acrylates are believed to be genotoxic primarily at their site of application because of their reaction with DNA to form appropriate positive control adducts. The genotoxicity (measured via micronucleated erythrocytes) in this study was not increased frequency of micronucleated erythrocytes, but rather the increased rate of erythrocytes with increased micronucleated erythrocytes [Nylander-French and French, 1991]. This increased rate of erythrocytes with increased micronucleated erythrocytes and may reflect either systemic or dermal mechanisms comparable to tumor burden or dose response in the epidermal keratinocyte population. In this study, the dermal application of EA, a monofunctional acrylate, or TPGDA,

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TNF- $\alpha$ : granulocyte-macrophage colony stimulating factor, GM-CSF; etc.) [Sauder and Pastore, 1993]. The significant difference in dose (equimolar consideration for the number of functional groups, i.e., mono- versus difunctional acrylate) observed in these studies and the skin tumorigenicity studies [Nylander-French and French, submitted] suggest that other inherent characteristics of TPQDA may be responsible for the increased toxicity relative to EA. TPQDA effects may be both qualitatively and quantitatively different from the known tumor promoter TPA (latency and tumor incidence), but the systemic effect on the %PCE as it reflects bone marrow activity is similar. Toxicity and the potential of acrylates to promote skin tumors may depend upon the number of functional groups, molecular structure, and molecular weight differences that affect their ability to be absorbed through and interact with the skin and influence the potential systemic genotoxicity of these electrophiles. Additional studies will be required to determine the significance of the role of TPQDA induced cellular proliferation, mutagenicity at the application site, and in the induction of skin tumors.

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# COMPARATIVE IN VITRO CYTOTOXICITY OF ETHYL ACRYLATE AND TRIPROPYLENE GLYCOL DIACRYLATE TO NORMAL HUMAN SKIN AND LUNG CELLS

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## Summary

The toxicology of the esters of acrylic acid (acrylates) is poorly understood, although the potential for occupational exposure is considerable. Confluent (70-90%) cultures of normal human epidermal keratinocytes (NHEK), dermal fibroblasts (NHDF), or bronchial epithelium (NHBE) were exposed to the monofunctional ethyl acrylate (EA), the multifunctional tripropylene glycol diacrylate (TPGDA), or TPGDA monomer in a radiation curable lacquer (Lacquer A) at equimolar dosages to determine their comparative *in vitro* cytotoxicity. Viability of the cells after 2-24 hours exposure to the acrylates or solvent control was used to calculate an index of acute cytotoxicity (50% inhibitory dose; ID<sub>50</sub>) and to determine the shape of the dose response curves. TPGDA, Lacquer A, and EA were equally cytotoxic (ID<sub>50</sub> ≈ 1 μmole/cm<sup>2</sup>) to NHEK at equimolar doses. TPGDA or Lacquer A were more cytotoxic (≈ 100x) to NHDF or NHBE than EA. Sequential exposure of UV A and TPGDA to NHEK indicate the potential for a synergistic cytotoxic response. These findings are consistent with decreases in free sulfhydryl groups (e.g., glutathione or cysteine) that parallel the dose response related decreases in viability. Together, these data suggest possible differences in toxicity between EA and TPGDA to NHEK, NHDF, or NHBE, possibly due to the difference in the number of functional acrylate groups and/or physicochemical differences between the acrylates investigated.

**Key words:** acrylates, cytotoxicity, human keratinocytes, fibroblasts, bronchial cells

## Introduction

Esters of acrylic acid are used in the manufacture of lacquers, paints, inks, etc. (22). Recently, the use of UV radiation cured acrylate based surface coatings has increased as fundamental changes in the industry have occurred (35). There are ≈ 200 acrylates listed on the United States Envi-

ronmental Protection Agency's Toxic Substances Control Act Inventory that are of potential industrial use (25) and most are of unknown potential biological activity or toxicity.

In general, simple monofunctional acrylates, like ethyl acrylate (EA), are considered to be more toxic, but less potent con-

tact sensitizers, than multifunctional acrylates, like tripropylene glycol diacrylate (TPGDA) [see Fig. 1 for chemical structures; (7)]. The acrylates have been classified based on a comparison of their structure activity relationships and potential biological activity (26). Acrylates are electrophiles and have an inherent potential for carcinogenicity. As potential direct alkylating agents, their toxicity may be expected to be limited by their ability to interact with biological systems. The major route of detoxification of acrylates is their conjugation with glutathione (13, 18, 21), via Michael's addition reaction or glutathione-S-transferase (10). Conjugation of acrylates with glutathione is expected to be proportional to the number of functional acrylate groups. The available data suggests that at the site of contact acrylates are detoxified by: (1) carboxylesterases, or (2) reaction with available glutathione (along with other potential critical cellular substrates) followed by carboxylesterase hydrolysis (11, 13, 14, 21, 28, 37).

EA, a monofunctional acrylate, is a carcinogen at the site of contact when administered orally by intubation, causing papillomas and squamous cell carcinomas in the forestomachs of rats and mice (34) and is considered a potential (IARC Class 2B) human carcinogen (22). However, under the experimental conditions employed, EA was not found to be carcinogenic to rats or mice by dermal application (15) or inhalation (29). 2-Ethyl hexyl acrylate (16, 38) and neopentyl glycol diacrylate (16) induced benign and malignant skin tumors in C3H/HeJ mice when administered topically. Tetraethylene glycol diacrylate or triethylene glycol diacrylate, but not trimethylol propane triacrylate, 1,6-hexanediol diacrylate, tripropylene glycol diacrylate (TPGDA), tetraethylene glycol dimethacrylate, or trimethylol propane trimethacrylate, induced a low incidence of either benign and/or malignant skin tumors in male C3H/HeJ mice (age not stated) when treated 2x/week for 80 weeks (2.5 mg/

mouse for acrylates or 25 mg/mouse for methacrylates in mineral oil) (1). Using a transgenic mouse model, we have recently demonstrated that TPGDA (alone or in a radiation curable lacquer), but not EA, induced a dose related increase in epidermal papillomas when topically applied to the skin (36).

In the studies reported here, normal human epidermal keratinocyte, dermal fibroblasts, or bronchiolar epithelial cell cultures were used to determine comparative *in vitro* cytotoxicity between a representative monofunctional (EA) and multifunctional acrylate (TPGDA). These data provide a basis for comparing the dose response relationships between human cytotoxicity and the rodent *in vivo* toxicology and short term carcinogenesis studies.

#### Materials and Methods

**Chemicals.** Ethyl acrylate (EA; CAS No. 140-88-5; 100.12 g/mole) was received from Aldrich Chemical Co. (Madison, WI, USA) as a colorless liquid with a pungent odor at 99% purity. Tripropylene glycol diacrylate (TPGDA; CAS No. 42978-66-5; 300.25 g/mole) and Lacquer A (reference lacquer containing only the base components for ultraviolet radiation curable wood surface coating lacquer) were gifts from Nobel Industrial Coatings (Malmö, Sweden). TPGDA was received as a pale yellow tinted liquid with a mild odor of 80% pure monomer. Lacquer A was a pale yellow tinted liquid with an acrid odor and according to the manufacturer contained 56.4% monomer (remaining components were proprietary ingredients). Due to the tendency of the acrylates to polymerize when exposed to light, hydroquinone (<200 ppm) was added as an inhibitor to each test article by the manufacturer. Samples were

stored in the dark at 4-6°C. Analyses of the stability of the test compounds were not carried out. All studies were conducted during the manufacturer's warranty for chemical stability. Acetone (CAS No. 67-64-1, Aldrich Chemical Co. Madison, WI, USA) was used as a vehicle control.

**Cell Culture.** Growth medium for each cell type used was composed of: (1) Keratinocyte Growth Medium (KGM); low calcium (0.15 mM) MCD8 153 supplemented with 0.4% bovine pituitary extract (BPE), 0.1 ng/ml human EGF, 5 ng/ml insulin, 0.5 µg/ml hydrocortisone, 50 µg/ml gentamycin, and 50 ng/ml amphotericin, (2) Fibroblast Growth Medium (FGM); 1 ng/ml basic fibroblast growth factor, 5 µg/ml insulin, and gentamycin and amphotericin, and (3) Bronchial Epithelial Cell Growth Medium (BEGM); 0.5 ng human EGF, 5 µg/ml insulin, 0.5 µg/ml hydrocortisone, 10 µg/ml transferrin, 0.5 µg/ml epinephrine, 6.5 ng/ml triiodothyronine, 0.4% BPE, 0.1 ng/ml retinoic acid, and gentamycin and amphotericin. All media and reagents, including subculturing reagents trypsin neutralizing solution (TNS), trypsin/EDTA solution (EDTA; 0.025% trypsin/0.01% EDTA in HBSS), and HEPES buffered saline solution (HBS) were obtained from Clonetics Corp. (San Diego, CA, USA). Cryopreserved primary or proliferating secondary normal human epidermal keratinocytes (NHEK), dermal fibroblasts (NHDF), and bronchial epithelial cells (NHBE) from 3 to 5 different adult female or male individuals were also obtained from Clonetics. Cryopreserved cells were received in a vial containing approximately 5x10<sup>5</sup> cells. Proliferating cells were received in a 25 cm<sup>2</sup> flask (5x10<sup>5</sup> cells) filled with growth medium. Cells were subcultured in growth medium in a loosely

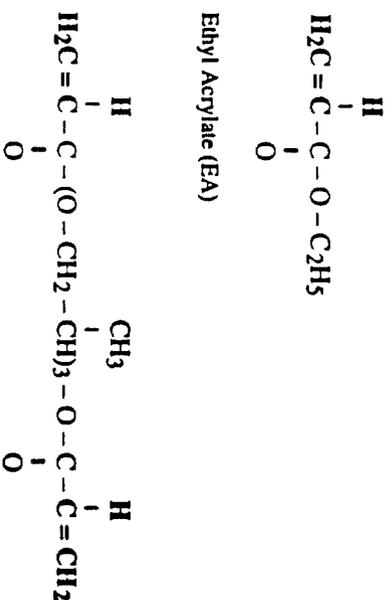


Fig. 1. Chemical structures of ethyl acrylate and tripropylene glycol diacrylate. Electrophilic structural alert of the acrylate functional groups are in bold.

capped 75 cm<sup>2</sup> tissue culture flasks in a humidified 5% CO<sub>2</sub> incubator at 37°C. Growth medium was changed every 48 h. For chemical exposure, cells were harvested from culture flasks (60-90% confluent) by standard trypsin digestion methods, counted, and seeded onto 96-well flat-bottomed plates at a density of ≈2500 cells/well or 60-100 cm<sup>2</sup> dishes at 500 cells/dish for determination of cloning efficiency. At confluence or ≈2/3 confluence, depending upon the assay, the culture medium was removed and replaced with growth medium containing the test chemical. Preliminary experiments indicated little difference in cytotoxicity as measured by the MTT assay, and cell number and/or morphology between log phase and stationary growth conditions employed. All experiments were performed on cells between the second and fourth passage from primary culture.

**Chemical Exposure.** Cell cultures were exposed to EA, TPGDA, Lacquer A, or acetone dissolved in the required growth mediums for 18 h in a humidified 5% CO<sub>2</sub> incubator at 37°C. Concentrations of EA, TPGDA, or Lacquer A varied from 5 to 0.0024 μmoles by 2 fold increments. Acetone (0.1% in medium) was used as a vehicle control. Chemicals were diluted in acetone before serial dilutions in medium to achieve the desired concentration. All exposures were performed with a total volume of 50 μl. All exposures and measurements were performed with two replicate plates for each treatment (N = 8 per plate) and were repeated twice (N = 32).

**UV exposure.** Epidermal keratinocyte cultures (eight 60 cm<sup>2</sup> dishes per treatment; ≈90% confluent) were exposed to: (1) 1 J/cm<sup>2</sup> UVA (600 μW/cm<sup>2</sup>) alone; (2) 1 J/cm<sup>2</sup> UVA (600 μW/cm<sup>2</sup>) followed by

3.54, 1.77, or 0.354 μmoles of TPGDA/dish; (3) 3.54 or 0.354 μmoles TPGDA alone; or (4) 0.1% acetone alone at 35-37°C. The experiment was replicated once. UVA radiation was generated using two Sylvania Blacklite Blue (#F15T8-BLB) lamps (Ultraviolet Products Inc., San Gabriel, CA, USA). Lamp output was determined using a Model IL570 spectral radiometer (International Light Inc., Newburyport, MA, USA). All exposures were performed in sufficient KBM to cover the cell layer and prevent desiccation during exposure. Chemical exposure cultures were sham irradiated under the same conditions minus UVA exposure. Medium was changed immediately to growth medium after radiation or sham-irradiation. After 48 h, cells were subcultured to 3 dishes (500 viable cells/dish) for determination of the cloning efficiency and transformation index. Cytotoxicity (viability) was determined from 3 aliquots from each dish by the MTT Assay (see below; 5000 cells/well) and percent viability (relative to control) calculated. Subcultured cells were held at 4°C until plating.

**Viability and Cell Growth.** The methylthiazol tetrazolium (MTT) assay (Cell Titer 96™; Promega, Madison, WI, USA) was used. After exposure, 15 μl 4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, was added to each well. After a 4 h incubation at 37°C, 100 μl stop/solubilization was added; except for individual wells with cells to serve as background controls, which had stop/solubilization solution added immediately after substrate. Cell cultures were incubated overnight in a humidified atmosphere at room temperature. Absorbance was determined at 570 nm using an ELISA plate reader (Molecular Devices Corp., Menlo

Park, CA, USA). Untreated and acetone vehicle control designated wells containing equal numbers of cells served as controls.

**Free Sulfhydryl Concentrations.** After exposure, free sulfhydryl content of control and treated cells in 96-well plates was determined by modification of Ellman's standard method and reagents (Pierce Chemical Co., Rockford, IL, USA). Cell cultures were washed twice with 100 μl PBS and 10 μl 0.1 Na<sub>2</sub>HPO<sub>4</sub> at pH 8.0 added. Culture plates were double wrapped in plastic and stored at -70°C for 24 h. Plates were allowed to equilibrate at room temperature (20-22°C) and 10 μl of 5,5'-dithio-bis-(2-nitrobenzoic acid) (4 mg/ml in 0.1 Na<sub>2</sub>HPO<sub>4</sub>) was added and mixed by pipette. A final volume of 190 μl 0.1 Na<sub>2</sub>HPO<sub>4</sub> was added and mixed. After 15 min, absorbance was determined at 412 nm in the ELISA plate reader and the free sulfhydryl concentration determined from a cysteine•HCl standard curve. A molar extinction coefficient of

EA12 = 1.36 x 10<sup>4</sup> cm<sup>-1</sup> M<sup>-1</sup> was used to determine concentration.

## Results

**Comparative cytotoxicity of EA, TPGDA, or TPGDA in a reference radiation curable lacquer (Lacquer A) to normal human cells in vitro (NHEK, NHDF, or NHBE)** was investigated using a methylthiazol tetrazolium (MTT) assay as a measure of cellular viability after acute exposure. The results indicate that EA, TPGDA, or Lacquer A were equally cytotoxic to NHEK at equimolar dose levels (Fig. 2). TPGDA and Lacquer A (81.5 and 70.5% TPGDA monomer, respectively; corrected to equimolar TPGDA concentration) were more cytotoxic to NHDF and NHBE than EA (Fig. 3 and 4). TPGDA is ≈100 times more cytotoxic than EA to NHDF and NHBE (Table 1). Both log-phase and stationary

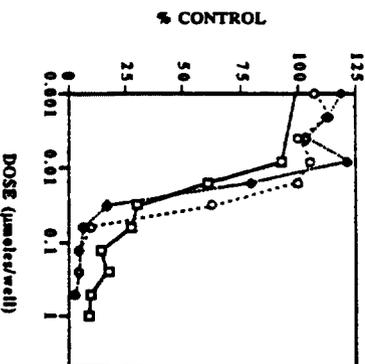


Fig. 2. Confluent cultures of normal human epidermal keratinocytes (NHEK) were exposed to ethyl acrylate (○), tripropylene glycol diacrylate (□), or Lacquer A (△) in 50.1% acetone for 18 h and cytotoxicity determined by measurement of MTT assay as an index of cytotoxicity (% control; N=32).

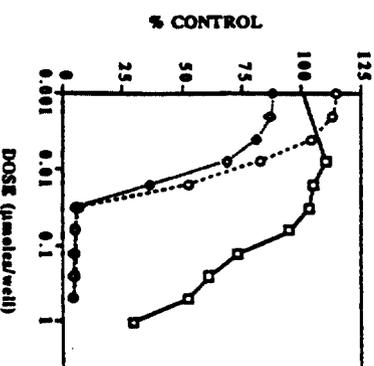


Fig. 3. Confluent cultures of normal human dermal fibroblasts (NHDF) were exposed to ethyl acrylate (○), tripropylene glycol diacrylate (□), or Lacquer A (△) in 50.1% acetone for 18 h and cytotoxicity determined by measurement of MTT assay as an index of cytotoxicity (% control; N=32).

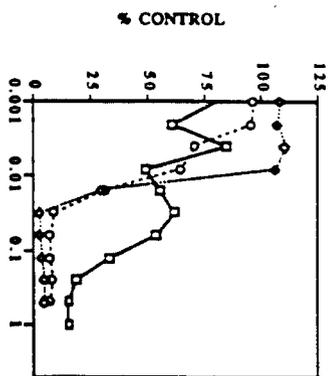


Figure 4. Confluent cultures of normal human bronchiolar epithelium (NHBE) were exposed to ethyl acrylate ( $\square$ ), tripropylene glycol diacrylate ( $\diamond$ ), or Laquer A ( $\circ$ ) in 50.1% acetone for 18 h and cytotoxicity determined by measurement of MTT assay as an index of cytotoxicity (% control;  $N=32$ ).

(70-90% confluent) growth population of NHEK and NHDF were equally sensitive to these acrylates in preliminary studies (data now shown).

Equimolar concentrations of TPGDA (81.5% monomer) alone or reference Laquer A (70.5% TPGDA and proprietary ingredients) yield similar ID<sub>50</sub> concentrations (Table 1). This suggests that ingredients other than the TPGDA are not toxic or are not present in sufficient concentration to exacerbate or potentiate toxicity and that TPGDA is the cytotoxic agent (Fig. 2-4, Table 1). Low doses of TPGDA or EA may be slightly mitogenic to both NHEK and NHDF (Fig. 3 and 4); TPGDA, but not EA, may be mitogenic at low doses to NHBE (Fig. 4). The free sulfhydryl concentrations in acrylate exposed human cells decreased with increasing acrylate dose (Fig. 5 and 6) and paralleled the cytotoxicity curves (Fig. 2 and 4).

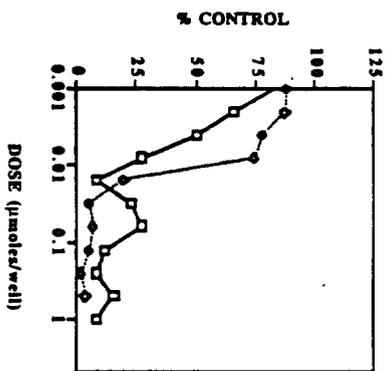


Figure 5. Confluent cultures of normal human epidermal keratinocytes (NHEK) were exposed to ethyl acrylate ( $\square$ ) or tripropylene glycol diacrylate ( $\diamond$ ) in 50.1% acetone for 18 h and concentration of free sulfhydryls were determined by Ellman's method using cysteine for generation of a standard curve (% control;  $N=16$ ).

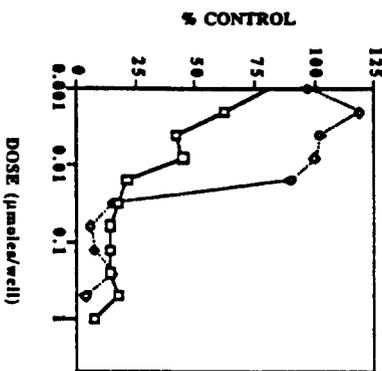


Figure 6. Confluent cultures of normal human bronchiolar epithelium (NHBE) were exposed to ethyl acrylate ( $\square$ ) or tripropylene glycol diacrylate ( $\diamond$ ) in 50.1% acetone for 18 h and concentration of free sulfhydryls were determined by Ellman's method using cysteine for generation of a standard curve (% control;  $N=16$ ).

Table 1. Acute toxicity of selected acrylates in vitro using normal human cells<sup>a</sup>. Estimated ID<sub>50</sub> concentration ( $\mu\text{mole}/\text{cm}^2$ ).

	NHEK $\mu\text{mole}/\text{cm}^2$	NHDF $\mu\text{mole}/\text{cm}^2$	NHBE $\mu\text{mole}/\text{cm}^2$
TPGDA	0.6	0.2	0.2
Laquer A	1.3	0.3	0.2
Ethyl acrylate	1.0	20.0 <sup>b</sup>	15.0 <sup>b</sup>

<sup>a</sup> Three to five different individual adult human early passage secondary cell cultures were used under the same conditions to calculate these estimates.

<sup>b</sup> Significantly different from other ID<sub>50</sub> values ( $p < 0.009$ ), but not to other values denoted the same.

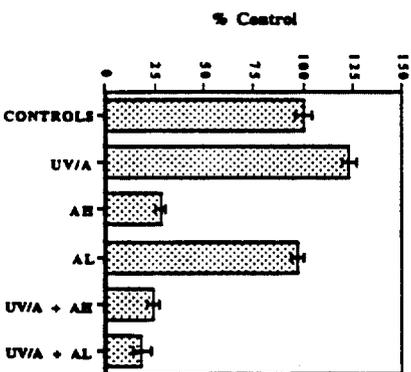


Figure 7. Confluent cultures of normal human epidermal keratinocytes (NHEK) were sequentially exposed to UV (5 J/cm<sup>2</sup>) and tripropylene glycol diacrylate (AH=60 or AL=6  $\mu\text{mole}/\text{cm}^2$ ; 50.1% acetone) for 2 h and cytotoxicity determined by MTT assay for cytotoxicity (% control;  $N=8$ ).

UV A exposure alone (1 J/cm<sup>2</sup> with 0.06% UV B) stimulated NHEK activity, whereas 0.06  $\mu\text{mole}/\text{cm}^2$  TPGDA was not cytotoxic (Fig. 7). UV A exposure (1 J/cm<sup>2</sup> with 0.06% UV B) followed by exposure to 6  $\mu\text{mole}/\text{cm}^2$  TPGDA resulted in a synergistic toxicity that was similar in magnitude to exposure to 60  $\mu\text{mole}/\text{cm}^2$  TPGDA (Fig. 7). Neither UV A and/or TPGDA increased

cloning efficiency or were observed to induce transformed colonies. An approximately 1% cloning efficiency (as expected for secondary passage diploid human cells) was observed in control or UV A treated NHEK populations (data not shown).

#### Discussion

Toxicity to monofunctional or multifunctional acrylates, which are chemical electrophiles and potent contact sensitizers (8) is poorly understood. Chemical electrophiles that induce contact sensitivity are also potentially carcinogenic (6). Recently, we have shown that TPGDA, but not EA induced epidermal papillomas when painted on the skin of TG $\times$ C transgenic mice at doses sufficient to induce epidermal hyperplasia (36). EA also failed to induce skin tumorigenesis (under the conditions of the studies) in C3H/HeI mice (15). In order to investigate the difference in toxicity between EA and TPGDA and potential human toxicity we conducted the studies presented here. Acrylates are cytotoxic in vitro to human cells (Fig. 2-4) that are potential target cells from occupational exposure (35). Furthermore, the potential for synergistic toxicity between low levels of ultraviolet radiation and multifunctional acrylates to

human cells is demonstrated (Fig. 7).

The MTT assay has been used widely to determine cellular proliferation and viability of cells in vitro after exposure to toxic agents (20, 23, 27). MTT is a pale yellow substrate that produces a dark blue formazan product when incubated with living cells. The MTT ring is cleaved in active mitochondria and the reaction occurs only in living cells (33). Our preliminary results compared favorably to both [3H]-thymidine incorporation and cloning efficiency assays. Use of the MTT assay greatly facilitated the number of cultures and replicates that could be performed in this comparative cytotoxicity study.

The ~100 fold difference between EA (20  $\mu\text{moles}/\text{cm}^2$ ) and TPGDA (0.2  $\mu\text{moles}/\text{cm}^2$ ) cytotoxicity observed in vitro for human cells is greater than the difference between the LD<sub>50</sub> skin paint dose for EA (2600  $\mu\text{moles}/8\text{ cm}^2$ ) and TPGDA (120  $\mu\text{moles}/8\text{ cm}^2$ ) in TG-AC mice when adjusted for the surface area exposed (36). Electrophiles, like the monofunctional EA and the multifunctional TPGDA, are believed to act directly at the site of application (18, 21). The absence of a significant difference in acrylate toxicity between human cells in vitro and mouse cells in vivo also supports a contact site toxicity and supports the in vitro and in vivo comparison. The increased toxicity of TPGDA, which is greater than a two-fold difference that could be attributed to the number of functional acrylate groups, suggests that other molecular characteristics may also contribute to its toxicity. Differences in toxicity may also be related to the significant difference in the relative low vapor pressure of a multifunctional acrylate, like TPGDA, compared to a monofunctional acrylate, like EA (2-5). This difference

could affect the availability of the compound in cell culture and in vivo skin exposure, compared to inhalation. Tissue specific differences in metabolizing enzymes (carboxylesterases versus glutathione-S-transferases) may also differ between skin and lung like the forestomach and liver (13, 21) and contribute to the differences observed.

Acrylate toxicity to human cells in vitro exhibited biphasic dose response curves (Fig. 2-4), which were similar to the free sulphydryl depletion curves (Fig. 5 and 6). This similarity suggests that free sulphydryls, like cysteine, glutathione, and sulphydryl containing cellular proteins or polypeptides are reduced proportionate to the acrylate dose in vitro. Although some loss of glutathione cannot be ruled out due to cytotoxicity and a decrease in cell number, cell loss (cells/cm<sup>2</sup>) was usually estimated at 10% or less with exposure conditions employed in these studies. Glutathione is known to react with and detoxify acrylates through Michael's addition reactions (18, 21). The potential of acrylates (electrophiles) to react with cells (nucleophilic biomolecules) as well as their potential to undergo polymerization demonstrates both their toxic potential and their industrial usefulness.

The potential exists for exposure to UV radiation (environmental or occupational) and multifunctional acrylates in the radiation curing surface coating industry (35). UVA radiation is known to induce reactive oxygen species (ROS), which results in the reduction of superoxide dismutase, catalase, and glutathione (9, 19). Acrylates are known to reduce glutathione upon contact and induce inflammation in vivo, which can also induce a prooxidant state with ROS (30). We speculate that sequential in vitro exposure to nontoxic dose levels of UVA

and TPGDA resulted in ROS and acrylate depletion of glutathione sufficient to induce cytotoxicity (Fig. 7). No effects of UV radiation and/or TPGDA on transformation was observed under these exposure conditions. This may be due, at least in part, to the use of near confluent cultures and the number of cells in division. Dose levels of UVA were chosen to be nonmutagenic (17) and mutagenicity studies of acrylates suggest a clastogenic mechanism of action (12, 31, 32) that requires cell division for acrylate reactivity (24).

In conclusion, an in vitro model system for investigating EA and TPGDA cytotoxicity using normal human cells that are potential targets from exposure was investigated. Estimation of the relative comparative cytotoxicity suggests that toxicity to some acrylates may be greater than can be accounted for by the number of functional acrylate groups, possibly involving other molecular characteristics. The relative difference in vapor pressure between monofunctional and multifunctional acrylates must also be considered to contribute to the relative differences in cytotoxicity observed. The synergistic toxicity between UVA and TPGDA in vitro warrants further investigation in vitro and in vivo to determine the potential importance to occupational exposure and the mechanistic basis for these observations.

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**IDENTIFICATION OF RISK TO WORKERS IN THE  
ULTRAVIOLET RADIATION CURING WOOD SURFACE  
COATING INDUSTRY**

*An Occupational Hygiene and Experimental Animal Study*

av

**Leena A. Nylander-French**

**AKADEMISK AVHANDLING**

som för avläggande av filosofie doktorsexamen vid Kungliga Tekniska Högskolan  
offentligen försvaras i sal E4, Lindstedtsvägen 5, Kungliga Tekniska Högskolan,  
fredagen den 3 juni 1994, kl 09.00

**STOCKHOLM 1994**

*From the Division of Industrial Hygiene, National Institute of Occupational Health, Solna and the Department of Environmental Technology and Work Science, the Royal Institute of Technology, Stockholm, Sweden*

**IDENTIFICATION OF RISK TO WORKERS IN THE ULTRAVIOLET RADIATION CURED ACRYLATE LACQUER WOOD SURFACE COATING INDUSTRY**

*An occupational hygiene and experimental animal study*

Leena A. Nylander-French

**ABSTRACT**

Use of surface coatings containing multifunctional acrylates that are cured with ultraviolet radiation (UVR) is rapidly increasing and the potential health effects to workers have not been adequately investigated. Occupational risks related to the surface coating of wood using UVR curable coatings were identified by using an industrial hygiene survey and medical examination of workers. In Sweden, workers are exposed to small amounts of acrylate containing aerosols, vapors, and/or dusts and organic solvents. The potential toxicity of tripropylene glycol diacrylate, the primary acrylate observed in use, was evaluated using rodents and human cells *in vitro*. The most significant findings are that: (1) an estimated 350 out of 8,500 wood surface coating workers are directly exposed to acrylates and UVR, (2) respirable dust particles (28% of the total dust concentration) are present, but overall dust levels are low ( $\approx 0.4 \text{ mg/m}^3$ ), (3) ozone concentration levels did not exceed the background concentration, (4) UVR exposure to unprotected skin at biologically effective dose levels from poorly shielded UV units resulted in erythema and pigmentation, (5) the potential exists for direct exposure to sensitizing agents (acrylates, initiators, and inhibitors) and development of contact dermatitis, and (6) most workers were inconsistent in their work practices and educational programs for workers were insufficient. Nasal, pharyngeal, and ocular symptoms of discomfort, but not for the lower airway, were common among all workers. These symptoms were most frequent in UV line workers and finishers of UVR surface coated wood products. Mucosiliary clearance and olfaction were impaired in UV line workers indicating changes in the nasal cavity which may be the result of toxicity. Irritant dermatitis was prevalent and a low frequency of contact allergy was observed. The principal findings from the toxicology studies indicated that tripropylene glycol diacrylate was moderately toxic and induced skin tumors when applied to the skin of genetically initiated mice and was toxic to normal human skin and lung cells *in vitro*. This technology does involve potential health risks for workers. Uncured or partially cured acrylate coatings must be considered as potentially harmful to airways, eyes, and skin. Control of the UVR curing process in respect to complete curing and shielding of UV units are important measures to reduce potential risk. Education and protective measures are required to reduce worker exposure. Additional research on exposure assessment, risk assessment, and managing risk by process, emission, and exposure control technology is required.

*Key words: occupational exposure, acrylates, dust, ultraviolet radiation, health effects, airway, skin, toxicity, surface coating, wood industry*

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**Triage of 8(e) Submissions**

Date sent to triage: \_\_\_\_\_

**NON-CAP**

**CAP**

Submission number: 12966A

TSCA Inventory:

**Y**

**N**

**D**

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO

AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX

SBTOX

SEN

w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX

CTOX

EPI

RTOX

GTOX

STOX/ONCO

**CTOX/ONCO**

IMMUNO

CYTO

NEUR

Other (FATE, EXPO, MET, etc.): \_\_\_\_\_

Notes:

**THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY**

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entire document: **0** 1 2 pages 1 pages \_\_\_\_\_

Notes:

Contractor reviewer: JW Date: 1/24/96

CECATS/TRIAGE TRACKING DBASE ENTRY FORM

CFCATS DATA: Submission # SEHQ-0894-12966 SEQ. A

TYPE: INT/SUPP FLWP

SUBMITTER NAME: Henkel Corporation

INFORMATION REQUESTED: FLWP DATE:

- 0501 NO INFO REQUESTED
- 0502 INFO REQUESTED (TECH)
- 0503 INFO REQUESTED (VOL. ACTIONS)
- 0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:

- 0639 REFER TO CHEMICAL SCREENING
- 0678 CAP NOTICE

SUB. DATE: 08/04/94 OTS DATE: 08/09/94 CSRAD DATE: 06/01/95

CHEMICAL NAME:

diacetyltriethylglycol

CAS#

42978-665

0401 VOLUNTARY ACTIONS:

- 0401 NO ACTION REPORTED
- 0402 STUDIES PLANNED/IN PROGRESS
- 0403 NOTIFICATION OF WORKING METHODS
- 0404 LABEL/MSDS CHANGES
- 0405 PROCESS/HANDLING CHANGES
- 0406 APP. USE DISCONTINUED
- 0407 PRODUCTION DISCONTINUED
- 0408 CONFIDENTIAL

INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C
0201 ONCO (HUMAN)	01 02 04	EPICLIN	01 02 04
<u>0202</u> ONCO (ANIMAL)	<u>01 02 04</u>	HUMAN EXPOS (PROD CONTAM)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0204 MUTA (IN VITRO)	01 02 04	HUMAN EXPOS (MONITORING)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	ECO/AQUA TOX	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	ENV. OCCUREL/FATE	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	EMER INCI OF ENV CONTAM	01 02 04
0208 NEURO (HUMAN)	01 02 04	RESPONSE REQUEST DELAY	01 02 04
0209 NEURO (ANIMAL)	01 02 04	PROD/COMP/CHEM ID	01 02 04
0210 ACUTE TOX. (HUMAN)	01 02 04	REPORTING RATIONALE	01 02 04
0211 CHR. TOX. (HUMAN)	01 02 04	CONFIDENTIAL	01 02 04
0212 ACUTE TOX. (ANIMAL)	01 02 04	ALLERG (HUMAN)	01 02 04
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	ALLERG (ANIMAL)	01 02 04
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	METAB/PHARMACO (ANIMAL)	01 02 04
<u>0215</u> CHRONIC TOX (ANIMAL)	01 <u>02 04</u>	METAB/PHARMACO (HUMAN)	01 02 04

TRIAGE DATA: NON-CBI INVENTORY

YES

NO

ONGOING REVIEW

YES (DROP/REFER)

NO (CONTINUE)

REFER:

SPECIES

MUS

TOXICOLOGICAL CONCERN:

LOW

MED

HIGH

USE:

CARCINOGENICITY

PRODUCTION:

Industrial

COMMENTS:

Non-Cap

#12966A

L

Carcinogenicity is of low concern based on the results of a skin painting study in mice. Mice were exposed to 1, 5 and 10  $\mu$ moles 3 times/week for 20 weeks. Focal areas of hyperkeratosis developed into papillomas, which increased in number/mouse over time. There was 100% incidence of papillomas at every dose level, and occurrence decreased in latency with 5 and 10  $\mu$ mole doses. There were no effects at 1  $\mu$ mole, and no dose-related tumor incidence.