Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:
2-hydroxyethyl methacrylate (HEMA)

EC Number: **212-782-2**
CAS Number: **868-77-9**
Index Number: **607-124-00-X**

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CONTENTS

1 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

1.1.1 [OASIS TIMES]

Metabolic map_HEMA_in vitro rat S9_Phase I, as predicted by OASIS TIMES.

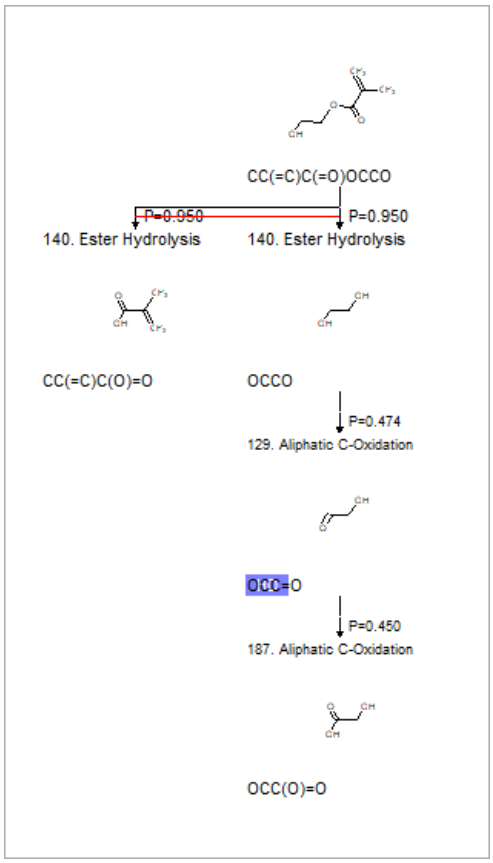
- **P (Prob., intrinsic)** is the probability of the current transformation from transformation table.
- **Quantity of metabolite** depends on both – probability to be obtained and probability to metabolize:

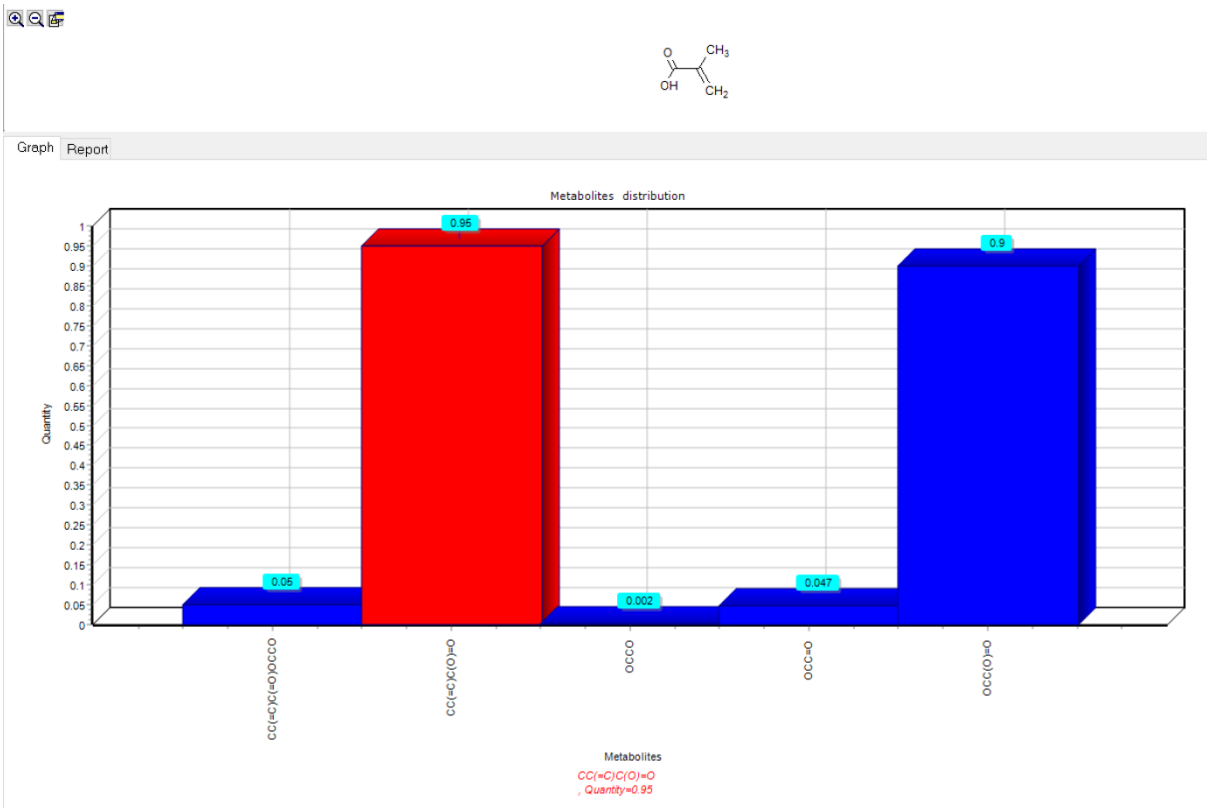
  Q = <probability to obtain> x (1 - <probability to metabolize>)

Quantity of parent is calculated under the assumption that the probability to obtain is equal to 1: <probability to obtain> = 1

Q(parent) = 1 - <probability to metabolize>

Hydrolysis is indicated with red horizontal line.





Metabolic map_HEMA_in vitro rat S9_Phase I, as predicted by OASIS TIMES.

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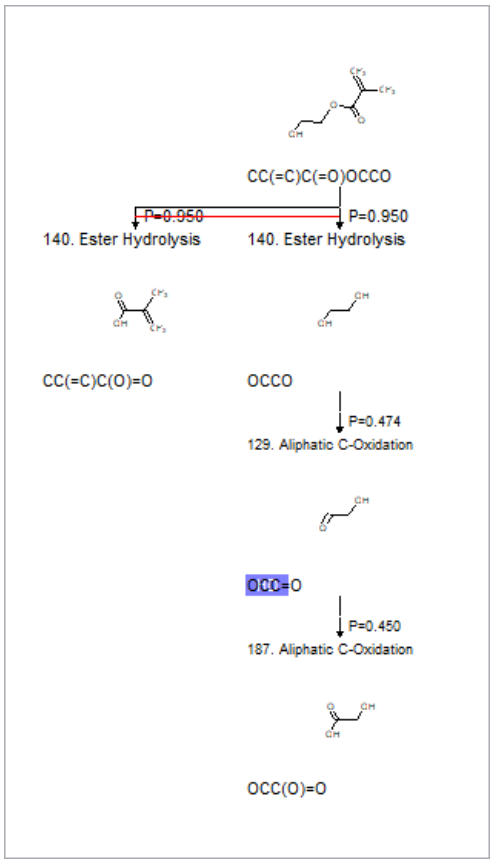

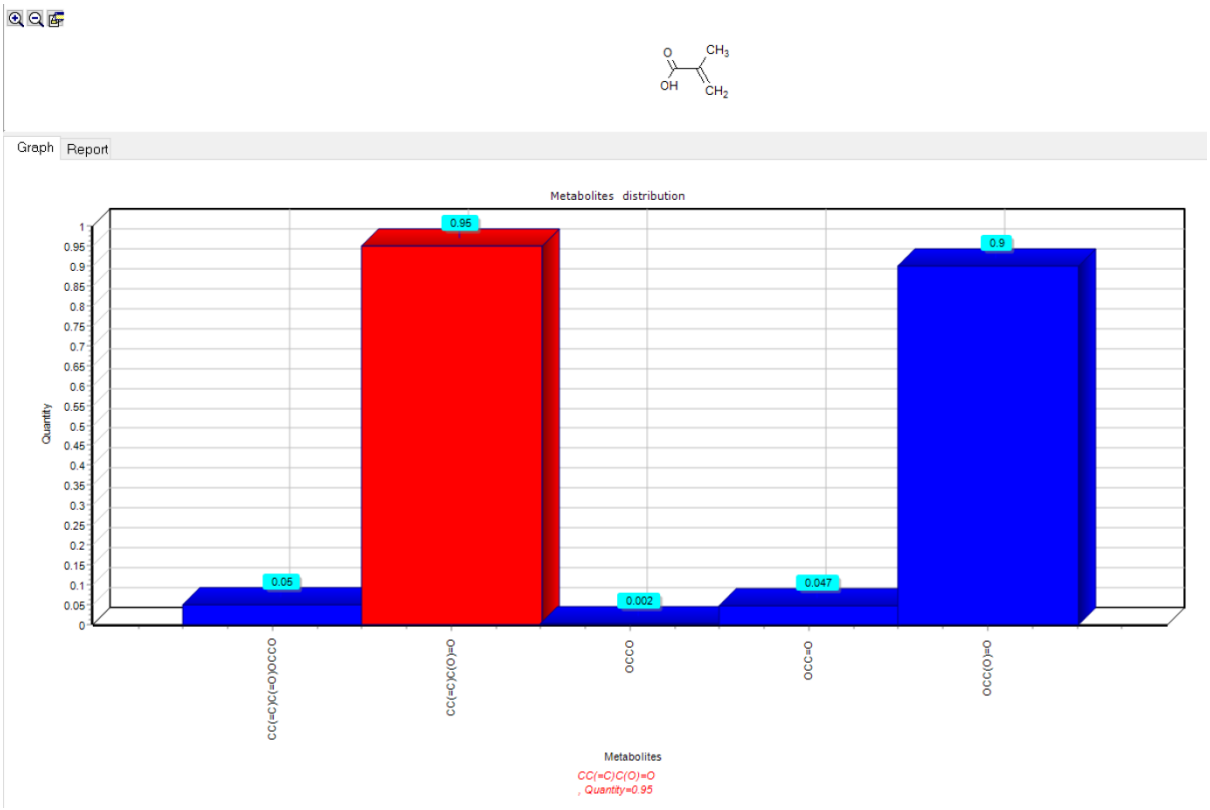

1.1.2 [Reichl, 2002]

Study reference:

Reichl, F.X., et al 2002: Toxicokinetic of HEMA in guinea pigs, Journal of Dentistry 30: 353–358.

Test type

Toxicokinetics *in vivo* in guinea pigs

Test substance

^{14}C-HEMA was purchased from NEN Life Science Products, Inc., 549 Albany Street, Boston MA 02118-2512, USA. Unlabeled HEMA was obtained from ESPE Dental AG, Seefeld, Germany.

Method

Three series of animal experiments were performed.

- Total ^{14}C excretion and ^{14}C distribution : For the first experimental series guinea pigs were allocated to four groups of four animals each. Each animal was put into a separate metabolic cage 3 days before the beginning of the experiment. Each animal received 0.02 mmol/kg HEMA, labeled with a tracer dose of radioactive ^{14}C-HEMA (0.3 kBq/g) either by subcutaneous injection (group 1) or via gastric tube (group 2). Control animals received 0.9% NaCl-solution by subcutaneous injection or by mouth instead of HEMA. Feces and urine were collected at 1, 2, 4, 6, 8, 12 and 24 h after ^{14}C-HEMA administration. Twenty four hours after the beginning of the experiment the animals were put to death in ether. Thereafter, the following organs and walls and/or contents of organs were removed: liver, kidney, blood, skin, brain, heart, spleen, lung, muscle, testes, eyes, bone, nerve tissue, spinal cord, wall of stomach, content of stomach, wall of ileum + jejunum, content of ileum + jejunum, wall of colon, content of colon, wall of caecum, content of caecum, wall of gall bladder, and fat tissue. Tissues were dissolved in tetraethylammoniumhydroxide (20%) in aqueous solution with Omni-Szintisol using the method described by Strugala and ^{14}C radioactivity determined.

- C excretion via the lungs : The second experimental series was aimed at the additional determination of exhaled CO_2. Again guinea pigs were allocated to four groups of four animals each and put into individual metabolic cages before the beginning of the experiment. Guinea pigs were treated with HEMA (+^{14}C-HEMA) as described for the first series. Exhaled air was captured during the 24 h-experimental period by flowing through seven bottles, one behind the other, filled with 250 ml 5N NaOH (ice-cold), each. $^{14}CO_2$ was captured as $Na_2{}^{14}CO^3$, and then the ^{14}C activity of the fluid in each bottle was measured. Urine, feces, organs and their contents were taken for analysis as described for the first experimental group.
- C excretion via the bile: For the third experimental series eight guinea pigs were randomized to two groups of four animals each. The animals were anesthetized with urethane (1 mg/kg, 4 m/kg; dissolved in 0.9% NaCl-solution). The ductus cysticus was ligated and a cannula was placed in the bile duct. HEMA, labeled with ^{14}C-HEMA, was injected via the jugular vein in the same dose as described above. Control animals received 0.9% NaCl-solution only. Samples of the bile (0.4 ml) were taken every 10 min. The experimental period was 60 min.
- Chromatography of ^{14}C-HEMA metabolites: In order to differentiate between the amounts of excreted ^{14}C-HEMA and individual ^{14}C metabolites, aliquots of the bile (0.1 ml) as well as unlabeled HEMA, unlabelled methacrylate, unlabeled pyruvate, and unlabeled L-malate were chromatographed on a thin liquid chromatography silica gel plate. Repeated runs were performed for all samples taken (six per experiment from four animals each). Between runs the respective silica gel plates were dried at 37°C for 12 h. Thereafter, the relevant spots were scratched off from the plates, given into a vial and then 0.5 ml 1N HCl were added and incubated at 60 °C for 24 h. The vials were centrifuged (5 min/10.000 U/min), the supernatant was taken off, then 0.1 ml distilled water and 4 ml Omni-Szintisol were added and the ^{14}C radioactivity was measured.

Results and discussion

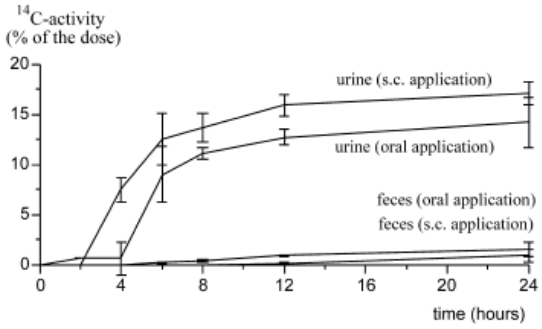

Fig. 2. Cumulative urinary and fecal ^{14}C excretion in guinea pigs. Guinea pigs received HEMA (0.02 mmol/kg by weight labeled with a tracer dose ^{14}C-HEMA 0.3 kBq/g by weight) s.c. or with a gastric tube, respectively. Urine and feces were collected during 24 h, and then the ^{14}C radioactivity was measured ($n = 4$; mean ± SEM)

Table 2
^{14}C excretion in guinea pigs via the urine, feces, and carbondioxide as well as ^{14}C distribution. Guinea pigs received HEMA (0.02 mmol/kg by weight labeled with a tracer dose ^{14}C-HEMA 0.3 kBq/g by weight) s.c. or with the gastric tube, respectively. Urine, feces, and exhaled $^{14}CO_2$ were collected, and the organs removed after 24 h, and then the ^{14}C radioactivity was measured ($n = 4$; mean ±SEM)

	^{14}C activity (% of the ^{14}C-HEMA dose administered)			
	Application subcutaneous		Application gastric tube	
	Mean	SEM	Mean	SEM
Urine	16.8	3.5	10.8	2.8
Feces	1.6	0.7	3.1	0.6
Exhaled $^{14}CO_2$	68.4	1.3	74.8	6.8
Wash water	1.2	0.1	1.0	0.1
Sum organs	7.5	1.2	8.1	1.0
Total ^{14}C recovery	95.5	4.9	97.8	4.1

Table 1
^{14}C activity in total organs and walls and/or contents of organs in guinea pigs at 24 h (% of the dose; $n = 4$; mean ±SEM) Guinea pigs received HEMA (0.02 mmol/kg by weight labelled with a tracer dose ^{14}C-HEMA 0.3 kBq/g by weight) s.c. or with the gastric tube, respectively. Organs were removed 24 h after the ^{14}C-HEMA application

	^{14}C activity (% of the ^{14}C-HEMA dose administered)			
	Application subcutaneous		Application gastric tube	
	Mean	SEM	Mean	SEM
Muscle	1.6	0.5	1.7	0.5
Content of caecum	1.6	0.6	1.5	0.3
Liver	1.4	0.2	1.7	0.4
Blood	1.2	0.6	0.4	0.2
Skin	0.5	0.2	0.6	0.3
Content of stomach	0.3	0.2	0.2	0.1
Content of colon	0.2	0.1	0.2	0.1
Kidney	0.2	0.1	0.2	0.1
Wall of jejunum	0.1	0.0	0.1	0.0
Testes	0.1	0.0	0.1	0.0
Content of jejunum	0.1	0.0	0.1	0.0
^{14}C activity in each other organ/content	<0.1	<0.02	<0.1	<0.02
Sum organs	8.3	1.2	7.8	1.4

Table 3
Total ^{14}C activities in the spontaneous bile (in % of the ^{14}C-HEMA dose administered) and ^{14}
activity in the spontaneous bile). Guinea pigs received HEMA (0.02 mmol/kg labeled with a tr
Samples from the bile were taken every 10 min during the 60 min, after which the ^{14}C activity w
substances were chromatographed on a thin liquid chromatography silica gel plate (mean ±SE

Min	10	20	30
Total ^{14}C activity (%)	1.85	0.75	0.44
SEM	0.20	0.10	0.05
Bile (ml)	1.57	1.46	1.49
SEM	0.2	0.2	0.2
^{14}C-pyruvate (%)	11.93	17.45	10.23
SEM	2.61	4.78	3.73
^{14}C-L-malate (%)	0.22	0.18	0.12
SEM	0.06	0.04	0.05
^{14}C-HEMA (%)	0.90	0.15	0.05
SEM	0.84	0.09	0.05
^{14}C-methacrylate (%)	7.59	10.15	11.09
SEM	4.15	6.42	9.00
^{14}C-pyruvate/^{14}C-L-malate	54.22	96.94	85.25

About 70% of the administered ^{14}C was excreted via the lungs as $^{14}CO_2$, while some 15% of the administered ^{14}C appeared in the urine. Clearance of 14C-label from the body appeared to be essentially complete one day following gastric or subcutaneous application. Peak tissue levels of HEMA were substantially below those known to be cytotoxic.

1.1.3 [Durner, 2009]

Study reference:

Durner, J., H. Kreppel, J. Kaspel, H. Schweikl, R. Hickel, and F. Reichl 2009: The Toxicokinetics and Distribution of 2-Hydroxyethyl methacrylate in Mice, Biomaterials 30, 2066-2071.

Test type

Toxicokinetics and distribution *in vivo* in mice

Test substance

HEMA was purchased from ESPE (Dental AG, Seefeld, Germany), tetraethylammonium-hydroxide (TEAH; 20%) and Omni-Szintisol from Merck (Darmstadt, Germany). All chemicals and reagents were of the highest purity available. ^{14}Clabelled HEMA was purchased from TNO Prins Maurits Laboratorium (Lange Kleiweg 137, 2280 AA Rijswijk, The Netherlands). The ^{14}C label was situated on the carbonyl group of the molecule.

Methods

56 male mice were randomized and allocated to fourteen groups of four mice. Each mouse was put into a separate cage for different time periods (5 min, 15 min, 0.5 h, 1 h, 12 h and 24 h). The food supply was stopped 12 h before the beginning of the experiment. Each mouse received HEMA (20 µmol/kg bw, dissolved in 0.9% NaCl solution, labelled with a tracer dose of radioactive ^{14}C-HEMA 0.7 kBq/g bw) either by subcutaneous injection beneath the shoulder skin or via gastric tube. The volume was 10 ml/g bw for both application forms. Control mice received 0.9% NaCl solution only. The mice were killed at corresponding time intervals by neck fracture. Organs, organ-wall and content of organs as well as blood were taken immediately. Tested organs were: liver, kidney, blood, skin, brain, heart, spleen, lung, muscle (from 3 areas), testis, eyes, bone, wall of stomach, content of stomach, wall of small intestine, content of small intestine, wall of large intestine, and content of large intestine. Organs were immediately washed with 2 x10 ml distilled H_2O and then the tissues were weighed and homogenized. Tissues were dissolved in TEAH.

A second set of 56 mice was treated as described above, with the addition that each mouse was kept in a closed chamber with controlled air flow. The exhaled air was captured during the total experimental period by flowing through 7 bottles, one behind the other, filled with 250 ml ice-cold 5 N NaOH. $^{14}CO_2$ was captured as $^{14}CNa_2CO_3$ and the total ^{14}C-activity determined. Urine and feces were collected at 0.5, 1, 2, 6, 12 and 24 h after the beginning of the experiment. The mice were killed by neck fracture. Organs, organ-wall and content of organs, blood, urine and feces were taken immediately. Organs were taken for analysis as described for the first in vivo experiment.

The clearance of ^{14}C-HEMA and the ^{14}C content in organs, wall and content of organs, blood, urine, feces and exhaled air were determined by measuring the ^{14}C activity. It was measured with TEAH in aqueous solution with Omni-Szintisol. The ^{14}C-activity was determined in a 2500 TR liquid scintillation analyzer (Canberra-Packard, Dreieich, Germany).

Results and discussion

Table 1
14C-activity up to 24 h following 14C-HEMA gastric administration in mice.

14C in percentage of the 14C-HEMA dose per total organ administered ± SEM	Time after administration					
	5 min	15 min	30 min	1 h	12 h	24 h
Liver	5.1 ± 0.4	2.1 ± 0.7	1.3 ± 0.1	1.2 ± 0.2	0.5 ± 0.1	0.3 ± 0.0
Kidney	1.3 ± 0.1	1.0 ± 0.1	0.9 ± 0.3	0.4 ± 0.0	0.1 ± 0.0	0.0
Heart	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0	0.0	0.0
Skin	4.3 ± 1.1	4.2 ± 0.9	1.7 ± 0.2	1.2 ± 0.1	0.4 ± 0.1	0.0
Brain	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0	0.0
Lung	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0	0.0	0.0
Muscle	11.3 ± 1.6	7.8 ± 1.5	3.7 ± 0.2	2.6 ± 0.4	0.8 ± 0.1	0.0
Stomach wall	10.5 ± 1.6	6.4 ± 1.8	1.5 ± 0.5	1.6 ± 0.9	0.0	0.0
Stomach content	19.8 ± 3.3	10.4 ± 5.1	2.4 ± 0.6	5.7 ± 1.7	0.0	0.0
Small intestine wall	3.9 ± 0.5	2.1 ± 0.6	1.3 ± 0.2	0.9 ± 0.5	0.1 ± 0.0	0.0
Small intestine content	0.5 ± 0.2	0.7 ± 0.2	0.9 ± 0.3	0.5 ± 0.1	0.0	0.0
Large intestine wall	0.8 ± 0.2	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	0.0
Large intestine content	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.6 ± 0.2	0.1 ± 0.0	0.1 ± 0.1
Spleen	0.1 ± 0.0	0.1 ± 0.0	0.0	0.0	0.0	0.0
Testis	0.1 ± 0.0	0.1 ± 0.0	0.0	0.0	0.0	0.0
Eyes	0.0	0.0	0.0	0.0	0.0	0.0
Blood	3.3 ± 0.3	1.5 ± 0.2	0.7 ± 0.1	0.3 ± 0.0	0.3 ± 0.3	0.0
Bone	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.0	0.1 ± 0.0
Sum	62.0 ± 1.3	37.7 ± 5.2	15.4 ± 0.6	15.6 ± 2.2	2.4 ± 0.1	0.5 ± 0.1

Table 2
14C-activity over time following 14C-HEMA subcutaneous administration in mice.

14C in percentage of the 14C-HEMA dose per total organ administered ± SEM	Time after administration					
	5 min	15 min	30 min	1 h	12 h	24 h
Liver	2.7 ± 0.3	1.8 ± 0.2	1.0 ± 0.1	0.9 ± 0.1	0.4 ± 0.0	0.2 ± 0.0
Injection area	3.7 ± 0.6	4.7 ± 1.8	0.5 ± 0.3	0.4 ± 0.2	0.3 ± 0.1	0.0
Kidney	1.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	0.5 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Heart	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0	0.0	0.0
Skin	5.6 ± 1.0	16.0 ± 6.9	1.7 ± 0.2	1.8 ± 0.3	0.3 ± 0.0	0.2 ± 0.1
Brain	0.6 ± 0.1	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.0	0.0
Lung	0.5 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0	0.0
Muscle	18.4 ± 1.4	10.5 ± 1.7	3.7 ± 0.4	3.4 ± 0.3	0.6 ± 0.1	0.4 ± 0.2
Stomach wall	0.4 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.0	0.0
Stomach content	0.1 ± 0.0	0.1 ± 0.0	0.0	0.3 ± 0.2	0.0	0.0
Small intestine wall	1.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	0.0
Small intestine content	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.0	0.0
Large intestine wall	0.6 ± 0.0	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.0	0.0
Large intestine content	0.6 ± 0.3	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.0	0.0
Spleen	0.1 ± 0.0	0.1 ± 0.0	0.0	0.0	0.0	0.0
Testis	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0	0.0
Eyes	0.1 ± 0.0	0.0	0.0	0.0	0.0	0.0
Blood	5.7 ± 1.4	1.6 ± 0.3	0.6 ± 0.1	0.3 ± 0.0	0.2 ± 0.2	0.0
Bone	0.2 ± 0.0	0.4 ± 0.2	0.2 ± 0.0	0.1 ± 0.0	0.0	0.0
Sum	43.4 ± 3.9	39.3 ± 8.0	10.6 ± 1.2	9.2 ± 0.6	2.0 ± 0.2	0.9 ± 0.4

The plasma half-life period of ^{14}C-HEMA is lower than 10 min after oral or SC administration.

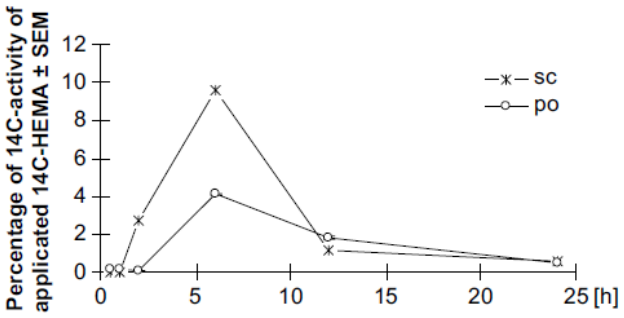

Fig. 1. 14C-excretion *via* urine in mice. Mice received HEMA (20 µmol/kg bw, dissolved in 0.9% NaCl solution, labelled with a tracer dose of 14C-HEMA 0.7 kBq/g bw) with a gastric tube (po) or subcutaneously (sc). Urine was collected within 24 h and the 14C-activity was measured ($n = 4$; mean ± SEM).

Table 3
14C-excretion in mice *via* urine, feces, and $^{14}CO_2$ as well as summed 14C-distribution in organs 24 h after 14C-HEMA administration with a gastric tube or by subcutaneous injection ($n = 4$; mean ± SEM).

	14C-activity (% of the 14C-HEMA dose administered)			
	Application with the gastric tube		Subcutaneous application	
	Mean	SEM	Mean	SEM
Urine	6.9	0.5	14.1	0.2
Feces	22.6	0.4	12.4	0.5
Exhaled $^{14}CO_2$	62.3	3.7	66.7	3.3
Organ wash water	1.5	0.2	1.3	0.2
∑ organs	1.3	0.2	1.0	0.3
Total [14C]-recovery	94.6	4.1	95.5	3.7

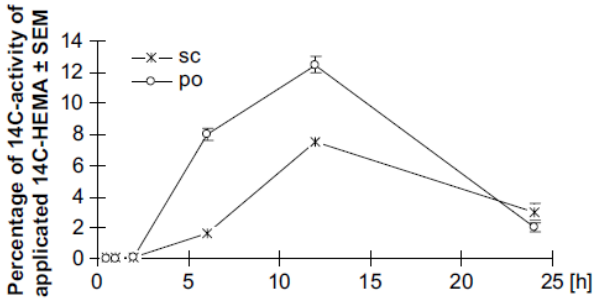

Fig. 2. 14C-excretion *via* feces in mice. Mice received HEMA (20 µmol/kg bw, dissolved in 0.9% NaCl solution, labelled with a tracer dose of 14C-HEMA 0.7 kBq/g bw) with a gastric tube (po) or subcutaneously (sc). Feces were collected within 24 h and the 14C-activity was measured ($n = 4$; mean ± SEM).

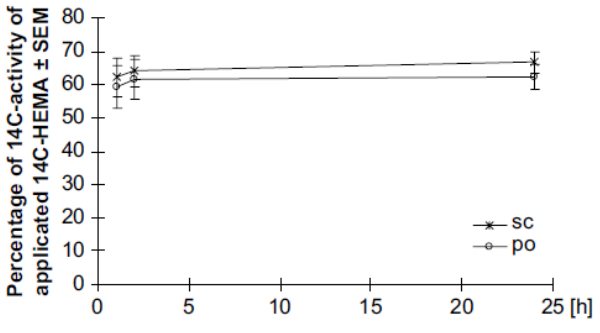

Fig. 3. 14C-excretion *via* exhalation in mice. Mice received HEMA (20 µmol/kg bw, dissolved in 0.9% NaCl solution, labelled with a tracer dose of 14C-HEMA 0.7 kBq/g bw) with a gastric tube (po) or subcutaneously (sc). Exhaled $^{14}CO_2$ was collected as 14C-Na_2CO_3 within 24 h and the 14C-activity was measured ($n = 4$; mean ± SEM).

^{14}C-HEMA was taken up rapidly from the stomach and intestines after gastric administration and was widely distributed in the body following administration by each route. Most ^{14}C was excreted within one day as $^{14}CO_2$. Two metabolic pathways of ^{14}C-HEMA can be described.

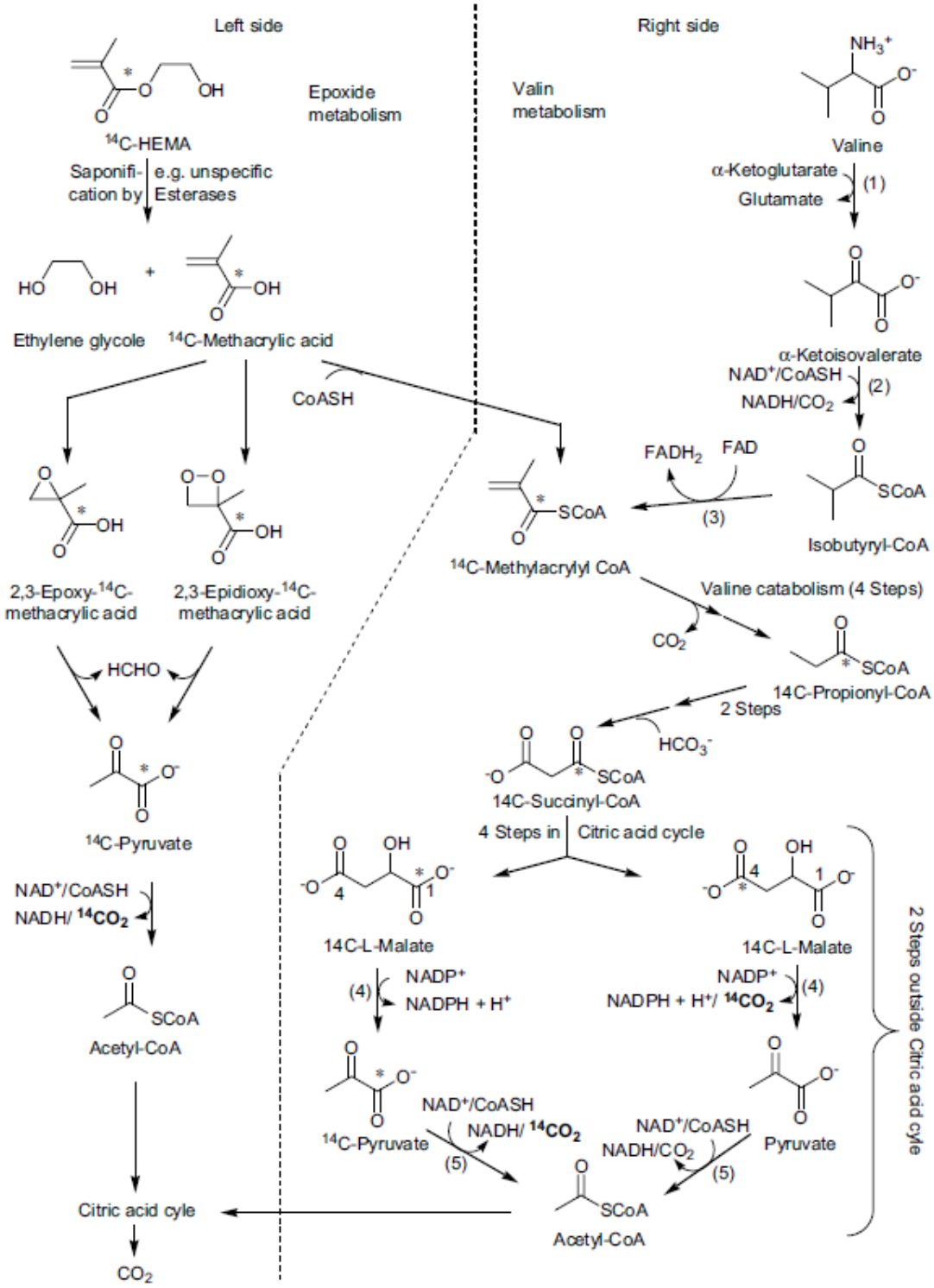

Fig. 4. Postulated metabolism of 14C-HEMA in mice. The pathway started with saponification of HEMA by e.g. unspecific esterases in saliva or blood. The emerging 14C-methacrylic acid can be oxidized by mono- or dioxygenases (left side of figure, epoxide metabolism) or it can be esterified to 14C-methylacrylyl CoA which follows metabolism of proteinogenic amino acid valine (right side of figure, valine metabolism). Thereby, two forms of 14C-L-malate can be built (14C labelling on C-atom 1 or on C-atom 4). Metabolisation of 14C-L-malate labelled on C-atom 1 can lead to 14C-pyruvate an intermediate of epoxide metabolism. Enzymes involved: (1) Aminotransferase for amino acids with branched chains (2) α-Ketoisovalerate dehydrogenase (3) Acyl CoA dehydrogenase (4) Malic enzyme (5) Pyruvate dehydrogenase. * Labelled with 14C.

1.1.4 [Durner, 2010]

Study reference:

Durner J., Walther UI., Zaspel J., Hickel R., Reichl FZ.. 2010. Metabolism of TEGDMA and HEMA in human cells. Biomaterials. Volume 31, Issue 5, 818-823

Test type

Metabolism *in vitro*

Test substance

^{14}C-labelled HEMA and ^{14}C-labelled TEGDMA were purchased from TNO Prins Maurit laboratorium (Rijswijk, The Netherlands). The ^{14}C-label was situated on the carbonyl group of the molecule. HEMA and TEGDMA were purchased from ESPE (Dental AG, Seefeld, Germany) and Omni-Szintisols from Merck (Darmstadt, Germany). Cell culture chemicals (DMEMmF12) [Dulbecco's modified Eagle's medium/HAM's F12 nutrient mix (1:1)], minimum essential medium (MEM) (Hanks'salts), penicillin/streptomycin, trypsin/EDTA were obtained from Gibco (Eggenstein, Germany), and fetal calf serum from Biochrom (Berlin, Germany). All other chemicals and reagents were from various suppliers and were of the highest purity available.

Methods

A549 cells were incubated with TEGDMA or HEMA (with a tracer dose ^{14}C-TEGDMA or ^{14}C-HEMA) and afterwards ^{14}C-TEGDMA or ^{14}C-HEMA, ^{14}C-methacrylate, ^{14}C-L-malate and ^{14}C-pyruvate were identified and quantified by thin layer chromatography at different time intervals from the extracellular and intracellular fluid.

Results and discussion

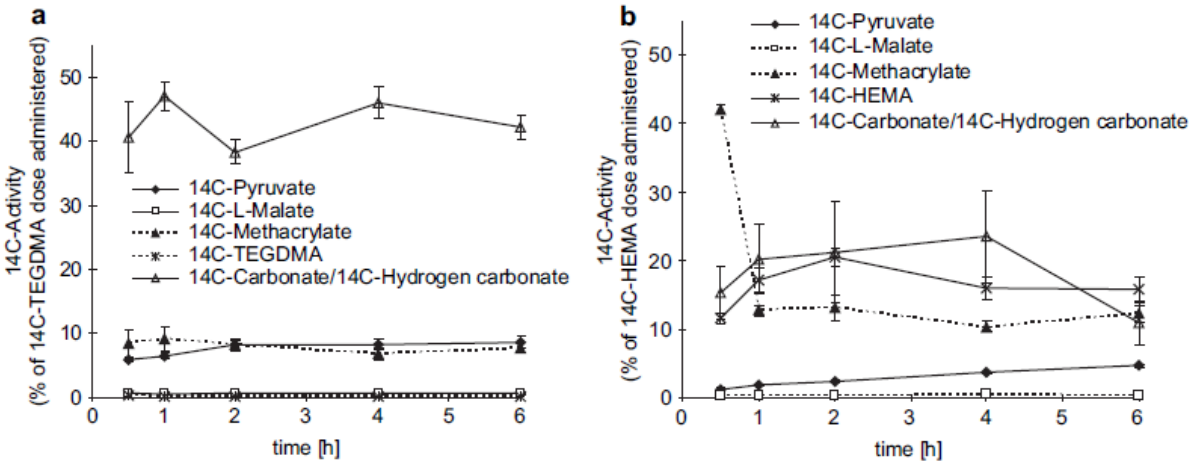

Fig. 1. Distribution of 14C labelled metabolites in the extracellular fluid at different time intervals. A549 cells were incubated with TEGDMA (a) or HEMA (b) (0.02 mM dissolved in modified DMEMmF12, labelled with a tracer dose (80 kBq/mL) of 14C-TEGDMA or 14C-HEMA) and 14C-activity was measured after isolation of 14C labelled metabolites by thin layer chromatography at different time intervals ($n = 4$; mean ± sem).

Table 2
Ratio of 14C-pyruvate/14C-L-malate in the different cell compartments and amount of the valine and epoxide pathway on the metabolisation of HEMA (in percent).

Time [h]	14C-HEMA exposure									
	Extracellular					Intracellular				
	14C-pyruvate/14C-L-malate			% Epoxide pathway	% Valine pathway	14C-pyruvate/14C-L-malate			% Epoxide pathway	% Valine pathway
0.5	5.0	:	1	80.0	20.0	2.9	:	1	65.5	34.5
1	4.7	:	1	78.9	21.1	3.7	:	1	72.9	27.1
2	6.5	:	1	84.6	15.4	2.5	:	1	60.0	40.0
4	7.1	:	1	85.9	14.1	2.8	:	1	64.6	35.4
6	12.9	:	1	92.2	7.8	3.1	:	1	68.3	31.7

More ^{14}C-pyruvate was formed compared to ^{14}C-L-malate for ^{14}C-HEMA metabolisation during 0.5 up to 6 h after ^{14}C-HEMA exposure. Therefore the epoxide pathway with formation of the epoxy-intermediate 2,3-epoxymethacrylic acid is the main route of metabolisation of HEMA.

1.1.5 [Anonymous 2017]

Study reference:

See confidential annex

Test type

Toxicokinetics *in vivo* in rats.

Non-GLP pharmacokinetic study of HEMA in rats via intravenous (IV) administration conducted to evaluate the potential quick hydrolysis of both HEMA *in vivo*.

According to the registrants to support the REACH registration for HEMA, a read-across approach can be applied if test materials can be quickly hydrolyzed to the methacrylic acid and the corresponding alcohols (glycols) *in vivo*.

Test substance

Non radiolabelled HEMA

Appropriate amounts of HEMA were added to sterile saline to obtain the appropriate dose of 5 mg/kg bw using aseptic techniques. The amount of dose solution administered was targeted at ~2.5 mL/kg bw and injected over a minimum of 45 seconds which corresponded to injection rates ranging from 0.7 to 0.8 mL/minute based on the averaged body weight of 0.2 kg.

Methods

Two male rats (Fischer 344/DuCrj) were intravenously administered HEMA at 5.0 mg/kg bw dose level with saline as the dose vehicle. After dose administration, blood samples (200 µl) were collected at 5, 10, 30, 60, and 180 minutes into individual glass vials containing ethyl acetate (600 µL) acidified with 1% formic acid. After vortexing, the levels of HEMA in the blood samples were quantitatively analyzed by GC/MS-MS.

Results and discussion

The results showed that levels of HEMA dropped rapidly after administration and were not quantifiable by 60 minutes with limit of quantitation (LOQ) of 45.0 ng/mL. The estimated half-lives for HEMA were less than or near 1 minute (0.84 and 1.06 min for each animal, respectively), indicating that the current study results support the assumption that both HEMA were quickly hydrolyzed after intravenous administration in rats.

1.1.6 [Anonymous, 2013]

Study reference:

See confidential annex

Test type

Basic toxicokinetics *in vitro / ex vivo*

Determination of *in vitro* hydrolysis rates of methacrylate esters; determination of half-lifes in rat liver microsomes and whole rat blood; determination of Km and Vmax values for ester hydrolysis in rat liver microsomes; these values were used for PBPK modeling to simulate *in vivo* blood concentrations.

Test substance

No-radiolabelled HEMA (Chemotechnique)

Methods

Duration of exposure:

- phase I: 120 min (samples collected at 0, 2, 5, 15, 30, 60 and 120 minutes)
- phase II: 5 min (samples collected at 0 and 5 minutes)

Doses / Concentrations:

- phase I: 0.25 mM
- phase II: 0.05, 0.1 and 5.0 mM

Positive control: MMA

METABOLITE CHARACTERISATION STUDIES

- Method type(s) for identification: liquid chromatography separation with accurate mass quadrupole/time-of-flight mass spectrometry detection (LC/QTOF-MS) to quantitate methacrylic acid concentrations

- Limits of detection and quantification: LLQ (phase I) = 0.0117 mM methacrylic acid; LLQ (phase II) = 0.00509 mM methacrylic acid

Results and discussion

Negative controls in the rat liver microsome experiments included incubations with heat-inactivated microsomes, no microsomes and no NADPH. Removal of NADPH made little or no difference in hydrolysis rates. Heat inactivation significantly reduced hydrolysis rates, and absence of microsomes resulted in no hydrolysis.

HEMA was rapidly converted to MAA in whole rat blood and rat liver microsomes with hydrolysis half-lives of 4.62 min (liver microsomes) and 99 min (blood).

Vmax (*in vitro*) = 111 nmol/min/mg

Vmax (*in vivo*) = 39 mg/hr/g liver

Km (*in vitro*) = 889 µM

Km (*in vivo*) = 116 mg/L

PBPK modelling showed rapid hydrolysis of HEMA.

2 HEALTH HAZARDS

2.1 Respiratory sensitisation

2.1.1 Human data

2.1.1.1 [Piirila, 1998]

Study reference:

Piirila P, Kanerva L, Keskinen H, Estlander T, Hytonen M, Tuppurainen M, Nordman H. Occupational respiratory hypersensitivity caused by preparations containing acrylates in dental personnel. Clinical and Experimental Allergy. 1998; Volume 28, pages 1404–1411

Detailed study summary and results:

Test type

Case reports

Flow-volume spirometry was performed with a rolling seal spirometer (Mijnhardt, Vicatest 3) connected to a microcomputer (Medicro MR-3), and Viljanen's reference values were used. Histamine challenge tests were performed according Sovijarvi's method following FEV1 (Forced expiratory volume in one second) values with a Vitalograph S bellow spirometer (Vitalograph, Buckingham, UK). A 15% reduction in FEV1 was considered significant and the provocative dose of histamine diphosphate causing a 15% reduction in FEV1 (PD15) was measured. The hyperresponsiveness was graded as strong with PD15 < 0.10 mg, moderate with 0.11–0.40 mg and slight with 0.41–1.6 mg. The long-term peak expiratory flow (PEF) monitoring at the workplace and on days off was made according to the method of Burge and the occupational effect was evaluated visually from the plotted presentation. Skin-prick tests to common environmental allergens were performed using a previously described method. All patients were also tested with natural rubber latex gloves, and a commercial latex extract (Stallergenes S.A. Fresnes Cedex, France); tests with chloramine T were done if there was a positive work history also. Skin prick tests with methyl methacrylate (MMA), 2-hydroxymethyl methacrylate (2-HEMA), bisfenol-A-diglycidylether-methacrylate (BIS-GMA), ethyleneglycol dimethacrylate (EGDMA) and triethyleneglycol dimethacrylate (TREGDMA) were done with human serum albumin (HSA) conjugates as previously described. The skinpatch tests to acrylates were done with the occlusive Finn Chamber technique.

Inhalation challenge tests with a placebo and dental acrylate compounds (mixtures in liquid or powder form) were performed in a 6m^3 challenge chamber according to the international guidelines. If the acrylate compound was in liquid form, the patient handled the compound for 30 min as follows: six drops of the dental primer and 6 drops of the dental adhesive were added simultaneously for 10 min; two drops of each were then added in turn for 5 min, and repeated once. If the challenge test was negative, the challenge test was repeated on another day with a double dose of both substances. The control challenge test was done with polyol (Hydrogenated decene oligomers, Nexbase 200 6FG, Neste PAO NV, Beringen, Belgium) if the agent tested was in fluid form, and with lactose if it was in powder form. The FEV1 and PEF values during the challenge test procedure were measured by a portable pocket-size spirometer (Micro Plus Spirometer, Micro Medical, UK). In case of patients 1 and 2, the PEF values were followed up with a Wright peak flow meter (Clement Clarke M286, Ferraris Medical) and the FEV1 values with a Vitalograph S bellow spirometer (Vitalograph, Buckingham, UK) according to a method reported earlier. The clinical symptoms and lung auscultation were recorded, as well. The mucosal response of the nose during the challenge tests was registered, and nasal blockage and rhinorrhea were scored before and after the provocation test according to a method by Hytonen and Sala. Physiological measurements were also used in the recording of the change in the nasal blockage either by rhinomanometry (Rhinomanometer NRG, Mercury Electronics, Glasgow, UK) or acoustic rhinometry (RHIN2000, SR Electronics APS, Lynge, Denmark). The nasal response was considered positive if the status change and physiological measurement criteria were fulfilled. A scoring method was used in the evaluation of the laryngeal and pharyngeal response. The ocular reaction was followed and evaluated by an ophthalmologist as described earlier.

Results

We diagnosed 12 patients in dental professions (six dental nurses and six dentists) to have respiratory and mucosal hypersensitivity caused by acrylates: nine had asthma, two rhinitis, and one laryngitis, caused by acrylate compounds during 1992–97. Most patients were involved with dental care, dental fillings and orthodontics. One dentist (patient no. 6) was also engaged with dental prostheses. The age of the patients was 47.3 +/- 9.5 years (mean +/-SD), the duration of exposure to acrylates in the dental profession was 21.8+/-7.4 years. The duration of acrylate exposure before the onset of the symptoms caused by acrylates was 13.5 +/- 8.9 years, and the duration of symptoms was 8.3 +/- 7.5 years (range 1–23 years). At the time of the diagnosis, spirometry was normal in nine of the patients, and three showed slight obstruction without significant response to bronchodilator. Histamine challenge test showed moderate bronchial hyperreactivity

in one and slight bronchial hyperreactivity in three, but the remaining eight did not display any bronchial hyperreactivity. Only one of the 12 patients (patient no. 5) had allergic contact eczema caused by ethyl acrylate caused by prosthetic work diagnosed 12 years before the present examinations. The other patients had no contact eczema associated with the use of acrylates. One patient had conjunctivitis caused by methacrylates in addition to the acrylate-induced asthma (patient no. 5). Also, contact urticaria caused by natural rubber (NRL) latex was verified in patient no. 4. Otherwise the patients did not show positive skin-test reactions to NRL.

In the patients, the duration of exposure was over 20 years, and the patients developed respiratory symptoms after about 14 years of exposure. The likely reason for the long latency period is the relatively sparce use of acrylates in the 1970s; their use did not become widespread until the 1980s. In any way, the latency period in occupational asthma caused by acrylates seems to be much longer than in asthma caused by other low molecular weight chemicals, such as di-isocyanates.

In three patients, occupational asthma was diagnosed based on the inhalation provocation tests (FEV1 reduction >=20%) combined with the patients' history of exposure and symptoms. Allergic rhinitis, laryngitis, pharyngitis or conjunctivitis were also diagnosed following tests in a provocation chamber. Eight out of nine patients diagnosed to have asthma showed a work-related effect of PEF recordings. In one patient with asthma, PEF monitoring was not performed. Thus, PEF monitoring seems to be valuable in the diagnostics of occupational asthma caused by acrylate compounds.

Patients 1 and 2 do not fulfil all the criteria of asthma (Table 1). Asthma was diagnosed, based on the patient history and the suggestive challenge test reaction, and also because previous moderate bronchial hyperreactivity had disappeared before the present studies, which were conducted after about 1 year's sick leave and daily inhaled beclomethasone dipropionate (1000 mg) medication. In the case of patient 2, simultaneous FEV1 measurement during the maximal PEF reduction is missing. In his case, the findings supporting the diagnosis of asthma were the suggestively positive challenge test reaction, the patient history, and the PEF work place monitoring with about 20% variation during working days and < 10% during days off.

In the present study, only three of the patients with occupational asthma reacted positively to histamine challenge test. According to Burge, 20% of patients with occupational asthma do not show bronchial hyperreactitivity during non-specific challenge testing. Six out of nine patients with asthma had received inhaled steroids before the inhalation provocation tests, which decreases bronchial hyperreactivity. In addition, most of the asthmatics had been on sick leave before the provocation tests, which also might have influenced on the result in the histamine challenge test.

All the present cases were skin-prick test negative. However, negative skin-prick test reactions do not exclude IgE-mediation.

Table 1. Characteristics of patients (1–5) suffering from respiratory diseases caused by acrylate compounds. p, primer; a, adhesive; Mm1, Methacrylate mixture: primer containing HEMA (2-hydroxy ethyl methacrylate) 40% and adhesive containing 62% BisGMA (Bisfenole-A-diglycidyl-ether methacrylate) and HEMA 37%; Mm2, Methacrylate mixture 2: TREDGMA (triethyleneglycol dimethacrylate) 40–50%, BIS-GMA 50–60%. *Clinical diagnosis, **major diagnostic criteria for asthma; d, drops. Prick positivity means one or more positive reactions in standard series.

Patient	1	2	3	4	5	6
Age	41	48	61	30	51	51
Gender	Male	Male	Female	Female	Female	Female
Profession	Dentist	Dentist	Dentist	Dental nurse	Dentist	Dentist
Smoker	No	No	No	Yes	No	No
Atopy history own/family	Yes/yes	No/Yes	No/Yes	Yes/No	No/Yes	No/Yes
Duration of exposure to acrylics (years)	20	22	24	5	30	27
Duration of symtpoms (years)	4	3	22	2	13	23
Prick positivity	Yes	No	No	Yes	No	No
IgE (kU/L)	296	83	289	105	14	17
Spirometry	Normal	Normal	Normal	Normal	Normal	Normal
Histamine challenge test (PD_{15})	>1.6 mg	>1.6 mg	>1.6 mg	>1.6 mg	>1.6 mg	>1.6 mg
Causative product	Scotchbond 7502A Schotchbond dc 7533R	Rely-A-Bond paste Scotchbond a	Scotchbond multipurpose p + a	Scotchbond dc 7533R; Scotchbond 7502A	Delton	Paladur Forestacryl
Ingredients	Mm2	BIS-GMA HEMA	Mm1	Mm2	TREDGMA EDGMA, DEGDA BIS-GMA	Methyl methacrylate
Challenge dose	10 d + 10 d	1 d + 12 d	20 d + 20 d	10 d + 10 d	TREDGMA 3d BisGMA 7d	Prosthesis dose of powder
Mode of FEV_1/PEF reaction	Late	Immediate	Late		Late	
Max FEV_1/PEF reduction	14%/13%	−/16%	20%/20%**		11%/28%	
Occupational effect in PEF	Not studied	Yes**	Yes	Not studied	Yes**	No
Diagnosis	Asthma Rhinitis*	Asthma Pharyngitis* Laryngitis*	Asthma	Rhinitis* Contact urticaria (latex)	Asthma Conjunctivitis*	Laryngitis*
Year of diagnosis	1992	1993	1994	1994	1994	1995

Table 2. Characteristics of patients 6–11. p, primer; a, adhesive; HEMA, 2-hydroxy ethyl methacrylate, BIS-GMA, Bisfenole-A-diglycidyl-ether-methacrylate, TREDGMA, triethyleneglycol dimethacrylate, EDGMA, Ethylene glycon methacrylate, DEGDA, Diethylene glycol diacrylate, Mm1, Methacrylate mixture: primer contianing HEMA 40% and adhesive containing 62% BisGMA and HEMA 37%, *Clinical diagnosis; **major diagnostic criteria for asthma, d, drops. Prick positivity means one or more positive reactions in standard series.

Patient	7	8	9	10	11	12
Age	53	48	61	41	34	49
Gender	Female	Female	Female	Female	Female	Female
Profession	Dental nurse	Dental nurse	Dentist	Dental nurse	Dental nurse	Dental nurse
Smoker	No	No	No	No	No	No
Atopy-history own/family	No/No	No/No	No/No	No/No	Yes/Yes	No/Yes
Duration of exposure to acrylics (years)	22	27	25	22	10	28
Duration of symptoms (years)	5	2	10	6	9	1
Prick positivity	No	No	No	No	Yes	No
IgE (kU/l)	59	203	93	270	260	43
Spirometry	Normal	Normal	Slight obstruction	Slight obstruction	Normal	Slight obstruction
Histamine challenge (PD_{15})	>1.6 mg	0.48 mg	0.15 mg	0.72 mg	0.79	>1.6 mg
Causative product	Scotchbond multipurpose a	Paladur	Scotchbond multipurpose p + a	Scotchbond multipurpose p + a	Scotchbond multipose p + a	Scotchbond multipurpose p + a
Ingredients	BisGMA HEMA	Methyl methacrylate	Mm1	Mm1	Mm1	Mm1
Challenge dose	4 d	Powder 10 ml, liquid 10 d	10 d + 10 d	20 d + 20 d	20 d + 20 d	20 d + 20 d
Mode of FEV_1/PEF reaction	Immediate	Late	Late		Late	Late
Max FEV_1/PEF reduction	5/18%	6%/20%	20%/16%		13%/17%	24%/13%**
Occupational effect in PEF	Yes**	Yes**	**Yes	No	Yes**	Yes
Diagnosis	Asthma	Athma Rhinitis* Pharyngitis*	Asthma	Rhinitis*	Asthma	Asthma
Year of diagnosis	1995	1995	1995	1995	1996	1997

2.1.1.2 [Sauni, 2008]

Study reference:

Sauni R, Kauppi P, Alanko K, Henriks-Eckerman ML, Tuppurainen M, Hannu T. Occupational asthma caused by sculptured nails containing methacrylates. Am J Ind Med. 2008 Dec;51(12):968-74.

Detailed study summary and results:

Test type

Case report.

One of the patients (Patient 1) was referred to Finnish Institute of Occupational Health (FIOH) for specialist examinations from a local central hospital and the other (Patient 2) from a local occupational health service unit.

Spirometry was performed with a rolling-seal spirometer (Mijnhardt, Vicatest 3, Bunnik, Netherlands) connected to a microcomputer (Medicro MR-3, Kuopio, Finland), and Viljanen's [1982] reference values were used. The histamine challenge test was performed according to the method of Sovijarvi et al. [1993], following the forced expiratory volume in 1 s (FEV1) values with a Vitalograph S bellow spirometer (Vitalograph, Buckingham, UK). Measurements of exhaled nitric oxide (NO) were carried out using a chemiluminescence gas analyzer (NIOX, Aerocrine AB, Solna, Sweden) according to ATS FENO guidelines; values of >30 ppb were considered to be over normal values [Piipari et al., 2002; ATS/ERS, 2005]. Peak expiratory flow (PEF) measurements were performed at home and at the workplace according to the method of Burge [1982].

Skin prick tests (SPTs) to common environmental allergens were performed as described earlier [Kanerva et al., 1991]. SPTs were also performed to formaldehyde, ammonium persulfate, and MAs. The tested MAs with test concentrations were 2-HEMA (1% pet; Trolab, Hermal, Germany), 2,2-bis-[4-(2-hydroxy-3-methacryloxypropoxy)-phenyl]-propane (bis-GMA) (2% pet; Trolab), EDGMA (2% pet; Trolab), MMA (2% pet; Trolab), TEGDMA (2% pet; Trolab), and triethyleneglycol diacrylate (0.1%; Chemotechnique Diagnostics, Vellinge, Sweden). Additionally, 2-HEMA and bis-GMA were also tested as human serum albumin (HSA) conjugates, which were prepared as previously described [Tupasela and Kanerva, 1997].

Bronchial provocation tests were performed in an 8-m^3 challenge chamber according to international guidelines [Allergy Practice Forum, 1992; Piirila et al., 1998]. As a reference challenge test, Coca solution and lactose powder were used in Patients 1 and 2, respectively. In the active challenge test, the patients simulated their work in the challenge chamber using their own products including MAs (methacrylates), i.e., they attached the plastic nail with a glue, and then iled and sculptured the nails. During the active challenge test, which took 30 min, three sculptured nails were produced. The patients were monitored for 24 hr after each challenge test. A portable, pocketsize spirometer (One Flow, STI MEDICAL, Saint-Romans, France) recorded the lung function measurements (FEV1, PEF); a drop of 20% in PEF or FEV1 was regarded as significant. An asthmatic reaction was defined as follows: an immediate reaction causing a decrease of 20% in the FEV1 or PEF during the first post-challenge hour; a delayed reaction causing a similar decrease in FEV1 or PEF after the first post-challenge hour; and a dual reaction as a combination of the afore-mentioned. Clinical symptoms and lung auscultation were recorded as well.

Acetone-soluble acrylates and methacrylates in gel nail materials and in gel nails were identified by gas chromatography-mass spectrometry (GC-MS) and quantified by liquid chromatography with ultraviolet (UV) detection at 210 nm. These were determinated in the case of Patient 2; in the case of Patient 1, the products were not available for analysis.

Results

The patient 1 was a 30-year-old female who had worked for 6 years as a manicurist and a nail technician. Her main job was to apply sculptured nails and artificial tips to nails.

About 4 years prior to the examinations at the FIOH, she experienced rhinitis, wheezing, and dyspnea during exercise. In pulmonary examinations at a local central hospital, spirometry was normal but the bronchodilatation test was positive. In the histamine challenge test, there was moderate bronchial hyperresponsiveness (PD15 0.226 mg), and the patient had typical asthmatic symptoms (dyspnea and wheezing). Asthma was diagnosed, and regular inhaled fluticasone medication with salbutamol on demand was started. Because she had respiratory symptoms when applying artificial nails, her asthma was also suspected to have an occupational origin. The patient was referred to FIOH for further respiratory

investigations. At FIOH, the patient had no respiratory symptoms, and lung auscultation was normal. SPTs to common environmental allergens, formaldehyde, and methacrylates were negative. X-Rays of the thorax and nasal sinuses were normal. Spirometry showed mild peripheral obstruction without a bronchodilatation effect. The exhaled NO was normal (17.1 ppb). In the histamine challenge test, mild bronchial hyperresponsiveness (PD15 0.649 mg) occurred. A significant variation was noted in the PEF measurements at home and at the workplace: the PEF values varying from 360 to 580 L/min with a maximal diurnal variation of 26% and frequent bronchodilating effects up to 43%. The reference bronchial challenge test was negative. In the active bronchial challenge test, a dual asthmatic reaction was noted: an immediate significant decrease of 25% in the FEV1, and 4 hr after the start, a delayed significant decrease of 37% in the FEV1. After the delayed significant decrease, the patient received short-acting bronchodilatating medication, after which the FEV1 returned to normal. On the basis on the work-related respiratory symptoms and findings in the workplace PEF follow-up, as well as the positive work simulation test, occupational asthma due to exposure to sculptured nails containing methacrylates was diagnosed. Minimizing the exposure to methacrylates was recommended, and asthma medication was continued with a combination of inhaled fluticasone and salmeterol. At the 6-month follow-up examination at FIOH, which is a normal procedure among patients in whom occupational disease is diagnosed at FIOH, the patient complained of nasal symptoms after long working days, but she had been able to continue her work as a nail technician.

The **patient 2** was a 27-year-old woman who had worked for 5 years both as a hairdresser and as a nail technician preparing artificial gel nails. The process of preparing gel nails and the used products were similar to that described in Patient 1.

About 5 years prior to examinations at FIOH, she had developed rhinitis, loss of voice, and recurrent sinusitis. The symptoms began during the first year after she started to apply gel nails. In pulmonary examinations at a local central hospital, the spirometry was normal, but there was significant diurnal variation in the PEF measurements and recurrent bronchodilating effects. In the histamine challenge test, there was mild bronchial hyperresponsiveness (PD15 0.154 mg). On the basis of these examinations, bronchial asthma was diagnosed and asthma medication was started with inhaled corticosteroids. In spite of the asthma medication, she experienced dry cough, wheezing, and shortness of breath, especially when preparing gel nails. The patient was referred to FIOH for further examinations due to the clinical suspicion of occupational asthma. This patient had no skin symptoms. At FIOH, the patient had no respiratory symptoms, and her lung auscultation was normal. Spirometry showed mild peripheral obstruction. Moderate bronchial hyperresponsiveness (PD15 0.29 mg) was noted in the histamine challenge test, and the exhaled NO value was increased (64.9 ppb). In the workplace PEF follow-up, there were no significant diurnal variations, but the patient did not prepare nails during the follow-up. SPTs to common environmental allergens showed allergy to animal epithelia (dog, cat) and to common pollens (alder, hay, mugwort) but no allergy to persulfates; methacrylates were not tested. The reference bronchial challenge test was negative. In the active bronchial challenge test, a dual asthmatic reaction occurred. An immediate significant decrease of 20% in the PEF (and a drop of 16% in FEV1) occurred 35 min after the start. After 8 hr, a delayed significant drop of 27% in the PEF (19% in FEV1) could be seen. The delayed drop fluctuated and was sustained until the patient received shortacting bronchodilatating medication, after which the PEF and FEV1 returned to normal. On the basis of the work-related respiratory symptoms and findings in the pulmonary investigations, including a positive bronchial provocation test, occupational asthma due to exposure to sculptured nails containing methacrylates was diagnosed. Minimizing the exposure to methacrylates by using respiratory protective equipment was recommended, and asthma medication was continued with inhaled budenoside. At the 6-month follow up at FIOH, the patient had been unable to continue her work as a nail technician because of respiratory symptoms.

The concentrations of methacrylates in the gel nail materials and in the gel nails themselves were determined after the active challenge test of Patient 2. The main methacrylate was HEMA (8%) in the bonding agent and bis-GMA(42%) in the sculpture resin. The sculpture resin also contained 7% of volatile hydroxypropyl methacrylate (HPMA). The identification of the main methacrylates in the sealing resin could not be confirmed. Hardened gel nails contained no detectable amounts of HEMA or aliphatic dimethacrylates.

TABLE I. Concentrations of Methacrylates Identified in Gel Nail Materials and in Gel Nails

Product	Identified methacrylates	Concentration (%, w/w)	Identification
Bonding agent	HEMA	7.5	GC-MS/LC
	EGDMA	0.8	GC-MS/LC
	TREGDMA	0.2	GC-MS/LC
	Ethyleneglycol acrylate methacrylate	1	GC-MS
	Ethyleneglycol-based dimethacrylates[a]	2	GC-MS
	Ethyleneglycol monomethacrylate	0.5	GC-MS
	MMA	<0.05	GC-MS/LC
Sealing resin	HEMA	<0.01	GC-MS/LC
	MMA	<0.01	GC-MS/LC
	EGDMA	0.1	GC-MS/LC
	Ethyleneglycol-based dimethacrylates[a]	20	GC-MS
Sculpture resin	HEMA	0.03	GC-MS/LC
	MMA	<0.01	GC-MS/LC
	HPMA	6.7	GC-MS/LC
	TREGDMA	≤0.1	GC-MS
	BUDMA	≤0.1	GC-MS
	Ethyleneglycol-based dimethacrylates[a]	12	GC-MS
	Bis-GMA	42	GC-MS/LC
Gel nails	HEMA	<0.01	GC-MS
	Aliphatic dimethacrylates	<0.01	GC-MS

HEMA, hydroxyethyl methacrylate; EGDMA, ethyleneglycol dimethacrylate; TREGDMA, triethyleneglycol dimethacrylate; MMA, methylene methacrylate; HPMA, hydroxypropyl methacrylate; BUDMA, butandiol methacrylate; Bis-GMA, 2,2-bis-(4-(2-hydroxy-3-methacryloxypropoxy)phenyl)-propane; GC-MS, gas chromatography with mass selective detection; LC, liquid chromatography.
[a]Tentatively identified according to the presence of the ions 69 and 113 in the mass spectrum.

The mechanism of occupational asthma (OA) induced by MAs is unclear. Both of the patients displayed a dual type of asthmatic reaction. In association with specific bronchial challenge tests, mainly late or dual asthmatic reactions has been reported to occur in dental personnel exposed to MAs [Piirila¨ et al., 1998] or in other occupations exposed to other acrylates (e.g., cyanoacrylates) [Savonius et al., 1993]. These modes of asthmatic reactions refer usually, but not necessarily, to reactions other than hypersensitivity type I. Taken together, although the results do not rule out a possible IgE-mediated mechanism, the pathophysiology of OA in relation to MA exposure probably involves other immunological mechanisms.

2.1.1.3 [Lindstrom, 2002]

Study reference:

Lindström M, Alanko K, Keskinen H, Kanerva L. Dentist's occupational asthma, rhinoconjunctivitis, and allergic con-tact dermatitis from methacrylates. Allergy. 2002;57:543–5

Detailed study summary and results:

Test type

Case report

Occupational asthma and rhinoconjunctivitis were diagnosed in a dentist according to patient history, PEF monitoring, and a work-simulated bronchial provocation test. ACD (allergy contact dermatitis) was diagnosed by skin-patch testing with methacrylates with the occlusive Finn Chamber-technique.

Patient: A 47-year-old non-smoking female dentist had been working in general dentistry for 22 years performing dental fillings, orthodontics, prosthetics and dental surgery. She had not had allergic symptoms as a child; however, her sister expressed an atopic constitution. When investigated at the authors' institute she had had symptoms of rhinoconjunctivitis and sneezing for 12 years, cough attacks for 10 years, and

shortness of breath for 2 years. Furthermore, she had had hand and face dermatitis for 3 years. The symptoms were work-related and disappeared during weekends and holidays. The patient associated the eye and respiratory symptoms to making dental fillings with photo-bonded resins and to working with dental prostheses. The dermatitis also got worse from disinfectants and natural rubber latex gloves. The patient had occasionally used inhaled epinephrine or salbutamol for nearly 10 years. During the past few years, she needed bronchodilating medication almost every day at work. Asthma medication with inhaled steroids was begun more than 1 year ago. During this medication PEF-flow monitoring at work and at home (3 weeks) showed values between 540 and 500 l/min during days off, whereas the lowest values during working days were 420 l/min. The results pointed towards occupational asthma. The patient was remitted mainly because of the work-related respiratory symptoms but also because her hand dermatitis had worsened. When seen by the authors, a fissured, purulent pulpitis was observed on her left thumb, and milder dermatitis was present on the sides of the left thumb and the left forefinger. No asthmatic rales were heard from her lungs. She had been on sick leave for 2 weeks.

Spirometry was performed with a rolling-seal spirometer (Mijnhardt, Vicatest 3, Bunnik, The Netherlands) connected to a microcomputer (Medicro MR-3, Kuopio, Finland), and Viljanen's reference values were used. The histamine challenge test was performed according to Sovijarvi's method, following FEV1 (forced expiratory volume in one second) values with a Vitalograph S bellow spirometer (Vitalograph, Buckingham, UK). A 15% reduction in FEV1 was considered significant, and the provocative dose of histamine diphosphate causing a 15% reduction in FEV1 (PD15) was measured. The hyper-responsiveness was graded as strong (PD15<0.10 mg), moderate (0.11–0.40 mg) or slight (0.41–1.6 mg).

Routine skin prick tests (SPTs) to common environmental allergens were performed. Prick tests were also performed with natural rubber latex (Stallergenes S.A., Fresnes Cedex, France), chloramine T (1% and human serum albumin (HSA) conjugate), and acrylates (Chemotechnique Diagnostics AB, Malmo, Sweden); 2-hydroxyethyl methacrylate (2-HEMA), methyl methacrylate, BIS-GMA, ethyleneglycol dimethacrylate, triethyleneglycol dimethacrylate (2%) and triethyleneglycol diacrylate (0.1%). 2-HEMA and BIS-GMA were also tested as HSA conjugates. Patch tests were performed according to the recommendations of the International Contact Dermatitis Research Group (ICDRG) with the occlusive Finn Chamber (Epitest, Tuusula, Finland) technique.

Inhalation challenge tests with a placebo (Coca solution) and dental liquid MAs were performed in a 6-m^3 challenge chamber according to the international guidelines. The products used by the patient in her work were used in the work simulating challenge tests (Scotchbond primer containing 40% of HEMA and adhesive containing 62% of BisGMA and HEMA 37%). No concentration measurements were carried out. The FEV1 and PEF values during the challenge test procedure were measured by a portable pocket-size spirometer (OneFlow tester ATS 94, Fuchs Medical, Saint Romans, France). The clinical symptoms and lung auscultation were recorded as well. The ocular reaction following the skin-patch tests to MAs was evaluated by an optometrist, as delayed conjunctivitis from MAs has earlier been reported.

Results

The patient's spirometry was normal and there was no significant response in the bronchodilation test (FVC 3.88 l, 108% of predicted, FEV1 3.12 l, 106% of predicted, and FEV1/FVC 80.55%, 98% of predicted). The histamine challenge test showed moderate bronchial hyper-reactivity with PD15 = 0.255 mg. The patient had been on sick leave for 2 weeks before the histamine challenge. There were no positive reactions in SPTs with common environmental allergens, natural rubber latex, chloramine-T, or acrylates. The total serum IgE was normal, 35 kU/l. The eosinophils in the peripheral blood were normal. The placebo challenge test was negative. In the first inhalation challenge test with metacrylates, the adhesive (20 drops altogether during 30 min) induced cough, rhinoconjunctivitis and a 10% decrease in FEV1 after 45 min. In the second test, with both the adhesive and the primer (40 drops during 30 min), an early late 23% FEV1 reduction was recorded, at a maximum at 3 h, as well as increased symptoms with dyspnea. Before the tests the inhaled steroids had been stopped for 8 days.

Patch testing with a MA series showed allergic reactions to several MAs, including 2-hydroxyethyl methacrylate (2-HEMA), present in Scotchbond (Table 1). In addition, patch testing induced itching, swelling and soreness of the eyelids, maximal during the 3-day patch test reading.

An optometrist's consultation indicated that the symptoms were in accordance to delayed allergic conjunctivitis.

She was ordered sick leave. On a control visit 10 months later, the patient did not have any symptoms of dermatitis or rhinoconjunctivitis. She still used inhaled steroids, and occasionally bronchodilators,e.g., when exposed to cigarette smoke. She did not use antihistamines, nasal steroids or eye drops. Two months before the control visit she had performed a work trial but developed strong respiratory reactions. It was concluded that she could not continue in her present work and needed retraining.

Table 1. The patient's positive patch test reactions with a (meth)acrylate series including 35 test substances

(Meth)acrylate	Source	Abbre-viation	Patch test concentration (%) (w/w) (all allergens in petrolatum)	Patch test result
Glycidyl methacrylate	O	GMA	0.1	1+
Ethyl acrylate	C	EA	0.1	1+
Ethyl methacrylate	C	EMA	2	1+
2-Hydroxyethyl methacrylate	T	2-HEMA	1	2+
2-Hydroxypropyl methacrylate	C	2-HPMA	2	2+
Ethyleneglycol dimethacrylate	T	EGDMA	2	2+
Methyl methacrylate	C	MMA	2	1+

C = Chemotechnique (Malmö, Sweden); T = Trolab (Reinbek, Germany), O = prepared by ourselves.

2.1.1.4 [Moulin, 2009]

Study reference:

Moulin P., Magnan A., Lehucher-Michel MP. 2009. Occupational allergic contact dermatitis and asthma due to a single low molecular weight agent. J Occup Health; 51: 91-96

Detailed study summary and results:

Test type

Study population consisted of all new cases of contact dermatitis (CD) diagnosed at the Occupational Health Medical Consultation from January to December 2005 (Marseille, France). To be eligible for the study, patients had to be workers of at least 16 year of age at the time of diagnosis. An eligible 234 subjects were asked about skin and respiratory symptoms. When these symptoms recurred during work exposure and improved during rest-days, they were considered as work-related contact dermatitis which was diagnosed according to the criteria of the American Contact Dermatits Society. Occupational asthma (OA) was diagnosed if the criteria proposed by the American College of Chest Physicians were met.

Skin-prick tests and patch tests: 10 patients, found both work-related CD and respiratory symptoms, underwent skin-prick tests and patch tests. Skin prick tests included common environmental allergens and were considered positive if the mean wheal diameter was 30 mm or greater. The patch test procedure included the European standard series and depended on the clinical examination and oriented anamnesis, supplementary allergen panels and specific patch testing. Two irritant contact dermatitis and 9 ACD cases were observed. Among them, 3 patients with both ACD and expiratory troubles caused by a single causative agent were found; with 2 caused by HEMA (patient No. 1 and 2).

Blood sampling, lung function and provocation tests: blood was sampled for total IgE measurement from the 3 subjects presenting ACD and expiratory troubles caused by a single substance. Lung function and provocation tests were carried out on 2 of them. Baseline lung function comprised measurement of FEV1 and forced vital capacity (FVC) with a dry seal spirometer according to the recommandations of the Eruoepan respiratory society. Bronchial hyperresponsiveness was assessed by inhalation of methacoline chloride. A 20% fall in FEV1 compared to baseline value was defined as bronchial hyperresponsiveness and the provocative dose (mg/ml) of methacholine chloride wausing a 20% reduction in FEV1 was measured. To

confirm the cause of the OA, specific inhalation challenge (SIC) tests were carried out for subjects 1 and 3 according to the existing recommandations. Subject 2 did not want to undergo respiratory investigations. The SIC test was considered positive if there was a fall of over 15% in FEV1 within 1 hour from the challenge (immediate reaction) or a fall of over 20% later (late reaction) compared to the values before the challenge and to the concurrent values of the placebo test.

Results

Table 1. Characteristics of three subjects suffering from skin and respiratory symptoms caused by resins

	Subjects		
	1	2	3
Personal			
Age	44	22	39
Gender	female	female	male
Work	beautician	beautician	resin applier
Smoker	no	no	no
Atopy own/family	no/no	no/yes	no/no
Prick positivity (standard series)	no	no	no
Total IgE	normal	normal	normal
Baseline spirometric measurements	normal		normal
Agent			
Kind of resin used	HEMA	HEMA	DGEBA
Time from first exposure to onset of symptoms (mo)	5	4	1
Duration of exposure before investigations (mo)	15	8	3
Disease			
Diagnostic (yr)	ACD+OA (2005)	ACD+OA (2005)	ACD+OA (2005)
Patch testing positivity (chemical)	Yes	Yes	Yes
Dose of methacholine (mg/ml) causing a 20% drop in FEV_1	0.2 mg/ml		0.4 mg/ml
Specific bronchial provocation (max. FEV_1 drop)	20%		

Table 2. Subject's positive patch test reaction with resins

Resin	Patch test concentration (%)	Subjects	Patch test results after 2 and 4 days
HEMA standard series	2	1	2+
Activated Ultraviolet gel*	2	2	2+
Acrylic liquid*	5	2	1+
DGEBA standard series	1	3	2+
DGEBA products*	2	3	1+

*patch test prepared by ourselves.

Subject 1: 44 year-old non smoking female beautician had been working in a hairdressing salon for 20 years. She had intolerance to pieces of costumes jewellery and developed urticaria when using make-up pencils. Her daughter had asthma. Authors investigated her fifteen months after she developed finger dermatitis and expiratory symptoms. The symptoms were work-related and improved during weekends and holidays. The patients associated the cutaneous and respiratory symptoms with the introduction of a nail prosthesis method using new solvents and resins. Her job required that she wore false nails for promotional purposes. The dermatitis did not improve with natural rubber latex gloves. When authors saw the patient, they observed a fissured purulent pulpitis on all her fingers. They did not hear any asthmatic rales from her lungs. Patch testing revealed that the patient had a patch reaction to HEMA in the methacrylate series. Due to the severity of her CD, she could not continue her work, so PEF monitoring could not be carried out. A SIC to HEMA without hardener was performed. She developed an early asthmatic reaction (20% drop in FEV1) 60 min after challenge with dysphonia. A placebo test performed with lactose was negative. These results lead to the diagnostic of ACD and OA induced by HEMA.

Subject 2: A 22 year-old beautician came to the consultation with cutaneous and respiratoy complaints that had started 4 months after she began her activity of nail prosthesis. She had had asthmatiform bronchitis and seasonal spring rhinitis in her childhood and her mother was asthmatic. However, there was a clear association with her occupational activities, since her symptoms decreased during weekends and cleared

completely on holiday. She worked during the days before the clinical examination which revealed sneezing, wheezing and dermatitis localized on the hands, forearms and low neckline and thighs. She attributed the troubles to activated ultraviolet gel and acrylic liquid used daily. A patch test with methacrylate series was negative. Positive patch tests were obtained from activated ultraviolet gel and from acrylic liquid. According to the material safety data sheets obtained from the manufacturer, the products contained HEMA. The patient did not wish to undergo further respiratory investigations because she decided to change occupation before these tests could be done. When patient changed job, all her symptoms and signs resolved. Thus, the association between work related wheezing and workplace exposure to agents known asthma defends the diagnosis of OA, even if it could no be proven.

2.1.1.5 [Esltander, 1996]

Study reference:

Estlander T, Kanerva L, Kari O, Jolanki R, Mölsä K. Occupational conjunctivitis associated with type IV allergy to methacrylates Case Reports. Allergy. 1996 Jan;51(1):56-9.

Detailed study summary and results:

Test type

Thirty cases of allergic contact dermatitis and one case of pharyngitis from MAS (methacrylates) in dental restorative and prosthetic materials were diagnosed during 1974-94 at the Finnish Institute of Occupational Health. Five of the patients worked in dental laboratories. In addition, one patient was diagnosed as having allergic contact dermatitis from the preparation of in-the-ear hearing aids. Two of the five dental laboratory workers were employed by the same dental laboratory (patients 1 and 2). The man (patient 1) had handled chemically curable MA for 3 years and light-curable MA for about 1 week (Table 1). The woman (patient 2) had used only chemically curable MAS for 8 years. The hearing aid worker (patient 3) had prepared in-the-ear hearing aids from chemically curable MA for 9 years and had covered the devices with a lightcurable acrylic lacquer for 3 years.

Skin tests und other investigations
Epicutaneous tests were conducted using the Finn Chambers (Epitest Ltd Oy, Finland) and read and scored as previously described. They included the European standard series, a dental screening series, and a (meth)acrylate series, all from Chemotechnique Diagnostics AB, Sweden. Patch tests were also carried out with acrylate and other products used at the workplaces. Prick tests included a series of environmental allergens and a latex allergen (Allergologisk Laboratorium A/S, Denmark). They were also done with two other latex prick test solutions prepared as described by Turjanmaa et al., and with methylmethacrylate (MMA) and 2-hydroxyethylmethacrylate (2-HEMA) conjugated with human serum albumin (HSA). Other investigations consisted of an ophthalmologist's examination of the eyes including conjunctival scrapings, and, in one case, demonstration of the activation of eosinophils with the monoclonal antibody (EG2, Pharmacia Diagnostics AB, Sweden) technique, and determination of eosinophilic cationic protein (ECP) in tear fluid. The investigations also included an industrial hygienist's inspection of the dental laboratory, determinations of airborne MMA concentrations with the charcoal method (SFS standard 3861), and gas chromatographic analyses of MMA in two cured hearing aids.

Table 1. Acrylate compounds used in work of patients according to information from material safety data sheets

Dental laboratory (patients 1 and 2)	Hearing aid laboratory (patient 1)
Chemically curable material	*Chemically curable material*
Liquid:	Liquid:
MMA	MMA
N,N-dimethyl-*p*-toluidine	Dimethacrylate
Ethyleneglycol dimethacrylate	Aromatic amine
Hydroquinone	
Terpinoline	
Powder:	Powder:
Polymethylmethacrylate	Polymethylmethacrylate
Dibutylphthalate	Dibenzoylperoxide
Light-curable material	*Light-curable material*
Liquid 1:	Acrylic lacquer:
Diacrylurethane monomer	MMA
1,6-Hexanediol dimethacrylate	
Initiators, accelerators	
Liquid 2:	
MMA	
Tetrahydrofurfuryl methacrylate	
1,6-Hexanediol dimethacrylate	
Initiators, accelerators	

The workers in dental and hearing aid laboratories are usually exposed to mixtures (1 1) of numerous chemicals (Table 1). The compounds are of commercial grade; i.e., they may also contain many impurities.

Results

The dental laboratory assistant (patient 1) had developed, after fingertip dermatitis, eye symptoms, which appeared soon after he had started to handle light-curable MA liquids. The redness, stinging, and itching of his eyes were worst during the last days of the working week and after he had polished light-curable materials. He had neither nasal nor chest symptoms. During holidays, his eyes were symptomless, without any medication. He continued in the same work, and the half-year follow up confirmed the clinical association of his eye symptoms with his work. An ophthalmologist's findings (Table 2) included follicular and papillary conjunctivitis in lower lid conjunctiva and hyperemic bulbar and tarsal conjunctivae. The corneas were quite clear, and no signs of corneal inflammation were seen. His conjunctival scrapings showed strong lymphocytic reaction and activated eosinophils. The tear ECP was elevated (7 pg/l) compared to the levels found in healthy nonatopic subjects. The patient was atopic and prick test positive to grass pollen, mugwort *(Artenzisiu),* and a housedust mite *(Dermatophugoides furinae).* He had had eye symptoms during the pollen season, but not during the past 15 years.

The dental technician (patient 2) had pulpitis on her fingers only. The skin symptoms of both dental laboratory workers disappeared when they used finger protectors made of 4H-Gloves (Safety 4 A/S, Denmark). On patch testing, in addition to MMA, the man (patient 1) reacted to many other MAS (Table 3), to a liquid containing MMA, and slightly to polymethylmethacrylate powder (Table 4). The dental technician (patient 2) was clearly 2 +positive only to 2-HEMA and the liquid containing MMA, but reacted with a weak ?+reaction to ethyleneglycol dimethacrylate (EGDMA). The hearing aid worker's (patient 3) eye symptoms had begun at the same time as her fingertip dermatitis; i.e., 3 years after she had started to use a light-curable

acrylic lacquer. She also continued her work after her sensitization to MAS was confirmed. Her eye symptoms were at first mild and occasional, but got worse during the 4-year follow-up period. No nasal or chest symptoms appeared. Her eye findings included eosinophilia and moderate neutrophilic and lymphocytic reaction in the conjunctival scrapings. She had neither follicular nor papillary conjunctival hypertrophy, and the tarsal conjunctiva was mildly hyperemic (Table 2). She was nonatopic and prick test negative to environmental allergens, but had atopic dermatitis in her family. She was patch test positive to several MAS' and dimethacrylate compounds (Table 3), as well as to a liquid containing MMA, a cured ear device (contained free MMA up to 3.3 mg/g), and lightcurable acrylic lacquer (Table 4). Prick tests with MMA-HSA and 2-HEMA-HSA were negative in all three patients.

Table 2. Ophthalmologist's findings on two conjunctivitis patients with type IV allergy to (meth)acrylates

Finding	Dental laboratory assistant (patient 1)	Hearing aid worker (patient 3)
Redness of conjunctiva	Yes	Yes
Negative conjunctival bacterial culture	Yes	Yes
Follicular and papillary reactions of conjunctiva	Yes	No
Eosinophilia and lymphocytosis in one or both eyes	Yes	Yes
Activation of eosinophils	Yes	ND
Elevated level of eosinophilic cationic protein in tear fluid	Yes (7 μg/l)	ND
Corneal changes	No	No
Lomudal® relieves the symptoms	Yes	Yes

ND: not done.

Table 3. Patch test results of two conjunctivitis patients with (meth)acrylate series

(Meth)acrylate	Dental laboratory assistant (patient 1)	Hearing aid worker (patient 3)
Methylmethacrylate (MMA)	3+	2+
Ethylmethacrylate	2+	1+
2-Hydroxyethylmethacrylate (2-HEMA)	3+	2+
2-Hydroxypropylmethacrylate (2-HPMA)	3+	2+
Ethylacrylate	NT	2+
2-Hydroxyethylacrylate	NT	2+
2-Hydroxypropylacrylate	NT	2+
Ethyleneglycol dimethacrylate	3+	2+
Triethyleneglycol dimethacrylate	3+	1+
Triethyleneglycol diacrylate	Neg	2+
Tetrahydrofurfuryl dimethacrylate	2+	NT
1,4-Butanediol dimethacrylate	2+	Neg
1,4-Butanediol diacrylate	Neg	2+
1,6-Hexanediol diacrylate	Neg	2+
Diethyleneglycol diacrylate	Neg	2+
Urethanediacrylate, aromatic	Neg	2+
Pentaerythritol triacrylate	Neg	2+

NT: not tested; Neg: negative.

Table 4. Positive patch test results of two conjunctivitis patients tested with (meth)acrylate products used in their work

Product	Dental laboratory assistant (patient 1)	Hearing aid worker (patient 3)
Methylmethacrylate (MMA) liquid 2% pet	2+	2+
Polymethylmethacrylate powder as such	?+	Neg
Cured in-the-ear hearing aid* as scrapings	NT	1+
Light-curable acrylic lacquer	NT	2+

NT: not tested; Neg: negative; pet: petrolatum.
* Analyzed by gas chromatography; contains up to 3.3 mg/g free MMA.

Most of the several patch reactions to acrylate compounds could be explained by cross sensitization, but concomitant sensitization was also possible because of impurities.

2.1.1.6 [Sala, 1996]

Study reference:

Sala E., Hytonen M., Tupasela O., Estlander T. 1996. Occupational laryngitis with immediate allergic or immediate type specific chemical hypersensitivity. Clin Otolaryngol Allied Sci;21(1):42-8.

Detailed study summary and results:

Test type

Twenty patients who were diagnosed as having occupationally induced laryngitis were examined at the author's institute during 1990-1993. There were 10 women (aged 27-53 years) and 10 men (aged 22-49 years). All of them were exposed to the suspected substances at their work and had work-related symptoms of hoarseness of voice, some also had cough, itching and discomfort or a feeling of swelling around the larynx.

The symptoms usually manifested themselves within one hour or even within some minutes of the beginning of the work and were relieved or disappeared within some hours or days after they had stopped working.

PROVOCATION AGENTS
The information of the materials to which the patients were exposed at their work places was based on patient inquiries, safety data sheets, and in one case also on measurements of aldehydes in the air of the workplace.

INVESTIGATIONS
Acute and chronic upper respiratory tract infections were excluded by clinical findings and sinus radiographs. Before the provocation test, the patients who had symptoms of allergic rhinitis were treated with local steroids, or those who had laryngitis avoided exposure until they were healthy. Before provocation, the erythema and oedema of the larynx and vocal cords was scored with 0 (no) or 1 (mild) in all patients except one smoking subject whose vocal cord oedema was scored 2 (moderate). The use of antiallergic drugs before the provocation test was restricted as follows: setiritzinedihydrochloride and acrivastine for 3 days, terfenadine for 2 weeks, astemizole for 8 weeks and local steroids for 3 days. Five of the patients had smoked within 1 year before the provocation test.

SKIN-PRICK TESTS
Standard skin-prick tests with 20 common environmental allergens including pollen, animal epithelia, house dust mite and moulds (Allergologisk Laboratorium, Denmark) and skin-prick tests with materials suspected of causing the symptoms were used, scored and evaluated as described previously. Each agent which was not included in the standard series was tested with 20 control subjects as described by Kanerva et al.

PROVOCATION TESTS
The provocation tests were performed either in a provocation chamber or as a local nasal test. The provocation was performed in a chamber if the suspected substance was a chemical, or if the patient was also suspected of having asthma. The chamber provocation test method was the same as that used for pulmonary provocation test. The exposure lasted for 30-45 min. All the patients were first exposed to placebo which was either polyol or lactose powder with a crystal diameter of 125 pm. Lactose fulfills the quality requirements of Ph. Eur. 2nd Ed.
In the nasal provocation test, a small piece of cotton wool (3 x 3 mm) was impregnated with the test material. The cotton wool was applied to both sides of the anteromedial surface of the inferior turbinate for 1 h. First all the patients were exposed to placebo, and the other tests were done on consecutive days.
The patient’s laryngeal status was observed immediately before and 45-60 min after the beginning of the provocation. The signs of erythema and oedema were both rated with a four point scale: 0 = no; 1 =mild; 2 = moderate, and 3 = abundant. The change in the status noticed after provocation compared to the status before provocation was interpreted as positive if both signs were increased by at least one point in the vocal cords.

CONTROLS
Forty-five subjects (34 women and 11 men aged from 24 to 60 years) who did not have occupational exposure and who had no laryngeal symptoms from the tested agents, served as controls. Each provocation material was tested with five control persons. Four women and one man were exposed to seven different agents and the other subjects only to one agent without any laryngitis reaction. The laryngeal status before the provocation was scored both in erythema and oedema from 0 to 1 point and the change after provocation was together 1 point or less.

Results

Table 5. Characteristics of the patients who had laryngitis with specific hypersensitivity to various chemicals

Patient	17-P.P.	18-A.V.	19-K.H.	20-L.T.
Age (years)/gender	40/male	49/male	45/female	45/female
Occupation	electrician	foreperson	dentist	packer
Duration of exposure (years)	13	20	7	5
Causative agent	MHHPA	formaldehyde	Scotchbond*) Scotchprep**)	Emuterm 10-130***)
Vocal cord status change	2	4	3	2
Skin-prick test to the causative agent	+++	negative	negative	negative
Specific IgE (kU/l)	8.3	ND	ND	ND
Own/family atopy	yes/no	no/no	no/no	no/no
Other hypersensitivity diseases from the same agent	rhinitis	no	no	asthma allergic contact eczema

ND, not done; *)Scotchbond 2 light-cured dental adhesive (7502A) contains 40–60 % bisphenol-A-diglysidylmethacrylate and 40–50 % 2-hydroxyethylmethacrylate; **)Scotchprep dentin primer (7502P) contains 30–65 % 2-hydroxyethylmethacrylate; ***)Emuterm is a hot melt glue which contains resin derivatives 40–60 %.