Some toxicological aspects of acrylic monomers, notably with reference to the skin

Theo Waegemaekers

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Promotores: Prof. Dr. K. E. Malten
Prof. Dr. J. M. van Rossum

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Proefschrift
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aan de Katholieke Universiteit te Nijmegen,
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door

Theodorus Henricus Josephus Maria Waegemaekers
geboren te Roosendaal

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THE RESULTS DESCRIBED IN THIS THESIS HAVE ALREADY BEEN PARTLY PUBLISHED IN THE FOLLOWING PAPERS

Chapter 2 : Waegemaekers THJM, Bensink MPM. Non-mutagenicity of 27 aliphatic esters in the Salmonella-microsome test. Mutation Res. 1984; 137: 95-102.

Chapter 3.2 : Waegemaekers THJM, van der Walle HB. The sensitizing potential of 2-ethylhexyl acrylate in the guinea pig. Contact Dermatitis 1983; 9: 372-376.

Chapter 3.4 : Waegemaekers THJM, van der Walle HB. α,β-Diacryloxypropionic Acid, a Sensitizing Impurity in Commercial Acrylic Acid. Dermatosen 1984; 32,2: 55-58.

Chapter 4 : Waegemaekers THJM, Seutter E, den Arend, JACJ, Malten KE. Permeability of surgeons' gloves to methyl methacrylate. Acta Orthop. Scand. 1983; 54: 790-795.

Chapter 5 of this thesis is the result of the cooperation with J.P.T. Burgers, Department of Pharmacology of the University of Nijmegen. On mutual agreement this chapter is included in this thesis, as well as in the thesis of J.P.T. Burgers.

C O N T E N T S

A BRIEF OUTLINE OF THIS THESIS

The first commercial synthesis of acrylate plastics in 1927 by Röhm & Haas in Germany marked the beginning of the production of a new class of synthetic polymers. Nowadays acrylate based polymers are used in still growing quantities for an increasing number of applications. From the 1940's on a gradual shift has taken place from a domination of large-scale production in closed systems of thermoplastic polymers, notably plexiglas, to a wide range of new ways of application resulting in a variety of risks of exposure, both in industrial and non-industrial settings. The new ways of exposure, together with the development and introduction of more precise toxicological test systems, set the need for further information on the toxicological profile of acrylic monomers.

In this thesis a number of experimental studies are described which may contribute to the evaluation of risks, possibly associated with the use of acrylic monomers. Attention is primarily paid to dermatotoxicological aspects. On theoretical grounds a certain similarity between mutagenic chemicals and allergenic chemicals can be expected. An allergic contact dermatitis to a chemical can only arise after the chemical has reacted covalently with a carrier protein in the skin. A mutagenic effect proceeds from a reaction of the chemical with the genetic information-carrying part of the cell, *viz.* DNA. In view of the known allergenic properties of acrylic monomers, an investigation of their mutagenic properties seemed necessary. In addition, such a short-term mutagenicity test can be used as a predictive tool to assess the probability of carcinogenic effects of acrylic monomers on the skin. Moreover, the intended experimental use of acrylic monomers on the skin of human volunteers seemed to justify adding to the dermatotoxicological research a study on the mutagenic effects of these substances.

After a short introduction on the principles of the chemical background of the synthesis of plastics, chapter 1 also deals with a number of applications of acrylic monomers. One of the most important applications is in the field of adhesives. The use of adhesives is, in general, asssociated with a relatively large risk of exposure, particularly of the skin. In oder to obtain some insight in the toxicological implications of the use of acrylics in adhesives, some basic principles of adhesive bonding are also described here. This is followed by a review of the toxicological literature on acrylic monomers.

Chapter 2 deals with the mutagenic properties of 27 monomeric acrylate esters and 5 acrylamides as tested in the Salmonella-microsome assay. The chapters 3-5 focus on dermatotoxicological aspects of certain acrylates, *viz.* allergenicity (ch. 3), permeation *in vitro* (ch. 4) and permeation *in vivo* (ch. 5). In the 6th chapter attempts to measure subclinical irritant effects on the human skin are described.

CHAPTER 1

INTRODUCTION

1.1. *PLASTIC MATERIALS*

1.1.1. *Definition*

Plastics can be defined as synthetic macromolecular substances built up by repetition of small simple chemical units.

1.2.3. *Principles of synthetic polymerization*

Plastic synthesis is based on repeated chemical binding between molecules, each with two or more reactive sites. In this way the starting molecules, the monomers, may react with each other to form a polymer. Monomers with two reactive groups form linear macromolecules while in polymerization reactions with participation of at least one chemical with more than two reactive groups networks are formed. In some cases it is possible to stop the polymerization process at a stage in which the reaction product consists of only a few monomers having reacted with each other. Such an intermediate product is called a synthetic resin or a prepolymer. At any desired moment the reaction may be restarted by heat, irradiation or the addition of catalysts or cross-linkers. This is done when the intermediate is used to produce the final product: application phase. Depending on the purpose which should be served by the endproduct several substances can be added during the polymerization reaction, *e.g.* fillers, plasticizers, U.V.-sensitizers, U.V.-protectants, lubricants and pigments.

Linear macromolecules are thermoplastic: when heated the intermolecular van der Waals forces diminish and the shape of the material can be changed by external force. After cooling the material stays in a new shape. Network macromolecules are thermoset plastics. Their shape cannot be changed after heating.

The polymerization reactions can be divided in two types (24):

1. condensation reactions
2. addition reactions: a. chain propagation < radical / ionic
   b. step reaction

ad 1. condensation reactions (fig. 1,2)

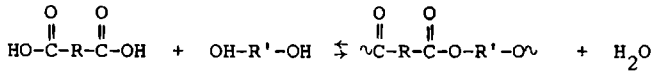
Historically the oldest way to produce plastics, polycondensation is the polymerization of two or more starting materials reacting with each other in an equilibrium reaction, in which a macromolecular main product is formed together with one or more by-products. The latter products are low molecular substances, which may remain present as an impurity in the (pre)polymer. They are not chemically bound to the (pre)polymer. Catching away such by-products promotes the reaction to proceed to the macromolecular endproduct. Examples of such by-products are water and formaldehyde.

Interruption of condensation reactions in an early phase to produce prepolymers is possible. In the subsequent phase of producing the endproduct, such resins are combined with a variety of auxiliary substances, which have a modifying influence on the endproduct. In this way the endproduct can be made to meet the needs of the consumer: taylor-made endproducts. There are several ways to restart further polymerization of resins:

- restoring the conditions which favour polymerization (and which had been altered in order to stop the reaction in the prepolymer phase). This, for instance, may be done by the addition of a catalyst, increasing the temperature or by changing the pH.
- addition of cross-linkers. These are substances able to react with prepolymer molecules to produce the endproduct. If either the prepolymer or the cross-linker or both are threefunctional, network macromolecules are produced.

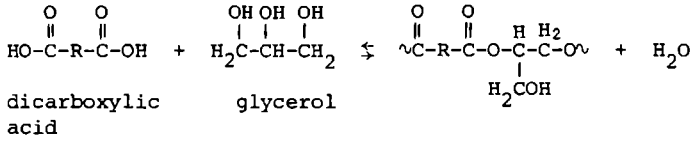
Examples of substances made by polycondensation reactions are epoxy resins, polyamides, polyesters and phenolformaldehyde resins.

figure 1: Example of a polycondensation reaction, resulting in a linear macromolecule (thermoplastic polymer).

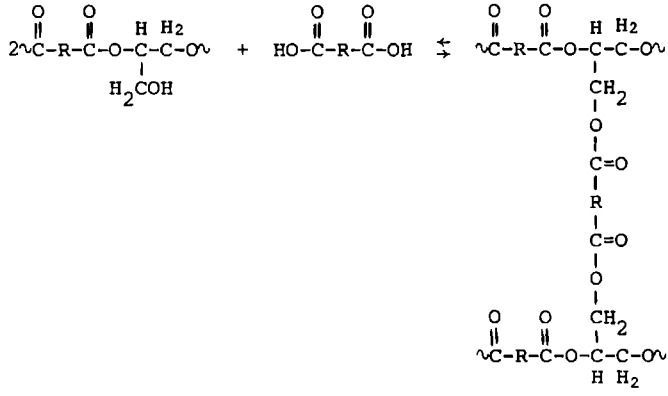


dicarboxylic acid dialcohol linear polyester

figure 2: Example of a polycondensation reaction, resulting in a network plastic (thermoset polymer).



dicarboxylic acid glycerol



network polyester

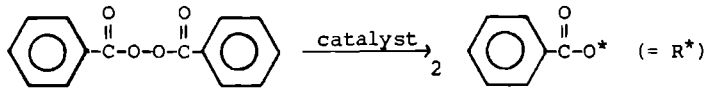
ad 2. addition reactions

a_1 : radical chain propagation reactions (fig. 3)

By tonnage, this is the most important way to produce plastics nowadays. The reaction is based on the activation of a double or triple bond in a monomer (predominantly C=C bonds, but C=O and C≡N are also possible) by free radicals. The first stage is called the initiation and this is considered to be the rate limiting step in this type of polymerization process. Free radicals are molecules or atoms with an unshared electron. They are formed when the addition of heat, irradiation, U.V.-light or a promotor results in the decomposition of radical donors like benzoyl peroxide and azodi-isobutyronitrile.

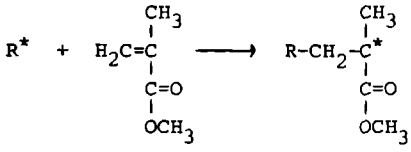
A radical thus formed reacts with a monomer by addition, producing a monomer radical, which can react again with another monomer and so on. This is the propagation stage or growth stage. Interruption of this process is not possible. One does have the possibility to influence the chain length of the macromolecular endproduct by a number of factors such as the

figure 3: Radical chain propagation reaction of methyl methacrylate with benzoyl peroxide as a radical donor.



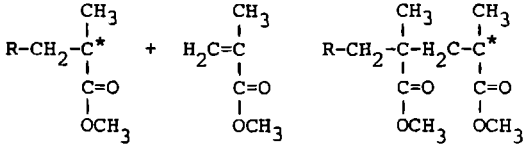
benzoyl peroxide benzoyl radical

Initiation

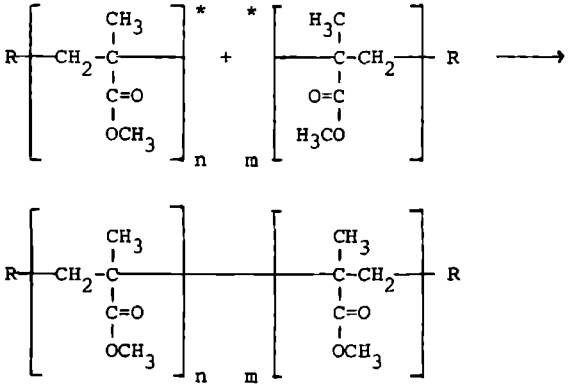


methyl methacrylate

Propagation



Termination



poly(methyl methacrylate)

temperature and the presence of so called modifiers like carbon tetrachloride and mercaptans.

This brings us to the third and final stage of the reaction: the termination stage. We can distinguish two types of reactions in the termination stage: 1. reactions which terminate the growth of a single chain, but which have no influence on the reaction rate; 2. reactions which terminate the growth of a single chain and slow down the reaction rate. Examples of the first type are chain transfer with a modifier (fig. 4) or with a monomer (fig. 5). Reactions of the second type are: mutual combination of two growing radicals (fig. 6) and disproportionation between two growing radicals (fig. 7).

figure 4: Chain transfer with a modifier, for instance a mercaptan.

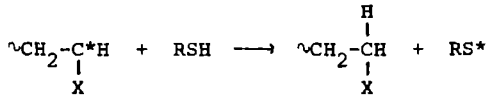


figure 5: Chain transfer with a monomer.

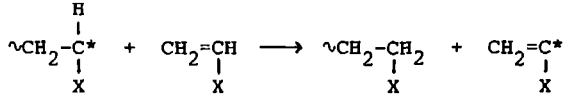


figure 6: Mutual combination of 2 growing radicals

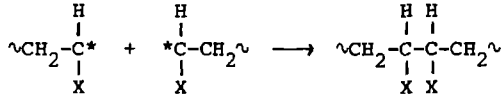
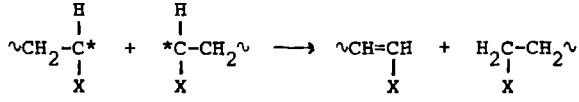


figure 7: disproportionation between 2 growing radicals.



Both propagation and termination reactions are essentially stochastic processes. Therefore the molecular weight of the endproduct is not uniform. To obtain information on the degree of polymerization, two parameters are used. The first one is the number average molecular weight which indicates the number of molecules per unit weight, *i.e.* the mean molecular weight.

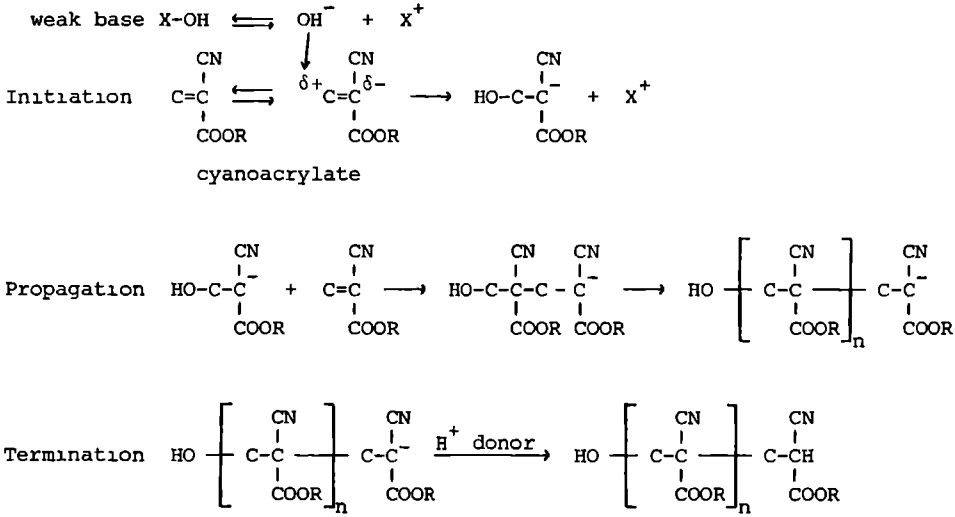
The second parameter is the weight average molecular weight which indicates the fraction of each molecular size by weight. The latter parameter is frequently used to describe the amount of residual monomer in the endproduct.

A plastic, made using a single type of monomer is called a homopolymer. When two different monomers are the starting materials, the endproduct is called copolymer; in the case of three monomers terpolymer. Examples of plastics obtained by radical chain addition polymerization are poly(vinyl-chloride), polystyrene, polyethylene, polypropylene, poly(methyl methacrylate) and ABS polymers (acrylonitrile, butadiene, styrene).

a_2: ionic chain propagation reactions (fig. 8)

This process is very similar to the radical chain propagation reaction involving initiation, propagation and termination stages. However, the growing molecule is an ion rather than a radical. The active center of chain growth may be a positively charged carbonium ion (cationic polymerization) or a negatively charged carbanion (anionic polymerization). The presence of catalysts and sometimes so-catalysts is necessary for the start of the reaction. Examples of polymers obtained by this type of reaction are: polycyano-acrylates, polyformaldehyde and butyl rubber.

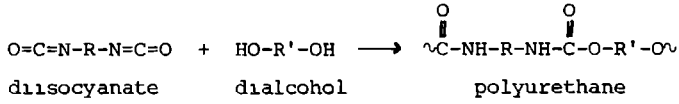
figure 8: Anionic chain propagation reaction of a cyanoacrylate.



b: step addition reactions (fig. 9)

This process, sometimes called rearrangement polymerization, is a stepwise reaction between two different monomers. A proton shift, *i.e.* a rearrangement of a hydrogen atom is the essential part of the reaction. Like in all addition polymerization reactions, no by-products are formed. In the polymer the two monomers are present in an alternating sequence. The kinetics of the reaction are more akin to those in condensation polymerization and it is possible to stop the reaction halfway by underdosing one of the two starting monomers. In that case the resulting prepolymer is terminated on both ends with the monomer present in the excess. Upon adding the consumed monomer the reaction starts again. Examples are the polyurethanes.

figure 9: Example of a step addition reaction.



1.2. *APPLICATIONS OF ACRYLIC MONOMERS*

1.2.1. *Introduction*

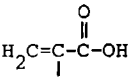
Many different polymers and resins are produced, using acrylic monomers. Polymerization occurs in a chain propagation reaction. The denominator of all acrylic monomers is the $H_2C{=}C\begin{matrix}\nearrow R\\ \alpha\\ \searrow R\end{matrix}$ structure, in which the double bond is polarized by an electron-withdrawing substituent at the α-carbon atom. In most cases this substituent is a carbonyl group (-C=O) but a nitrile group (-C≡N) is also possible.

The different classes of acrylic monomers and their main applications are described in this section. Because of their easy polymerization with each other and with other monomers like styrene and butadiene, a large number of copolymers exists. Only a few of them are mentioned below.

1.2.2. *Acrylic acid, methacrylic acid* (fig. 10)

Polymers of the sodium and ammonium salts of these acids have been used as emulsion thickening agents in latexes. Acrylic acid can be copolymerized with rubbers to produce carboxyl-containing elastomers. These are excellent adhesives for more polar adherends like wood and metals. For the same reason

acrylic acid is added as a copolymerizing substance to certain (meth)acrylic ester compositions.

figure 10: 

R = H : acrylic acid

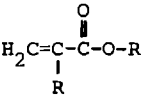
R = CH_3: methacrylic acid

1.2.3. *Acrylic esters*

1.2.3.1. *mono(meth)acrylates* (fig. 11)

The homopolymer of methyl methacrylate is one of the plastics most frequently used. Display signs, lamp housings, wind screens and glazing material in aircraft applications are some examples in which the high light transmission and the good outdoorweathering properties of thermoplastic sheets of poly(methyl methacrylate) are used.

Although - in view of the impossibility to interrupt a chain propagation reaction - one would only expect bulk production of such substances, this certainly does not hold true for acrylic esters. Along with the bulk production of materials as described above, there is also a craft-like production of tailor-made products, with more risks of exposure. Examples of the latter are the manufacturing of dentures, hearing aids, noise protectors and also the glueing of prostheses to bone in orthopaedic surgery. In these applications a two-component system is used. The first component is a polymer powder, with a radical donor, for instance benzoylperoxide. The second component is the monomeric liquid methyl methacrylate containing an accelator (necessary to promote decomposition of the radical donor into radicals), for instance dimethyl paratoluidine, and possibly additives such as antibiotics and of course an inhibitor. The two components are mixed together to form a doughy mass and this dough is applied for example to cement a prosthesis to the bone. Advantages of monomer-polymer systems are: 1. less shrinkage of the endproduct, resulting in lower strains, because the polymer powder does not shrink any further; 2. faster curing, since a part of the mixed substance has already polymerized. The newly formed polymer is attached to the polymer powder by means of the van der Waals intermolecular forces.

figure 11: 

R = H : monoacrylate

R = CH_3: monomethacrylate

Other polymers of mono(meth)acrylates, for instance poly(ethyl acrylate) and poly(methyl acrylate), are used as leather finishes, textile size and surface coatings. In pressure-sensitive adhesives, 2-ethylhexyl acrylate is frequently used, but a wide range of other acrylates is also used in this field.

1.2.3.2. *multifunctional acrylates* (fig. 12, 13)

Molecules with at least 2 reactive acryl groups belong to the class of the multifunctional acrylates. Di(meth)acrylate esters of dialcohols and tri- or tetra(meth)acrylate esters of polyalcohols are multifunctional acrylates generally used. They are applied in the production of photopolymer printing plates, U.V.-curable or electron beam curable inks and coatings, paints, glues and anaerobic sealants.

They function by cross-linking other unsaturated, so called backbone molecules to each other, or by polymerization with themselves. The polymerization reactions can be initiated by free radical formation, by radicals resulting from photoactivators, decomposing under the influence of U.V.-light, by electron beam irradiation or "spontaneously". In the latter case the reaction starts when oxygen, which prevents autopolymerization during storage, is excluded. Hence the name: anaerobic sealants. In addition, the reaction is accelerated by metal ions, coming off the surface of the substrate.

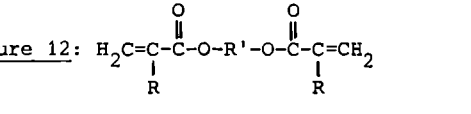


figure 12: R = H : diacrylate; R = CH_3: dimethacrylate

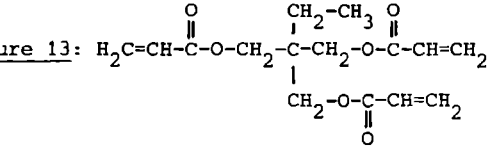


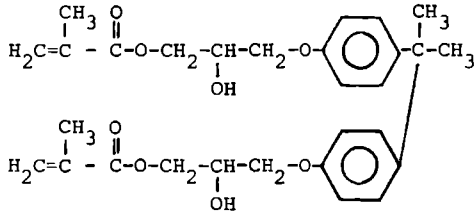
figure 13: trimethylolpropane triacrylate

1.2.3.3. *acrylated resins* (fig. 14)

Acrylated resins are composed of prepolymers of a different chemical constitution, which have reacted with 2 or more acryl molecules which now form reactive sites. Important examples are acrylated epoxies , acrylated polyurethanes and acrylated polyesters.

Their main use is in the field of U.V. or electron beam curable inks and coatings. The simplest formulation of such inks and coatings consists of a photoinitiator (only for U.V.-curable products), a diluent system of multifunctional acrylate esters and an unsaturated resin, for instance an acrylated resin. Methacrylated resins are also applicable in this field, although they require a slightly longer curing time.

figure 14:



a methacrylated epoxy resin

1.2.4. *Acrylonitrile* (fig. 15)

This monomer is widely used as a copolymer in manufacturing synthetic fibers. Approximately 20-25% of all synthetic fibers contain acrylonitrile as a copolymer. It is further used for rubbers and for the production of ABS terpolymers (acrylonitrile-butadiene-styrene) and styrene-acrylonitrile copolymers. These co- and terpolymers are low-cost thermoplastics used in *e.g.* the automobile industry and the production of suitcases and shoes.

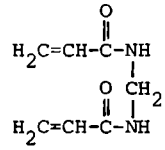
figure 15: $H_2C{=}CH{-}C{\equiv}N$ acrylonitrile

1.2.5. *Acrylamides* (fig. 16, 17)

Polyacrylamide is used as a flocculating agent, *i.e.* for the separation of solids, suspended in water. Furthermore it is used as a papermaking strengthener, as an emulsion thickening agent and as a grouting agent. A specific purpose is gel electrophoresis in biochemistry. Acrylamide as well as the structurally related substances are also used as copolymers in adhesives. Secondary acrylamides are used in the production of photopolymer printing plates.

figure 16: $H_2C{=}CH{-}\overset{\overset{O}{\|}}{C}{-}NH_2$ acrylamide

figure 17:

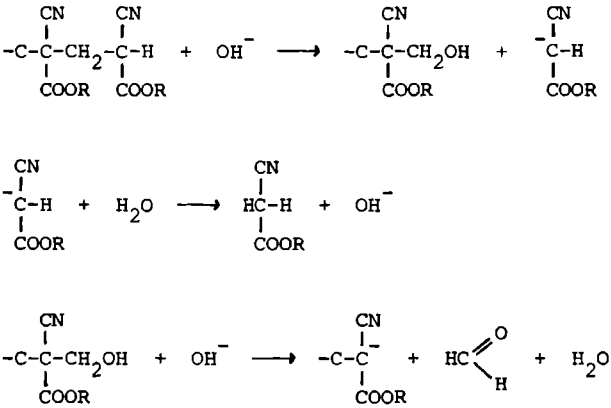


N,N'-methylene-bis-acrylamide

1.2.6. *Cyanoacrylates*

The excellent and rapid adhesion of cyanoacrylates to various adherends has given them the name of "super glues". The polymerization is an ion chain propagation reaction in which weak bases, generally water, function as ion donors. Within a few minutes the polymerization is completed. Cyanoacrylates are used as adhesives for plastics, rubber, glass, metals and in medicine for sealing wounds and as an alternative for surgical "suturing" of internal organs such as the liver and the spleen. In an alkaline environment polycyanoacrylates can degrade by hydrolysis, in which case formaldehyde is formed as one of the degradation products (fig. 18).

figure 18· Degradation of polycyanoacrylate in an alkaline environment.



1.3. *PRINCIPLES OF ADHESIVE BONDING*

An adhesive can be defined as a substance, capable of holding materials together by surface attachment.

Skeist (141) describes adhesives as social substances."They unite materials, creating a whole that is greater than the sum of its parts" (141). To bring about this union adhesive and both adherends must be compatible.

Failure of the adhesive bond can be either at the interface between adhesive and adherend or in the adhesive itself. The strength within the adhesive is referred to as cohesion, while adhesion denotes the bonding power between adhesive and adherend. A good adhesion is achieved when the glue molecules are able to come into close contact with the substrate molecules. In order to achieve this they must first of all be able to move freely so that they can make their reactive sites orientate on corresponding sites in the molecules of the adherend (95). Free movement of glue molecules is promoted by using a solution, a dispersion or a latex and also by heating. Close contact, the so-called wettability of the substrate, is possible when the surface tension of the glue is not higher than the critical surface tension of the adherend.

A stable state will occur when the joining of glue and substrate has caused a decrease in free energy. This free energy change (ΔF) is described with the following formula: $\Delta F = \Delta H - T.\Delta S$, where ΔH is the heat of mixing, T the temperature (°K) and ΔS the change in entropy. When 2 materials are combined the entropy change is in general positive, so there is a decrease in free energy when the heat of mixing is negative or not greater than $T.\Delta S$. When hydrogen-bonding or any form of chemical bonding occurs, ΔH is negative, but for most weak- or non-polar pairs of materials ΔH is positive. It has been shown that when the solubility parameters of adhesive and adherend are approximately the same, the heat of mixing is lowest. Hence such combinations are the most stable ones.

So we have 2 parameters which are useful in selecting an adhesive for a specific adherend, *viz.* the critical surface tension of adhesive and adherend, and the solubility parameters of both adhesive and adherend.

The cohesive strength of the glue, which is in most instances smaller than the adhesive strength, is also developed by approach and orientation of sensitive spots in the glue molecules themselves. In addition chemical bondage may take place in certain adhesives namely by polymerization *in situ* or by cross-linking. Together with the transition from a fluid state to a viscous or solid state by cooling, also solvent removal and/or crystallization, the result is a decrease of free energy and hence a stable state. One or more of the following mechanisms are active in adhesive bonding:

1. mechanical bonding
2. bonding at the atomic level: primary (= chemical) bonds
3. bonding at the molecular level: secondary bonds

The secondary bonds are by far the most important in adhesives technology. There are 4 types of these intermolecular forces:

1. dispersion forces
2. dipole forces
3. hydrogen forces
4. induction forces

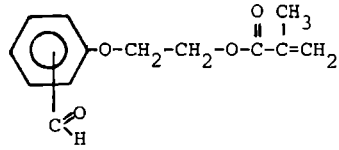
Dispersion forces are important when large molecules are in close proximity to each other and they fall off rapidly as the sixth power of the distance between molecules. Flexibility of the molecules enables them to approach each other more closely, hence dispersion forces are greater in such molecules. For non-polar adhesives these forces account for virtually all the bonding power.

Dipole forces are slightly less dependent on the proximity of the molecules. They are active in molecules bearing certain functional groups like an alcohol group $-\overset{|}{\underset{|}{C}}-OH$, carboxyl group $HO-\overset{|}{\underset{|}{C}}=O$, ketone group $O=\overset{|}{\underset{|}{C}}-$, ester group $RO-\overset{|}{\underset{|}{C}}=O$ and an amine group $-NH_2$. Never in such functional groups does the center of their negative loads coincide with the center of their positive loads. The resulting electrostatic force between the positive center of a functional group in one molecule and the negative center of a functional group in another molecule works as an attracting force.

Hydrogen bonds are also important in the field of adhesives. They occur when a hydrogen atom of *e.g.* a carboxyl group O=C-OH in the adhesive material is situated opposite a double bonded oxygen atom of a carboxyl group in the adherend material and vice versa.

Induction forces arise when a electromagnetically loaded atom or molecule induces a non-permanent dipole in a neighbouring molecule. They are usually very small compared to the other forces.

Of the primary bonds especially the covalent bonds are important in the adhesives technology. Chemical bonds are quite common in the adhesive itself but between adhesive and adherend they are less frequently encountered. Cellulose adherends (-OH groups) are partly bonded by covalent bonds when urea-formaldehyde (-NH groups) or phenol-formaldehyde (-OH groups) resins are used as adhesives. More recent examples are found in dentistry where zinc-polycarboxylate cement is used. The zinc atom is able to adhere to calcium phosphate-containing surfaces such as tooth enamel by weak chelate bonding. Another example is the addition of benzaldehyde glycol methacrylate to a modern dental composite resin (fig. 19) (96). This monomer can bind 2

figure 19: 

benzaldehyde glycol methacrylate

large prepolymers in the resin, but then an aldehyde group still remains in the new chain. This aldehyde group serves as an adhesion promoting agent for dentin. So here, too, we have a technically fast ideal situation in which adhesive and adherend are bonded both by chemical and secondary bonds.

1.4. *TOXIC EFFECTS OF ACRYLATE ESTERS*

1.4.1. *Early studies*

Reports on toxicological aspects of acrylate esters were published at first in the 1940's. At that time production of acrylates was mainly restricted to bulk-polymerization processes in industry. Therefore attention was focused on acute toxic effects of relatively high doses of monomers, as might occur during accidents in the production.

Deichmann (37) investigated methyl, ethyl and *n*-butyl methacrylate in rats, guinea pigs and rabbits and found oral LD_{50}'s in the range of 10 g/kg bodyweight. A concentration of 3600 ppm methyl methacrylate in air proved lethal to rats, guinea pigs and rabbits with 5 hours of exposure (37). Application of methyl methacrylate in a dose of 38 g/kg bodyweight to the skin of rabbits did not result in any deaths (143). The oral LD_{50} of methyl acrylate in rabbits was 0.2 g/kg bodyweight, while a concentration of 237 ppm in air caused death to rabbits and guinea pigs after an exposure of 7 hours (152). Ethyl acrylate was studied by Pozzani *et al.* (127) and they found an oral LD_{50} in rats of 1.0 g/kg bodyweight. Repeated exposures for 7 hours to a concentration of 250 ppm ethyl acrylate in air caused death in rats.

In short these studies indicated that a) the acrylate esters studied exhibit a higher acute toxicity than the methacrylate esters studied, and b) the longer the alkyl chain of the ester group, the lower the acute toxicity.

1.4.2. *Tumorigenesis, teratogenesis*

Implantation of poly(methyl methacrylate) films subcutaneously in the abdominal region appeared to cause fibrosarcomas in mice (77) and in rats (78,119,120), although negative results in mice were published too (76). Painting the back of the neck of 10 rats with undiluted methyl methacrylate 3 times a week for 4 months did not cause a single local tumor during their lifespan (120). Ethyl acrylate and methyl methacrylate were tested for carcinogenicity by oral feeding in rats and in dogs for 2 years. Doses for rats were 2000 ppm in drinking water, for dogs the maximum dose was 1000 ppm for ethyl acrylate and 1500 ppm for methyl methacrylate. No treatment-related tumors could be detected (17).

Singh *et al.* (140) reported teratogenic effects of methyl, ethyl, *n*-butyl, isobutyl and isodecyl methacrylate in rats, after intra peritoneal administration of doses ranging from 0.1 to 0.8 g/kg bodyweight. In another study (116) only some unspecific teratogenic effects were noted in rats, exposed to 24,400 ppm methyl methacrylate in the air. The lack of teratogenic potential of inhaled ethyl acrylate (50 or 150 ppm in air, 6 hours a day) in rats was described by Murray *et al.* (110). The difference in routes of administration is a possible explanation for these conflicting results. A direct absorption from the peritoneum into the fetuses is postulated as an explanation of the observations of Singh *et al.* (65). The high doses, used in the 2 former studies (116,40) are likely to exert an unspecific teratogenic effect, due to the toxicity *per se* of the administered compound and not because of the teratogenic activity of the compound.

Embryotoxic effects of methyl methacrylate and trimethylolpropane trimethacrylate were detected in three-day chicken embryos (75), while 1,3 butyleneglycol dimethacrylate caused no significant level of malformations. Unlike the other chemicals tested in this study, methyl methacrylate caused no regular dose-response curve. A poor penetration into the embryo was postulated as a reason for the relatively low acute toxicity of methyl methacrylate for chicken embryos. Therefore it is difficult to classify methyl methacrylate among the other compounds tested with this assay. Conflicting results have also been reported regarding the mutagenicity of methyl methacrylate. In the S. typhimurium-microsome assay and in the cell transformation assay negative results were obtained (7). Poss *et al.* (126) detected methyl methacrylate as a mutagen using a forward bacterial mutation assay with S. typhimurium. They noted only a mutagenic effect at doses

which killed 80% or more of the bacteria. Under such extreme circumstances the assessment of mutagenicity becomes less reliable.

So, apart from the occurrence of sarcomas after implantation of poly(methyl methacrylate) films - a phenomenon which is observed with virtually all plastics - the available evidence for carcinogenicity, teratogenicity or mutagenicity of acrylate esters is not overwhelming. At this place it may be noted that the older carcinogenicity reports do not meet today's requirements for a carcinogenicity study. A number of pitfalls in carcinogenicity studies (61) are enumerated in table 1.

table 1: Some pitfalls in carcinogenicity studies.

- test compound not analysed: impurities
- actual given dose not checked
- too toxic a dose
- dose level not constant
- other compounds tested in the same room
- insufficient number of animals at risk
- insufficient number of control animals
- no concurrent control animals
- no dose-response curve

1.4.3. *Biotransformation*

Biotransformation of acrylate esters is believed to start along two lines (38) *viz.* hydrolysis and addition to sulfhydryl groups. Hydrolysis of methyl methacrylate to methacrylic acid and methanol appears to be the major pathway (28,33). After oral or intravenous administration (5.7 mg/kg bodyweight) of methyl methacrylate to rats up to 88% was excreted as CO_2 within 10 days and up to 65% even within two hours (21), suggesting a very rapid biotransformation. The excretion of methylmalonic acid in the urine made it likely that biotransformation of methyl methacrylate may proceed after hydrolysis by the valine pathway of catabolism to the citric acid cycle (21,34).

The reactivity of acrylate esters to glutathione was shown *in vitro* (18,63) and *in vivo* (19). Acrylate esters are more reactive with glutathione and other free sulfhydryl groups than methacrylate esters (38). Silver and Murphy (139) reported a significant increase in toxicity of methyl and ethyl acrylate in rats after blocking the esterases by means of triorthotolyl phosphate. The depletion of sulfhydryl groups was also significantly larger

in animals, pretreated with triorthotolyl phosphate. The excretion of thioethers, resulting from the reaction between acrylate esters and glutathione, in the urine after intra-peritoneal administration of methyl acrylate or methyl methacrylate, was shown in rats (38) and for methyl acrylate also in guinea pigs (137).

1.4.4. *Miscellaneous effects*

The effects of methyl methacrylate on drug-metabolizing enzymes in the liver have also been studied. Short term (2.7 to 13.5 minutes) high exposures (40,000 ppm in air) of mice extended the pentobarbital sleeping time (79), while long term (up to 160 hours) exposures of 100 or 400 ppm methyl methacrylate in air shortened the pentobarbital sleeping time in mice (148). The former results may be interpreted as an acute depressive effect on enzyme activity, while the long term low level exposure is more likely to induce the synthesis of liver enzymes. A recent study (43) demonstrated transient decreases in activity of drug-metabolizing enzymes after intraperitoneal administration of 1.0 g/kg bodyweight methyl methacrylate for 3 consecutive days.

Histological examination of rats exposed to 116 ppm methyl methacrylate in air revealed only slight and inconsistent evidence of liver changes (148). Such slight liver changes were also detected in mice exposed to 100 or 400 ppm methyl methacrylate in air (148). McLaughlin *et al.* (104) found no histological evidence of damage to lungs, liver or kidneys in mice after an exposure of 40 hours in 10 days to 1,500 ppm methyl methacrylate in air. After 6 months of exposure, 5 days a week, 7 hours a day, to 116 ppm methyl methacrylate in air (only slightly above the current Threshold Limit Value for humans of 100 ppm), histological examination of the exposed rats showed a focal haemorrhage in the tracheal mucosa and a reduced amount of cilia and microvilli on the tracheal cells (148).

Inhalation of 400 ppm methyl methacrylate in air had little effect on the E.E.G. recordings of rats: only a depression of activity in the lateral hypothalamus and the ventral hypocampus was noted (71). Exposure of dogs to 2000 ppm methyl methacrylate in air resulted in reduced gastro-intestinal motor neuron activity and in a transient decrease in the blood pressure (147). The fall in blood pressure after intravenous administration of methyl methacrylate is noted by several authors (37,68) and this may lead to serious consequences in humans during hip surgery, when the monomer-polymer mixture

is used to cement a prosthesis.

A local neurotoxic effect of methyl methacrylate was described in humans after acquisition of a contact allergy to the same substance. The resulting paraesthesia heals only after months (50,57). Recently, Verkkala *et al.* (165) have investigated the local neurotoxicity of methyl methacrylate in rats. They exposed rat tail skin to undiluted methyl methacrylate under occlusion for 3 hours daily during 8 weeks. Daily absorption averaged to 0.78 g/animal. After 4 weeks of exposure abnormal responses of the motor nerves were found and these effects were progressive until the end of the experiments.

In a few reports the structure-activity relationship of (meth)acrylate esters was studied (80,146). The acute LD_{50} in mice was selected as the parameter for toxicity. The best correlation was found with the octanol-water coefficient, the hydrophilic acrylates appearing to be most toxic. The reactivity with glutathione appeared to have a lower predictive value. This finding may be an explanation for the fact, already noted by Autian (9), that the toxicological literature concentrates round the short-chained (meth)acrylates. Of course the extensive use of these monomers is another important factor.

1.4.5. *Dermatotoxicology*

Three aspects of dermatotoxicology of acrylate esters are discussed here: permeation, irritant capacity and sensitizing capacity.

- *permeation*

Both acrylate esters and methacrylate esters can be absorbed through the skin in sufficient quantities to cause the death of the animals tested. A standard dermal LD_{50} in rabbits was reported to be 1.2 g/kg bodyweight for methyl acrylate and more than 9 g/kg bodyweight for methyl methacrylate (46). The ability of methyl methacrylate to penetrate through rubber gloves is well known (122), although only qualitative data at room temperature were given by these authors.

- *irritant capacity*

As a "by-product" of sensitization studies in guinea pigs, the irritant capacity of the compounds on shaven skin of that species is assessed. Depending on the sensitization method used, open or occluded applications on the

flank of the animal are used. In both methods of application van der Walle (158) found a decreasing irritant potential in his series from diacrylates-monoacrylates-dimethacrylates to monomethacrylates. Björkner (15) noted the strong irritant potential of the polyfunctional acrylates pentaerythritol triacrylate and trimethylolpropane triacrylate and a lower irritant potential of trimethylolpropane trimethacrylate. These effects were seen in patch testing both humans and guinea pigs. The application of diacrylates and other polyfunctional acrylates as cross-linkers for industrial purposes such as printing or painting, resulted in several cases of irritant contact dermatitis in exposed workers. Beurey *et al.* (13) reported irritant effects up to bullous skin reactions in workers exposed to tetraethyleneglycol diacrylate. In subsequent human patch testing a 1% (v/v) concentration in acetone proved to be irritant. Ethanediol diacrylate and triethyleneglycol diacrylate appeared to be at least as irritant in human patch testing. In another report (112) irritant reactions in workers exposed to pentaaerythritol triacrylate, hexanediol diacrylate and trimethylolpropane triacrylate were described. Whether one or more of these acrylates was the cause for these skin reactions remained unclear. Emmett (44) observed 4 workers with an allergic contact dermatitis and one worker with an irritant contact dermatitis, due to contact with pentaerythritol triacrylate and trimethylolpropane triacrylate. Butanediol diacrylate and hexanediol diacrylate were reported (92) to be the cause of an irritant contact dermatitis which developed 12-14 hours after ephemeral work contact. As is the case with all these occupational exposures described in these studies (13,44,92,112) no quantitative data regarding exposure were available.

The delayed irritation observed by Malten *et al.* (92) was confirmed in an experiment with a volunteer, who was exposed to 87% hexanediol diacrylate in methyl ethyl ketone on the volar side of his forearms. The site exposed for 20 minutes became red after 15 hours, proceeding to bulla formation the next two days. The sites exposed for 5 and 10 minutes only showed redness (92).

- *allergic reactions*

There is extensive literature concerning allergic reactions to acrylate esters both in humans and in experimental animals. A summary is given in the thesis of Van der Walle (158). The comparisons between the publication of Bradford (20) of one patient sensitized to one substance, *viz.* methyl meth-

acrylate, virtually the only acrylic in industrial use at that time, and the theses of Van der Walle (158) and Björkner (15) is illustrative for the changes through which the acrylate technology has gone. However, the applicability of, for instance, the U.V.-curable acrylates is not as extensive as was previously expected. This is partly due to skin problems in exposed workers (113). Thus a reliable risk assessment of all available acrylics and methacrylics still seems of the utmost importance.

CHAPTER 2

NON-MUTAGENICITY OF 27 ALIPHATIC ACRYLATE ESTERS AND 5 ACRYLAMIDES IN THE SALMONELLA-MICROSOME TEST

2.1. *INTRODUCTION*

Except for some viruses, DNA is the genetic material for all living organisms. This universal function of DNA is the scientific basis of virtually all short-term mutagenicity tests. A substance which causes damage to DNA in lower organisms like bacteria or yeasts, is believed also to be able to induce damage to DNA in more complicated organisms. These changes in the DNA might have several effects, of which point mutations and chromosome breaks are the most important ones. A general term for all permanent changes in DNA is mutation.

If a mutation occurs in a germ cell the result will be a change in the genome of the germ cell and hence possibly in that of a next generation. Since this mutation is an at random change in a finely tuned system the resultant effect is most likely to be deleterious. There are over 1000 known human diseases which are of genetic origin and several diseases with a high prevalence like diabetes and epilepsy are presumed to have a genetic component.

If mutation occurs in a somatic cell there are no effects on future generations. Somatic mutation however may well be the first step in the process leading to cancer. Some authors (4,145) state that somatic mutations by environmental mutagens are the major cause for cancer, although this is disputed by others (27). There is evidence that mutations may be responsible for the activation of oncogenic viruses (131). The fact that the majority of known carcinogens are also mutagens (102,132) supports the theory that a somatic mutation is a key event in the origin of cancer. So both the prevention of genetic diseases in the future and the prevention of cancer in the existing generation seem to be partly dependent of the identification and subsequent elimination of mutagens from our environment.

A substance is tested in a mutagenicity assay primarily to assess its capacity to produce damage to DNA. The extrapolation of these data into a categorization of the risk of mutagenicity to man is the next necessary step, which however is attended by substantial uncertainties. Although several attempts have been made to minimalize these uncertainties, *e.g.* by the parallellogram model (142) no widely accepted method is known. The parallel

use of several mutagenicity assays, which measure different forms of genetic damage is necessary before trying to categorize the mutagenic risk of a chemical.

Because of their high sensitivity, mutation tests in bacteriae are particularly useful for the detection of mutagens. The salmonella-microsome test as developed by Ames and co-workers is the most widely used and also the most thoroughly validated bacterial mutation assay. Although there has been some dispute about it (3,8) it is now widely recognized that the test provides qualitative rather than quantitative information on the mutagenicity of a chemical (62).

Esters and amides of acrylic acid and methacrylic acid are widely used in modern plastic technology. Over 500 million kg of methyl methacrylate alone is produced annually (70). The resulting acrylic resins and polymers are used for a variety of applications, such as prostheses, contact lenses, photopolymer printing plates, adhesives and coatings. Workers in industry and craftsmen are exposed to various levels of acrylic monomers. Skin problems are frequently encountered in some trades (93). Exposure to acrylamide may lead to neurotoxic symptoms, notably a periferal neuropathy (42,144).

The potentially harmful effects of plastic monomers became a major cause of concern to public health officers, especially since the discovery of vinylchloride as a mutagen (129) and a carcinogen to animals (166) and humans (32). Compounds which are structurally related to acryl esters and acrylamides like acrylonitrile and acrolein have been shown to be mutagenic (14,84,107,164).

Glycidyl methacrylate was found to be mutagenic in the Ames test (154) and in a fluctuation test with Kl. pneumoniae (167). Methyl 2-cyanoacrylate was also detected as a mutagen in the Ames test (6) and Poss *et al.* (126) reported the mutagenicity of methyl methacrylate in a forward mutation assay with S. typhimurium. Acrylamide was reported to induce chromatid exchanges and breaks in spermatogonia of mice (138).

The reactivity of acrylate esters (18,19) and of acrylamide (63) with glutathione was clearly demonstrated. Delbressine (38) was able to establish experimentally that this reactivity was based on a Michael-type addition to the olefinic bond, *i.e.* a direct alkylating effect. Acrylamide was bound apparently irreversible to the red blood cells of rats *in vivo* (106). Singh *et al.* (140) reported teratogenic effects in rats after intraperitoneal administration of esters of methacrylic acid. A further review of teratolog-

ical effects of the investigated substances is given in chapter 1.

In view of the observed alkylating potency of these esters and acrylamides we became interested in the possible genotoxic effects of these substances. Therefore we decided to assess the mutagenic potential of 27 acrylate esters and 5 acrylamides with the Ames test (see Table 1).

2.2. *MATERIALS AND METHODS*

chemicals

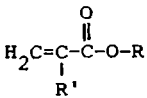
Dimethyl sulphoxide (DMSO), 9-aminoacridine hydrochloride monohydrate, 2-aminoanthracene, 4-nitro-*o*-phenylenediamine, benzo[α]pyrene and glycidyl methacrylate were purchased from Aldrich Europe (Belgium). Sodium azide and acrolein were obtained from Merck (F.R.G.). Nicotinamide adenine dinucleotide phosphate sodium salt (NADP), D-glucose-6-phosphate sodium salt and D-biotin were purchased from Sigma (U.S.A.). L-Histidine monohydrochloride came from BDH (Great Britain). Bacto agar was obtained from Difco Lab. (U.S.A.) and nutrient broth No. 2 from Oxoid Ltd. (Great Britain).

Names, manufacturers and purity of the tested compounds are shown in Table 1. The acrylate esters, synthesized in our own laboratory (Drs. E. Seutter) were prepared by treatment of acryloyl chloride or methacryloyl chloride with the required alcohol in the presence of triethylamine and the acyl transfer catalyst 4-(dimethylamino) pyridine (64). N-*t*-butyl acrylamide was synthesized by the reaction of acrylonitrile with tertiary butanol and sulphuric acid in glacial acetic acid (124). The acrylamides were recrystallized from an appropriate solvent (methanol, 2-propanol or methylene chloride). Purity was checked on a Hewlett Packard 402 gas chromatograph, equipped with a glas column of 1.8 m length, internal diameter 3 mm, containing 3% OV.101 on Gaschrom Q 80-100 mesh, in connection with an Atomaire Flame Ionisation Detection. Conditions of use were: injection block 240°C, detector 300°C, column 70°C, isotherm 4 min increasing from 70 to 300°C at 7.5°C/min, helium carrier flow 30 ml/min. To prevent spontaneous polymerization all esters contained hydroquinone or *p*-methoxyphenol in quantities ranging from 1 mg/l to 2.5 g/l.

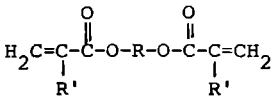
mutagenesis assay

Basically, the procedure as described by Ames *et al.* (2) was followed, unless otherwise indicated. We used the S.typhimurium strains TA1535, TA1537

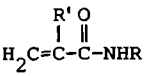
TABLE 1: Names, purity, structure and concentration range of compounds tested

Mono(meth)acrylates: 

R' = H monoacrylate (1-7)
R' = CH_3 monomethacrylate (8-15)

Di(meth)acrylates : 

R' = H diacrylate (16-21)
R' = CH_3 dimethacrylate (22-27)

(Meth)acrylamides : 

R' = H acrylamide (28-31)
R' = CH_3 methacrylamide (32)

Compound	Manufacturer	Purity	R	Tested concentration range (µg/plate) in Ames test
1. Methyl acrylate	Aldrich	>99%	$-CH_3$	40- 2500
2. Ethyl acrylate	BDH	>99%	$-CH_2-CH_3$	30- 2000
3. *n*-Butyl acrylate	Merck	>99%	$-(CH_2)_3-CH_3$	30- 2000
4. *t*-Butyl acrylate	UCB	>99%	$-C(CH_3)_2-CH_3$	30- 2000
5. *n*-Pentyl acrylate	own synthesis	>99%	$-(CH_2)_4-CH_3$	25- 1600
6. Neopentyl acrylate	own synthesis	>99%	$-CH_2-C(CH_3)_2-CH_3$	30- 2000
7. *n*-Hexyl acrylate	Polyscience Inc.	>99%	$-(CH_2)_5-CH_3$	25- 1600
8. Methyl methacrylate	Merck	>99%	$-CH_3$	40-10000
9. Ethyl methacrylate	Merck	98%	$-CH_2-CH_3$	40- 2500

TABLE 1 (continued)

Compound	Manufacturer	Purity	R	Tested concentration range (µg/plate) in Ames test
10. *n*-Butyl methacrylate	Koch-Light	>99%	$-(CH_2)_3-CH_3$	40-2500
11. *t*-Butyl methacrylate	Polyscience Inc.	>99%	$-C(CH_3)_2-CH_3$	40-2500
12. *n*-Pentyl methacrylate	Polyscience Inc.	>99%	$-(CH_2)_4-CH_3$	40-2500
13. Neopentyl methacrylate	own synthesis	>99%	$-CH_2-C(CH_3)_2-CH_3$	40-2500
14. *n*-Hexyl methacrylate	Polyscience Inc.	97%	$-(CH_2)_5-CH_3$	40-2500
15. 2-Hydroxyethyl methacrylate	Merck	>99%	$-CH_2-CH_2-OH$	40-2500
16. 1,2-Ethanediol diacrylate	Polyscience Inc.	87%	$-CH_2-CH_2-$	30-2000
17. 1,4-Butanediol diacrylate	Merck	98%	$-CH_2-(CH_2)_2-CH_2-$	30-1000
18. 1,5-Pentanediol diacrylate	Polyscience Inc.	96%	$-CH_2-(CH_2)_3-CH_2-$	30-2000
19. Neopentanediol diacrylate	Polyscience Inc.	97%	$-CH_2-C(CH_3)_2-CH_2-$	30-2000
20. 1,6-Hexanediol diacrylate	Polyscience Inc.	96%	$-CH_2-(CH_2)_4-CH_2-$	30-1000
21. Diethylene glycol diacrylate	Polyscience Inc.	89%	$-CH_2-CH_2-O-CH_2-CH_2-$	40-2500
22. 1,2-Ethanediol dimethacrylate	Merck	95%	$-CH_2-CH_2-$	40-2500
23. 1,4-Butanediol dimethacrylate	Polyscience Inc.	97%	$-CH_2(CH_2)_2-CH_2-$	40-2500
24. 1,5-Pentanediol dimethacrylate	Polyscience Inc.	96%	$-CH_2(CH_2)_3-CH_2-$	40-2500

TABLE 1 (continued 2)

Compound	Manufacturer	Purity	R	Tested concentration range (µg/plate) in Ames test
25. Neopentanediol dimethacrylate	Polyscience Inc.	97%	$-CH_2-\overset{\overset{CH_3}{|}}{\underset{\underset{CH_3}{|}}{C}}-CH_2-$	40-2500
26. 1,6-Hexanediol dimethacrylate	Polyscience Inc.	97%	$-CH_2-(CH_2)_4-CH_2-$	40-2500
27. Diethylene glycol dimethacrylate	Polyscience Inc.	95%	$-CH_2-CH_2-O-CH_2-CH_2-$	40-2500
28. Acrylamide	Aldrich	>99%	$-H$	5-5000
29. N-hydroxymethyl acrylamide	BDH	98%	$-CH_2OH$	5-2500
30. N-tertiary butyl acrylamide	own synthesis	98%	$-\overset{\overset{CH_3}{|}}{C}-CH_3$	5-5000
31. N,N'-methylene bis acrylamide	Aldrich	98%	$-CH_2-HN-\overset{\overset{CH_3}{|}}{\underset{\underset{O}{\|}}{C}}-CH=CH_2$	5-2500
32. Methacrylamide	Aldrich	98%	$-H$	5-2500

TA1538, TA98 and TA100, which were a gift of Dr. Ina Mattern (TNO, Rijswijk, The Netherlands). The strains were grown overnight in the nutrient broth in a shaking water bath at 37°C until an optical density of 0.4 at 700 nm was reached. Dilution experiments indicated that this corresponded to $0.5\text{-}1.10^9$ bacteria/ml. The trace amount of histidine (0.09 µmole/plate) was incorporated in the plates instead of in the top agar. The Aroclor-1254-induced rat liver S9 fractions were purchased from Litton Bionetics Inc. (U.S.A.). Phenobarbital-induced S9 fractions were prepared from male Wistar rats weighing approximately 200 g, who had received 0.1% (w/v) sodium phenobarbital in their drinking water during the 5 days preceding their sacrifice. Further preparation was performed according to Ames *et al.* (2). Protein content of the homogenate was 25-40 mg/ml, estimated by the method of Lowry *et al.* (83). S9 mix contained 0.1 ml homogenate per ml.

All tests were performed on triplicate plates. Compounds were once tested with phenobarbital-induced S9 mix, once with Aroclor-1254-induced S9 mix and twice without any additional metabolizing system. Compounds were diluted in DMSO prior to use and the required amount was delivered in 0.1 ml DMSO to the top agar. At least 4 concentrations up to 2500 µg/plate or toxic levels were tested. To minimize loss of the volatile test substance, the petri dishes were transferred quickly after plating into air-tight glas jars. Solvent controls, positive controls and sterility controls for S9 mix were run with each experiment. The plates were counted manually after incubation in the dark at 37°C for 48-72 h.

Methyl methacrylate, methyl, *n*-butyl, and hexyl acrylate were also tested in a modification of the liquid suspension test described by Rannug *et al.* (130). Bacteria (TA100 only) were grown in nutrient broth as described above. Of the obtained suspension 0.9 ml was added to either 1.0 ml S9 mix (Aroclor-induced) or 1.0 ml 0.1 M phosphate buffer pH 7.4. To this mixture 0.1 ml of the test compound in DMSO was added. The tubes were tightly closed and placed in a shaking water bath at 37°C for 90 min. Then 0.8 ml of this mixture was added to 3 ml molten top agar and poured in duplicate onto standard petri dishes, which were incubated and counted as described above.

2.3. *RESULTS*

The concentration range of the test substances is shown in Table 1; a stepwise increase of 4 times the previous concentration was used, *e.g.* 40-160-625-2500 µg/plate. When the background lawn of bacteria was sparse, the

TABLE 2: Control values observed during these studies.

Strain	S9[a]	Spontaneous revertants (mean±S.D.)	Positive controls (µg/plate)	Revertants (mean±S.D.)
TA1535	-	24± 8	Sodium azide (1)	481± 60
			Glycidyl methacrylate (25)	137± 24
TA1535	PB	15± 4	2-Aminoanthracene (0.7)	192± 24
TA1535	Aro	13± 3	-	-
TA1537	-	9± 3	9-Aminoacridine (70)	340± 51
TA1537	PB	8± 4	2-Aminoanthracene (0.7)	224± 59
TA1537	Aro	11± 5	-	-
TA1538	-	16± 4	4-Nitro-*o*-phenylenediamine (3)	596± 53
TA1538	PB	23± 6	2-Aminoanthracene (0.7)	1123±368
TA1538	Aro	30± 5	Benzo[α]pyrene (5)	130± 6
TA98	-	32± 5	4-Nitro-*o*-phenylenediamine (3)	779± 90
TA98	PB	43± 6	2-Aminoanthracene (0.7)	1461± 47
TA98	Aro	49± 5	Benzo[α]pyrene (5)	470± 30
TA100	-	125±19	Sodium azide (1)	749± 61
			Glycidyl methacrylate (125)	500± 25
TA100	PB	120±15	2-Aminoanthracene (0.7)	1326±111
TA100	Aro	127±25	Benzo[α]pyrene (5)	814± 76

[a]PB, phenobarbital-induced S9 mix; Aro, Aroclor-1254-induced S9 mix.

chemical was considered to be toxic in that concentration. In such a case also half that concentration was tested. All monoacrylates and diacrylates were toxic in the highest concentration tested, but none of the methacrylates or dimethacrylates. Acrylamide exerted a toxic effect in the highest concentration tested only in TA100. All other acrylamides tested, appeared to be non-toxic to the bacteria. None of the tested substances produced a significant increase of revertants, *i.e.* a number of revertants which is more than 2-fold compared to the spontaneous, in any of the 5 Salmonella typhimurium strains.

The use of glass plates instead of plastic plates had no influence on the results. Control values are shown in Table 2. Glycidyl methacrylate also contained *p*-methoxyphenol as an inhibitor. The addition of 2 µg hydroquinone to benzo[α]pyrene or 2-aminoanthracene had no influence on the response of TA100 to these mutagens.

The results of the liquid suspension tests are presented in Table 3. Also with this method the acrylate esters failed to show a mutagenic response. No acrylamides were tested with the liquid suspension method, since their volatility is very low.

2.4. *DISCUSSION*

During the last few years a number of investigations have been published on the mutagenic activity of olefinic compounds. Several halogenated alkenes (10), allylic compounds (114), haloacroleins (133) and also acrylonitrile (107,164) were reported to be mutagenic. On the other hand, styrene (26,41,84) gave conflicting results regarding mutagenicity. Acrylamide was found non-mutagenic to TA100 (109) but it appeared to produce chromosome damage in mice spermatogonia (138). Although generally substances which cause chromosome abnormalities also cause point mutations (60) there are some exceptions to this rule, of which benzene is the best known. Other mutagenicity tests with acrylamide need to be performed before a reliable classification of the genotoxic risk of acrylamide can be made. Of the other tested acrylamides no further data on their mutagenic potential were known to us. As for neurotoxicity, acrylamide itself appears to be more toxic than the other tested acrylamides (42). Methyl methacrylate was negative in a standard Ames assay and in a cell-transformation assay (7). However, Poss *et al.* (126) detected methyl methacrylate as a mutagen in a forward bacterial mutation assay, using Salmonella typhimurium TM677. They only observed a rise in the mutation

TABLE 3: Mutagenicity data in the liquid incubation modification of the Ames assay with strain TA100

Compound	Concentration (µg/2 ml incubation volume)	Number of revertants[a] -S9	+S9 (Aroclor-induced)
None	-	136±13	165± 8
Methyl acrylate	6000	100±10	113± 9
	600	128±10	148± 7
	60	129±14	160±10
Butyl acrylate	1500	115± 7	137±11
	150	122±13	144±12
	15	131±12	156± 8
Hexyl acrylate	300	100±12	101±21
	30	117±15	142±11
	3	121±15	158± 6
Methyl methacrylate	10000	121± 7	143±10
	1000	130±11	145±14
	100	136± 6	161±11
Acrolein	10	322±16	-
	15	378±35	-

[a]Mean±S.D. of 2 independent experiments of 2 selection plates each.

frequency with additional rat liver homogenate at concentrations of methyl methacrylate which killed 80% or more of the bacteria. Under such extreme circumstances the assessment of mutagenicity becomes less reliable. Brusick (23) gives an example of a possible artefact with the mechanism of preferential selection. When the revertants are more resistant to the toxic effect of the test substance, a rise in the mutation frequency is observed, which, however, is not a mutagenic effect.

It is a well-known fact that acrylate esters and other monomers can be bound rapidly to several plastics, but the use of glass plates instead of plastic plates had no influence on the results. Therefore we suppose that the binding of these chemicals to the plastic petri plate is not important under the conditions of the Ames assay. False negative results may occur because of the volatility of the compounds. Therefore we also used a liquid incubation test with the highly volatile acrolein as a positive control. In view of the control experiments performed, it seems unlikely that hydroquinone or *p*-methoxyphenol are able to suppress a mutagenic response under these circumstances.

In addition to the possibility of a direct mutagenic effect due to the electrophilicity of these unsaturated esters (84) we could also think of an indirect mutagenic effect *via* the biotransformation to epoxides. Since several epoxy compounds are well-known mutagens and carcinogens (47,160,167, 168) it is an important question whether such metabolites are formed during the metabolism of acrylic compounds. No such epoxy intermediates were detected *in vivo* or *in vitro* both with phenyl-substituted acrylate esters (40) and with alkyl-substituted acrylate esters (38). These findings are in agreement with the observed lack of mutagenic potency of these esters.

CHAPTER 3

THE SENSITIZING POTENTIAL OF ACRYLIC CONSTITUENTS OF PRESSURE-SENSITIVE ADHESIVES

3.1. *INTRODUCTION*

Pressure-sensitive adhesives are adhesives which are permanently in the tacky stage and they can be used without application of solvents or heat. They adhere to most ordinary surfaces when only slight pressure is applied. Their adhesion to most adherends is lower than their cohesion and the adhesion to the backing, so when a pressure-sensitive adhesive tape is removed from a surface, the glue molecules stick together on the tape, leaving only faint traces of glue on the adherend. Therefore the integrity of the adherend is almost completely preserved, which is a reason why this type of adhesives is very suited for use on the human skin.

The application of adhesives on the human skin also means that in such a situation there is an occlusive contact of the chemicals in the adhesive layer with the skin. The occurrence of an irritant or an allergic reaction of the skin to a substance is stimulated by such an occlusive contact. Because of the generally short induction time of acute irritant contact dermatitis (94) most likely adhesives with acute irritant properties are detected before massive introduction of such an adhesive has taken place. It is much more difficult to recognize and rate chronic irritant properties of such pressure-sensitive adhesives which are involved when for instance stoma bags are affixed to an often already irritated skin around artificial orifices. Such rating should be possible with instrumental investigative procedures such as described in chapter 6.

A third possible untoward effect of adhesive tapes is allergic contact dermatitis based on a very specific immunologic reaction which in practice only occurs after repeated contact with a given substance. It may take anything between one week and a lifetime before an allergic contact sensitization has developed. A enumeration of factors which play an important role in the induction of an allergic contact dermatitis is given in chapter 4.1. Once being sensitized, the only solution to prevent recurrence of the dermatitis is to avoid any further contact with the sensitizing substance, since in many cases even ephemeral contact is already sufficient to bring about a revival of the dermatitis. In addition, the use of other products containing chemically

related substances may also lead to recurrences of an allergic contact dermatitis (group-specific reaction).

Consequently it is important to test the materials used in the production of pressure-sensitive adhesives, on their sensitizing potential, before they are introduced on the market.

Because of their high clarity and good resistance to oxidation and UV-radiation, acrylic monomers are often used to produce these adhesives, especially for applications on the human skin. The adhesive layer consists usually of a copolymer of 2-ethylhexyl acrylate (2-EHA) with polar monomers such as acrylic acid, acrylamide or N-alkyl maleamic acids (96). These acrylic-based pressure-sensitive adhesives are often marketed with the recommendation "hypo-allergenic". There are some reasons to view this latter recommendation with suspicion. Firstly, in several so-called hypo-allergenic tapes the occurrence of allergenic derivatives of colophony was demonstrated (72, 136). These allergenic constituents of the adhesive tapes were able to cause a contact sensitization (136). Colophony is one of the top 20 common allergens (89). Secondly, the application of such adhesives to diseased skin is likely to be associated with a greater risk of contact sensitization. The easy sensitization of ulcerated or eczematous skin to topical medications is notorious (55). Fisher (51) even noted a patient, who had developed an allergic contact dermatitis to parabens in a bandage, applied to diseased skin, while the same patient continued to use the parabens in other applications on other, healthy skin sites without any difficulty.

At present it remains unclear which conditions need to be fulfilled, before the term "hypo-allergenic" can be used. A prerequisite before using this term should be at least the obligation to enumerate all ingredients on the label.

To our knowledge 2 reports regarding human sensitizations after use of modern pressure-sensitive adhesives have been published (73, 66). In both publications a Curad^{R} adhesive was incriminated as the causative factor. In the first report (73), the 7 patients appeared to be sensitized to 2-ethylhexyl acrylate and 3 of them also to N-*t*-butyl maleamic acid (table 6). In the other report (66), N-dodecyl maleamic acid (table 6) was described as the sensitizing substance for the 2 patients. Human sensitizations to acrylamide and derivatives (table 5) are described in workers engaged in photopolymer printing processes (128, 91, 121), but until today not in people exposed to pressure-sensitive adhesives, containing acrylamides.

In order to obtain some insight in the sensitizing potential of the above mentioned possible constituents of pressure-sensitive adhesives, we decided to investigate 2-ethylhexyl acrylate, several acrylamides, alkyl maleamic acids and acrylic acid with guinea pig test methods, designed to evaluate contact sensitizing properties. A quantitative estimation of the sensitizing potential is also important for the occupational dermatologist, who wants to be able to advise industrial technicians on replacement of allergens by technically possible alternatives with a lower sensitizing potential.

3.2. *THE SENSITIZING POTENTIAL OF 2-ETHYLHEXYL ACRYLATE IN THE GUINEA PIG*

3.2.1. *Material and methods*

chemicals

2-Ethylhexyl acrylate (2-EHA) was obtained from Ciago (The Netherlands), containing 120 mg/l hydroquinone as inhibitor. Purity was 94% (gas chromatographically). Butyl acrylate and methyl methacrylate were from Merck (FRG). Hexyl acrylate and hexyl methacrylate were from Polyscience Inc. (USA), ethyl acrylate from BDH (England) and *t*-butyl acrylate from UCB (Belgium). Except for hexyl methacrylate, which was 97% pure, the remaining acrylates after distillation were over 99% pure (gas chromatographically). They contained either hydroquinone or *p*-methoxyphenol as inhibitor in quantities ranging from 30 to 160 mg/l. Freund's complete adjuvant was from Difco (USA).

animals

Female Dunkin Hartley outbred guinea pigs (Olac Ltd., England) were used for the study. They were housed in steel cages in pairs and fed on a pellet diet supplemented ad libitum with water.

Freund's Complete Adjuvant Test

A modification of Freund's Complete Adjuvant Test (159) was used, in which 3, instead of 5 intradermal injections were given on days 0, 5 and 9, respectively, in the shaved nuchal area of the guinea pig. The injected emulsion consisted of equal amounts of distilled water and Freund's complete adjuvant, in which the test substance was suspended to a final concentration of 0.5 M or 0.17 M (equal to about 9, and 3% respectively). Controls received the same

treatment without the test substance. The control group consisted of 10 animals; the 2 experimental groups consisted of 16 guinea pigs each.

Challenges were performed every 2 weeks from day 21 by open epicutaneous applications of 0.025 ml of the test substance in Aramek (1 part peanut oil, 2 parts methyl ethyl ketone by volume) on a circular 2 cm^2 area of the shaved flank of the animals. Reactions were read after 24 and 48 hrs. An animal was considered to be sensitized if it showed a clear confluent redness at 24 and 48 hrs to a concentration of the test substance which did not give a visible reaction in control animals.

In addition, we used in the first challenge 1 irritant concentration of 2-EHA, *viz.* 3 M, while in all other challenges, only non-irritant concentrations were used. Hydroquinone was tested in the 3rd challenge at a concentration of 1 M, using the same method as used for 2-EHA.

cross reactions

On day 63, after 3 consecutive challenges at day 21, 35 and 49, we determined the cross-sensitivity in 8 test animals, 4 from each group which had reacted in all 3 former challenges to 2-EHA (1 M), and in 6 control animals which up to that time had not reacted to 2-EHA (1 M) in any challenge. The challenge procedure was the same as described above. To verify the reactivity of the animals to 2-EHA, they were tested on days 77 and 105 with a 1 M and a 0.3 M solution of 2-EHA. The control animals were only tested on day 105.

adhesive tapes

Testing of the adhesive tapes was carried out in several ways on days 77, 91 and 105 with the same animals as used in the cross reaction challenge.

(a) We applied a tape directly to the guinea pig skin, removed it after 48 hrs and read the reactions after 50 and 72 hrs.

(b) We applied 0.025 ml of 1 of 4 solvents (ethanol, olive oil, methyl ethyl ketone, distilled water) to the Whatman paper of a Silverpatch (89) and covered each of them with one of the tapes to be investigated. The wetted Silverpatches plus tape were then fastened to the guinea pig skin with an elastic bandage (Coban, 3 M Co., USA) encircling the trunk. After 48 hrs this dressing was removed and reactions were read after 50 and 72 hrs. In this way a situation similar to human patch-testing was created.

(c) We immersed 100 cm^2 of a tape in 3 ml of ethanol or Aramek in a closed beaker for 24 hrs and used this solution for an open elicitation test

as described above.

Methods (a) and (b) were used for the tape brands Scanpor (Norgesplaster, Norway) and Leukopor (Beiersdorf, FRG). Method (c) was used for these 2 brands and for Band-aid (Johnson & Johnson, USA), Curafix (Lohmann, FRG) and Sparaplast (Lab. de pansement et d'hygiène, France).

3.2.2. *Results*

With both induction concentrations (0.17 M and 0.5 M), sensitization of guinea pigs to 2-EHA was readily demonstrated. The lower induction concentration gave a slightly higher response (Table 1). In the second challenge on day 35, 3 out of 10 control animals also appeared to be sensitized. Cosensitizations to the inhibitor hydroquinone occurred in 4 experimental animals, 3 from the group induced with 0.5 M 2-EHA and 1 from the group induced with 0.17 M 2-EHA.

TABLE 1: Sensitizing potential of 2-EHA in the modified Freund's Complete Adjuvant Test.

Induction concentration	No. of animals	Challenge concentration*	Number of sensitized animals day 21	day 35	day 49
0.5 M	16	1 M	6	11	9
0.17 M	16	1 M	13	13	11
controls	10	1 M	0	3	1

*In Aramek (1 part peanut oil, 2 parts methyl ethyl ketone v/v).

TABLE 2: Cross reactions to other acrylate monomers of animals sensitized to 2-EHA.

Monomer	Concentration*	No. of positively reacting animals out of the total number of tested animals	Control animals
ethyl acrylate	3 M	3/8	0/6
n-butyl acrylate	3 M	7/8	0/6
t-butyl acrylate	undiluted	0/8	0/6
hexyl acrylate	1 M	2/8	0/6
methyl methacrylate	undiluted	0/8	0/6
hexyl methacrylate	3 M	0/8	0/6

*In Aramek (1 part peanut oil, 2 parts methyl ethyl ketone v/v).

No control animals appeared to be sensitized to the inhibitor. Cross sensitizations are shown in Table 2. With the exception of *t*-butyl acrylate, all tested monoacrylates (ethyl, *n*-butyl, hexyl acrylate) gave at least some positive reactions. Reactions to the 2 methacrylates (methyl and hexyl) were not observed.

On day 77, all test animals reacted to 2-EHA in both concentrations. On day 105, 5 out of 7 animals (1 died) reacted to the 1 M concentration of 2-EHA, while none of the 6 control animals reacted.

All tests with the adhesive tapes gave negative results. The immersion extracts of Band-aid with ethanol as well as with Aramek proved to be mildly irritant to the guinea pig skin.

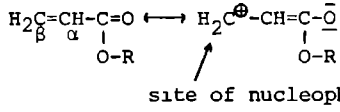
3.2.3. *Discussion*

The strong sensitizing potential of 2-EHA in guinea pigs is in accordance with the findings of Van der Walle *et al.* (156), who showed that monoacrylates were potent sensitizers in Freund's Complete Adjuvant Test. The higher sensitization incidence obtained with the lower induction concentration is in contrast to the findings of Magnusson & Kligman (85), who observed an increasing sensitization incidence with increasing induction dose. This was also reported in human test methods (99). A possible explanation of the effect observed by us may be that with the higher dose, a significant amount will not be bound in the skin, but will reach the central part of the lymphatic system unchanged, possibly entailing the production of suppressor lymphocytes (11). The strong sensitizing potential of 2-EHA is also demonstrated by the fact that 3 out of 10 control animals showed allergic responses 2 weeks after a single application of the challenge test concentrations on day 21.

As shown by Van der Walle (155), concomitant sensitization to hydroquinone interfered with neither the sensitizing potential of the acrylate under investigation nor the cross reaction pattern.

The observed cross reactions with other monoacrylates together with the non-reactivity of the monomethacrylates, correlate well both with the findings of Van der Walle & Bensink (157) on guinea pigs and with the observation of Jordan (73) in 5 human beings, of a broad cross reaction pattern with monoacrylates, but only with one methacrylate, *viz.* hydroxypropyl methacrylate. This methacrylate was unfortunately not available to us. The non-reactivity of our guinea pigs to *t*-butyl acrylate might be due to the high volatility of this monomer, which was applied undiluted and not occluded. With guinea

pig test methods, monoacrylates are more potent sensitizers than monomethacrylates (156). This is reflected in the number of reported sensitizations in humans(134). A theoretical base for the observed differences in sensitizing potential of monoacrylates vis-à-vis monomethacrylates, can be postulated. Generally, a covalent binding in the skin to a carrier protein is necessary to induce contact sensitization (125, 153). Acrylate esters can react easily in a Michael-fashion with nucleophiles like -SH or $-NH_2$ groups in proteins, due to the electron-withdrawing effect of the functional ester group (39). The presence of a methyl group at the α-carbon may diminish this electron-withdrawing effect with a consequent fall in reactivity towards nucleophiles. The lower sensitizing potential of methacrylates is compatible with this theory.



The negative results of testing with the adhesive tapes were not totally unexpected, as previous testing had indicated that the sensitized animals reacted to 2-EHA only in concentrations of 0.3 M or higher. We only knew that Scanpor tape contained 2-EHA, which is why we tested this brand in the most elaborate way. Obviously, the concentration of free penetrable 2-EHA in the tape was so low that no reactions could be observed. This seems to be in contrast with Jordan's observation (73) of 7 patients sensitized to 2-EHA present in an adhesive tape. Technical improvements in the manufacturing of pressure-sensitive adhesive tapes may also be responsible for the observed lack of reactivity of the tapes in the guinea pigs. Several years ago, the adhesives were made by the polymer solution method, in which case solvent impurities, low molecular fragments and small quantities of monomers could be found in the end product. Nowadays, solution polymerization has been replaced by emulsion polymerization. The use of this latter method gives a more complete polymerization, resulting in a lower concentration of reactive low molecular substances in the end product (96). Furthermore, this method results in an environmental improvement, as only water and a detergent are needed in the production of aqueous acrylic dispersions, without any toxic, highly inflammable solvents.

The observed irritant reaction to the extract of 1 of the tapes is presumably due to the presence of such a detergent. For minimalizing allergological risks

the emulsion polymerization method appears to be substantially better than the solution polymerization method. In the classic adhesives colophonium (30) turpentine peroxides (31) and rubber chemicals (29) are recognized as rather frequent sensitizers. Compared to them, adhesives made by emulsion polymerization seem to have a low allergenic potential. Although introduced about 5 years ago very few "plaster irritation" patients have been encountered so far. A disadvantage of the modern adhesives is, generally, their lower adhesive strenght, compared to the classic adhesives.

3.3. *SENSITIZING POTENTIAL OF ACRYLAMIDES AND ALKYL MALEAMIC ACIDS IN THE GUINEA PIG*

3.3.1. *Materials and methods*

chemicals

Acrylamide, methacrylamide and N,N'-methylene bis acrylamide were from Aldrich (Belgrium). N-hydroxymethyl acrylamide came from BDH (U.K.), while maleamic acid was obtained from Koch-Light (U.K.). N-*t*-butyl acrylamide, N-*t*-butyl maleamic acid and N-dodecyl maleamic acid were synthesized in the department of organic chemistry of the section of occupational dermatology (drs. E. Seutter). All substances were recrystallized from an appropriate solvent in order to obtain a purity, better than 98% (gas chromatographically).

animals

Female Hartley outbred guinea pigs (Broekman Institute, The Netherlands) weighing approximately 400 g at the beginning of the experiments were used. They were housed in pairs in plastic cages in airconditioned rooms of our animal laboratory and fed on a pellet diet supplemented ad libitum with water.

sensitization procedures

The modified Freund's Complete Adjuvant Test was used as described in chapter 3.2. In the injected emulsion the final concentration of test substance was 0.5 M, which equals to 3.5% for acrylamide, 4.2% for methacrylamide and 5.0% for N-hydroxymethyl acrylamide. Only these 3 substances were investigated with the Freund's Complete Adjuvant Test. Challenges were performed on day 21 and day 35 as described in chapter 3.2. Only non-irritant concentrations were

used. On day 49 the sensitized animals were tested for cross-reactivity to related substances by open epicutaneous application of 0.1 ml of the substance in petrolatum, in the same concentration as used in the challenges in the Split Adjuvant Test. Maleamic acid was tested in a 2.5 M concentration. The Split Adjuvant Test as described by Maguire (86) was used by us with some minor modifications. Chronologically the procedure was as follows:

Day 0 : 1. To reduce damage to the skin by scratching, adhesive tape (Leukopor, Beiersdorf, FRG) is wrapped around the rear feet of the guinea pigs. They remain in place during the whole study and are replaced if lost.

2. The nuchal area of the guinea pig is clipped.
3. An elastic bandage, 3.8 cm width (Coban, 3 M, USA) is wound around the shoulder area of the animal. By means of scissors a window of 2x2 cm is made in this dressing in the nuchal area. The bandage is allowed to settle into position for a few hours.
4. Dry ice is applied for 5 seconds to the exposed site in the nuchal area.
5. On a circular (Ø 2 cm) filter paper 0.2 ml of the test substance in petrolatum is dispensed and the ointment is applied through the window, with the edges of the filter paper tucked under the dressing.
6. The window is occluded with impermeable adhesive tape (Leukosilk, Beiersdorf, FRG).

Day 2 : The window covering and the filter paper are removed and steps 5 and 6 of day 0 are repeated.

Day 4 : After removing the window covering and the filter paper two 0.1 ml intradermal injections of Freund's complete adjuvant/saline (50/50 v/v) are given on either side in the epicutaneously exposed nuchal area. Thereafter steps 5 and 6 of day 0 are repeated.

Day 7 : The procedure of day 2 is repeated.

Day 9 : All dressings are removed.

Day 21: An open epicutaneous challenge is performed with a non-irritant concentration of the test substance in petrolatum as a vehiculum; 0.1 ml is rubbed in, with a gloved finger, on 2 cm^2 of the clipped left flank of the guinea pig. Reactions are read after 24, 48, 72 and 96 hrs.

Day 35: The procedure of day 21 is repeated on other sites of the left flank of the guinea pig. Only for acrylamide a closed patch test is performed, using Silver patches (89) and a Coban occlusive dressing. Amount, concentration and vehiculum are identical to the open applications. After 24 hrs the dressings and the Silver patches are removed.

Day 49: Repetition of the procedure of day 21, again using the left flank. In addition the test substance is tested in another vehiculum. N,N'-methylene bis acrylamide is tested in ethanol/distilled water (80/20 v/v), N-dodecyl maleamic acid is not tested in another vehiculum. All other substances are tested in Aramek.

Day 63: Cross-reactivity of the sensitized animals is determined by open applications in petrolatum on the right flank. Concentrations are identical to the highest ones, used in the challenges. The original sensitizing substance is always retested. Only those animals who still reacted to that substance are included. Control animals, who had also received 2 Freund's complete adjuvant injections on day 4, are also tested.

Criteria for sensitization are described in chapter 3.2. In advance of all experimental procedures the maximal non-irritant concentration for open epicutaneous application was estimated on 4 untreated guinea pigs, not participating in the induction procedure. This was done both with petrolatum and with Aramek as a vehiculum. With petrolatum the maximum elicitation concentration was limited to that concentration which could be spread easily with good adherence to the guinea pig skin.

3.3.2. *Results*

No irritant reactions were observed to any of the substances in the tested concentrations. In addition to the highest concentration, a concentration 3 times and 10 times lower was tested in the challenges. The 2 higher concentrations in petrolatum and the highest concentration in Aramek were applied in the challenges. Only the results of the guinea pigs to the highest concentrations used for challenges are shown in Tables 3 and 4.

In the Freund's Complete Adjuvant Test (Table 3) with an induction concentration of 0.5 M, acrylamide and N-hydroxymethyl acrylamide appeared to be moderate sensitizers, according to the classification of Magnusson & Kligman (85). The incidence of sensitizations on day 35 was used for this grading.

TABLE 3: Results of the Freund's Complete Adjuvant Test

Substance	Number of animals	Induction concentration	Challenge concentration*	Number of sensitized animals day 21	day 35
Acrylamide	8	0.5 M (=3.5%)	0.3 M	4/8	3/8
controls	4		0.3 M	0/4	0/4
Methacrylamide	8	0.5 M (=4.2%)	0.3 M	0/8	0/8
controls	4		0.3 M	0/4	0/4
N-hydroxymethyl acrylamide	8	0.5 M (=5.0%)	0.1 M	1/8	4/8
controls	4		0.1 M	0/4	0/4

*Open epicutaneous applications in Aramek.

Methacrylamide in the same induction concentration did not sensitize any of the tested animals.

With one exception no cross-reactions were observed in the sensitized animals to the substances listed in Table 5. One animal, sensitized to N-hydroxymethyl acrylamide showed a cross-reaction to acrylamide.

In the Split Adjuvant Test (Table 4) acrylamide, N-hydroxymethyl acrylamide and N,N'-methylene bis acrylamide were moderate sensitizers according to the same above-mentioned classifications (85). The incidence of sensitizations on day 35 was used for this grading. For all 3 substances no positive reactions were observed in the challenge on day 21. With N-hydroxymethyl acrylamide a somewhat higher incidence of sensitizations was observed with Aramek as a vehiculum in the challenge. The substances methacrylamide, N-dodecyl maleamic acid appeared to be weak sensitizers with this test method, while N-*t*-butyl acrylamide did not sensitize a single animal.

Only one animal showed cross-reactions to the substances, named in Table 5. This guinea pig, sensitized to N,N'-methylene bis acrylamide cross-reacted to maleamic acid, acrylamide and N-hydroxymethyl acrylamide. The animals sensitized to one of the two alkyl maleamic acids were tested for cross-reactions with the substances listed in Table 6 in their respective concentrations in petrolatum. No cross-reactions to these substances were observed.

TABLE 4: Results of the Split Adjuvant Test.

Substance	Number of animals	Induction[1] concentration	Challenge concentration	Number of sensitized animals day 21	day 35	day 49
Acrylamide	10	3 M (=21%)	3 M[1]	0/10	5/10[2]	5/10
			0.3 M[3]	NT	NT	5/10
controls	6		3 M[1]	0/6	0/6[2]	0/6
			0.3 M[3]	NT	NT	0/6
Methacrylamide	10	3 M (=25%)	3 M[1]	0/10	1/10	1/10
			0.3 M[3]	NT	NT	1/10
controls	6		3 M[1]	0/6	0/6	0/6
			0.3 M[3]	NT	NT	0/6
N-hydroxymethyl	9	2.5 M (=25%)	2.5 M[1]	0/9	3/9	2/9
acrylamide			0.1 M[3]	NT	NT	4/9
controls	6		2.5 M[1]	0/6	0/6	0/6
			0.1 M[3]	NT	NT	0/6
N,N'-methylene	10	2 M (=31%)	2 M[1]	0/10	4/10	4/10
bis acrylamide			0.3 M[4]	NT	NT	2/10
controls	6		2 M[1]	0/6	0/6	0/6
			0.3 M[4]	NT	NT	0/6
N-*t*-butyl	10	2 M (=25%)	2 M[1]	0/10	0/10	0/10
acrylamide			0.3 M[3]	NT	NT	0/10
controls	6		2 M[1]	0/6	0/6	0/6
			0.3 M[3]	NT	NT	0/6
N-*t*-butyl maleamic	8	2.5 M (=43%)	2.5 M[1]	1/8	1/8	1/8
acid			0.1 M[3]	NT	NT	2/8
controls	4		2.5 M[1]	0/4	0/4	0/4
			0.1 M[3]	NT	NT	0/4
N-dodecyl maleamic	10	1 M (=28%)	1 M[1]	0/10	2/10	1/10
acid						
controls	6		1 M[1]	0/6	0/6	0/6

[1] = in petrolatum; [2] = closed challenge; [3] = in Aramek; [4] = in ethanol/water (80/20 v/v).

TABLE 5: Structure formulas of the test substances used to determine the cross-reactivity of animals, sensitized to any of the acrylamides.

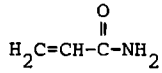
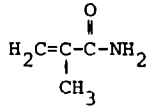
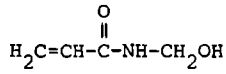
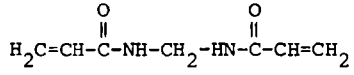
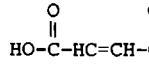
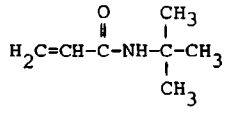
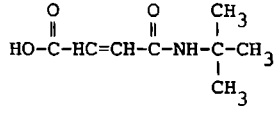
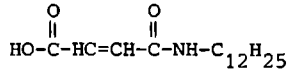
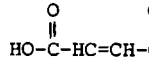
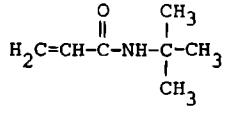
Acrylamide	:	
Methacrylamide	:	
N-hydroxymethyl acrylamide	:	
N,N'-methylene bis acrylamide	:	
Maleamic acid	:	
N-*t*-butyl acrylamide	:	

TABLE 6: Structure formulas of the test substances used to determine the cross-reactivity of animals, sensitized to any of the alkyl maleamic acids.

N-*t*-butyl maleamic acid	:	
N-dodecyl maleamic acid	:	
Maleamic acid	:	
N-*t*-butyl acrylamide	:	

3.3.3. *Discussion*

In a pilot study in our laboratory we did not observe allergic reactions in guinea pigs, induced with acrylamide, N-*t*-butyl acrylamide or *t*-butyl maleamic acid. In this pilot study with 8 experimental and 4 control animals for each substance, we used the Freund's Complete Adjuvant Test with an induction concentration of 0.1% (w/v) for all 3 substances. The poor solubility of especially N-*t*-butyl maleamic acid in both water and paraffin oil forced us to use this low induction concentration. To obtain a higher yield of sensitizations we decided to apply the more time-consuming Split Adjuvant Test, in which higher induction concentrations could be used. For comparison purposes the water-soluble test substances, *i.e.* acrylamide, methacrylamide and N-hydroxymethyl acrylamide, were also investigated in the Freund's Complete Adjuvant Test using a equimolar induction concentration. We did not use the same induction concentration on a molar basis for all substances in the Split Adjuvant Test. Instead we used the highest concentration in petrolatum which could be spread easily with good adherence to the guinea pig skin. This might be a draw-back in comparing the sensitizing capacities of the tested chemicals. However, we feel that in case of induction by epicutaneous application of the allergen the influence of the vehicle on the actual dose, *i.e.* the dose penetrating through the skin, is very great. With higher concentrations than applied by us, the properties of the ointment change drastically. So, using the same high concentration for all substances would create a false sense of mutual comparability in our opinion, while lowering all concentrations for all substances would unduly diminish the yield of sensitizations of the test for those, sufficiently stable in the higher concentrations.

In our experiments a good correlation was found between the results obtained in the Freund's Complete Adjuvant Test and those in the Split Adjuvant Test. In one article (45) the Split Adjuvant Test was reported to be only slightly inferior to the Guinea Pig Maximization Test and better than the other 9 investigated animal tests. Tests in which the application of Freund's Complete Adjuvant is part of the induction procedure, appear to be superior to the other, non-adjuvant tests (101).

The challenges on day 35 and day 49 frequently resulted in higher numbers of sensitized animals than the challenge on day 21. This is suggestive for a function of the challenge on day 21 in inducing sensitization by both methods used.

In the comparison between the 2 published reports on human sensitizations

to pressure-sensitive adhesives (73, 66) some discrepancies are noted. Jordan (73) stated that N-*t*-butyl maleamic acid was present in the Curad adhesive, whereas Heskel *et al.* (66) contended that only alkyl maleamic acid was present, in which the alkyl group ranged from 8 to 16 carbon atoms. A patent, assigned to the producer of Curad, describes the application of N-*t*-butyl maleamic acid as a copolymer in the production of a porous pressure-sensitive adhesive (111). However, changing the chemical composition of a product without changing the name of that product is not uncommon in industry. This may be an explanation for the different statements regarding the chemical composition of Curad adhesive in the 2 reports. Unfortunately Heskel *et al.* (66) did not test their 2 patients with N-*t*-butyl maleamic acid.

The cross-reaction pattern of the patients described in these 2 reports (73, 66) cannot be simply compared with each other since the tested substances, the vehicles and the concentrations were not the same. Jordan (73) did not observe cross-reactions to N-*t*-butyl acrylamide (5% in petrolatum), but only to several other acrylate esters. No other acrylamides were tested by him. 2-EHA (4.8% in petrolatum) gave negative results in the 2 patients of Heskel *et al.* (66). They (66) observed cross-reactions to N-octadecyl maleamic acid (1%) and to octyl maleic acid ester (1%) with a remarkable vehicle specificity: the former substance elicited positive reactions in water/isopropanol (50/50) but not in petrolatum, while the reverse was observed for the latter substance. Such a vehicle specificity was also noted in an experimental study of Björkner and Niklasson (16). They observed more allergic reactions to acrylate monomers when the guinea pigs were challenged with the monomer in petrolatum as a vehicle than when challenged with acetone as a vehicle. By means of HPLC-analysis of these solutions they showed that this phenomenon was due to a rapid polymerization of the monomer in acetone, resulting in false-negative responses because of too low an actual concentration of the monomer.

In our animal experiments both N-*t*-butyl maleamic acid and N-dodecyl maleamic acid were weak sensitizers in the Split Adjuvant test. A cross-reactivity could not be detected, but only 2 animals were available for the determination of the cross-reactivity. On the basis of our results no preference can be given for one of these alkyl maleamic acids with respect to their sensitizing potential in animals. Of the acrylamides 3 substances appeared to be moderate sensitizers, *viz.* acrylamide, N-hydroxymethyl acrylamide and N,N'-methylene bis acrylamide. Human sensitizations to these substances are reported: Fregert and Dahlquist (54) described a worker in the plastic

industry, who appeared to be sensitized to N-hydroxymethyl acrylamide. Malten *et al.* (91) described 2 patients sensitized after handling Nyloprint printing plates. Both patients showed positive reactions when patch-tested with N,N'-methylene bis acrylamide (5% in ethanol), and one patient also reacted to N-hydroxymethyl acrylamide (1% in water) and to acrylamide itself (5% in water). Pedersen *et al.* (121) noted that his 7 patients, sensitized to Nyloprint, were all positive to N,N'-methylene bis acrylamide, 6 also to N-hydroxymethyl acrylamide and 2 to acrylamide. Since they could not detect the latter 2 substances in the Nyloprint plates, they suggested that cross-allergy was responsible for the positive results to these substances. In our animal experiments we only observed cross-reactions to other acrylamides in 2 out of 18 animals sensitized to one of the acrylamides.

The absence of reports of clinical sensitizations to methacrylamide or N-*t*-butyl acrylamide correlates well with our findings in the guinea pigs.

The well-known neurotoxicity of acrylamide (117) remains the prime reason for keeping the concentration of this monomer in endproducts as low as possible. However, if a person is sensitized to acrylamide even a dose of monomeric acrylamide substantially below the minimum dose to elicit neurotoxic symptoms, might be high enough to provoke an allergic contact dermatitis. At present the use of acrylamides in pressure-sensitive adhesives does not seem to be associated with great allergological risks. Our results suggest that if dermatological problems occur, replacement by methacrylamide or N-*t*-butyl acrylamide might be considered. When 2-EHA would cause sensitizations to pressure-sensitive adhesives, replacement by pentyl methacrylate might be a solution. Pentyl methacrylate appeared to be a non-sensitizer in guinea pigs (156).

3.4. α,β-*DIACRYLOXYPROPIONIC ACID, A SENSITIZING IMPURITY IN COMMERCIAL ACRYLIC ACID*

3.4.1. *Introduction*

The presence of carboxyl groups (-C(=O)-OH) in adhesives and dental filling materials improves adhesion to substances such as metals, nylon, enamel and dentin (96, 115). Therefore acrylic acid is frequently used as a copolymerizing monomer in the production of pressure-sensitive adhesives; other uses of acrylic acid are described in chapter 1.2.2. Van der Walle *et al.* (156) obtained a strong allergic response in guinea pigs to a commercial

sample of acrylic acid. No reactions were observed to a distilled sample of acrylic acid. This pointed to a strongly sensitizing impurity in this commercial grade acrylic acid. Should this impure acrylic acid be further used to produce a variety of endproducts, these endproducts might still contain this sensitizing substance. Especially when used in adhesives meant for application on the human skin, the risk of sensitization to this impurity would be considerable.

Therefore we decided to try to isolate and identify this sensitizing substance by a combination of chemical analysis (Drs. E. Seutter) and a bio-assay with guinea pigs. The latter bio-assay also gave us the opportunity to look for possible cross-reactions of the sensitized animals to related compounds.

3.4.2. *Materials and methods*

chemicals

Samples of acrylic acid were obtained from various sources: Merck Schuchardt (FRG), Aldrich (Belgium) and BDH (England). After a complaint on the impurity of the sample obtained from Merck Schuchardt we received from that supplier a second sample with a gas chromatogram, illustrating its purity (better than 99%). Diazald (N-methyl-N-nitroso-*p*-toluenesulfonamide, precursor to diazomethane), ε-caprolactam, 4-methoxyphenol, methacrylic acid, 2,2-dimethoxypropane and glyceric acid were obtained from Aldrich, while 4-toluenesulfonic acid, diethylether, methanol and inorganic chemicals were analytical grade Merck products. Hexyl acrylate and 1,2-ethanediol diacrylate were obtained from Polyscience Inc. (USA). Freund's Complete Adjuvant was from Difco Lab. (USA).

chemical preparations

Methylation was performed either by diazomethane in ether or by 2,2-dimethoxypropane in methanol with a catalytic amount of 4-toluenesulfonic acid, followed by evaporation *in vacuo*. Hydrolysis of the esters was performed by heating one hour with excess of NaOH in water, acidifying with HCl and evaporating *in vacuo*. 5-Hexenoic acid was prepared from ε-caprolactam according to Linstead and Rydon (82).

gas chromatography

Analytical gas chromatography (g.c.) was performed with a Hewlett Packard 402 gas chromatograph with integrator 3373 B flame-ionization detector and nitrogen as a carrier (2,5 $cm^3.min^{-1}$) with 6' columns containing 5% silicone OV 101 or 5% silicone OV 17 on Gaschrom Q. Preparative gas chromatography was performed with a Hewlett Packard 700 instrument with thermal conductivity detector and helium as a carrier (30 $cm^3.min^{-1}$) on 6' columns containing 5% silicone OV 101 on Gaschrom Q. The Standard program was 70-300°C at 7.5°C min^{-1}, injection port 80°C, detector 325°C. Fractions from preparative gas chromatography were collected in cooled glass tubes.

spectra

Mass spectra were obtained with a LKB gas chromatograph mass spectrometer 9000 at 150°C ion source temperature and 20 eV (ion source pressure $1.5 \cdot 10^{-4}$ Pa) or with a Varian SM Ib at 130°C ion source temperature and 70 eV.

animals

Female guinea pigs of the Hartley outbred strain (Broekman Institute, The Netherlands), weighing approximately 400 g at the beginning of the study, were used for the sensitization experiments. They were housed in pairs in steel cages with free access to water and pellet food (Hope Farms, The Netherlands).

sensitization procedures

The modified Freund's Complete Adjuvant Test with identical challenge procedures and identical criteria for sensitization was used as described in chapter 3.2.1. A maximal non-irritant concentration of the test substances in Aramek was estimated as described in chapter 3.3.1. This maximal non-irritant concentration and 2 lower concentrations were used in the challenges. Groups of 8 animals each were induced with one of the following substances: the first impure Merck Schuchardt sample of acrylic acid, 5-hexenoic acid (confer results) from our own synthesis and α,β-diacryloxypropionic acid (DAPA), obtained by distillation *in vacuo* of the first Merck Schuchardt acrylic acid sample. These test substances were mixed each with distilled water to a concentration of 0.34 M and to this solution an equal volume of Freund's Complete Adjuvant was added and mixed well. Because of a severe ulceration

at the site of the first injection with DAPA we were forced to change the induction procedure for the animals of this group. After intradermal injection at day 0 these animals received on day 7, also in the nuchal area, a 0.1 ml intradermal injection of Freund's Complete Adjuvant without test substance, together with a single open epicutaneous application of 0.025 ml of a 0.3 M solution of DAPA on the site of this latter injection. No injections were given in animals of this group on day 5 or 9.

The inhibitor 4-methoxyphenol, which had been added to all acryl containing substances under investigation to prevent polymerization was tested in all animals on day 49 in a 1 M concentration. Cross-reactions to other acryl containing compounds were investigated on day 63 in the sensitized animals with the same method as used in the challenges.

3.4.3. *Results*

In the first Merck Schuchardt sample analytical gas chromatography on the OV 101 phase of samples of acrylic acid showed a large fraction in addition to the acrylic acid fraction, with a peak area ratio of 2:3 and a number of smaller peaks. In the Aldrich and BDH products no deviations from the purity specified by the manufacturer (99%) were observed.

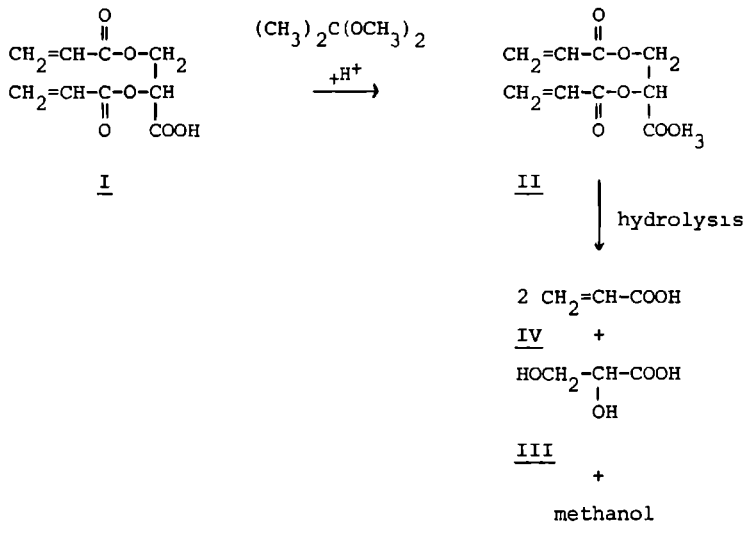
Preparative gas chromatography of the impure Merck Schuchardt product gave the same main fractions. Using this technique it was evident that part of the substance introduced on the column decomposed and part of it was retained and could only be removed by long heating. Of the smaller fractions, obtained by preparative gas chromatography, the largest one, after methylation, could be identified by gas chromatography mass spectrometry as hexenoic acid, presumably 5-hexenoic acid.

Upon vacuum distillation of the large unidentified fraction a distillate was obtained, boiling both at 145°C-1.6 kPa and at 88-89°C-0.13 kPa, which always partly decomposed during distillation into acrylic acid. When the latter distillate was methylated, we obtained a stable ester. With vacuum distillation of this ester we got a distillate, boiling at 95-96.5°C-1.6 kPa, that in analytical g.c. was stable on the OV 101 phase and better than 99.5% pure, but which was unstable on the OV 17 phase. Its mass spectrum showed a small parent mass at m/e 228.

Upon complete hydrolysis of this ester acrylic acid could be removed and a syrup remained, that, after methylation, showed the same decomposition pattern in the g.c. as methylated glyceric acid. Hence, the unknown compound

in this commercial acrylic acid sample was glyceric acid, esterified with 2 molecules of acrylic acid. The reaction scheme we followed is shown in Scheme 1.

Scheme 1



Reaction scheme in the identification of α,β-diacryloxypropionic acid (DAPA). I = α,β-diacryloxypropionic acid; II = methyl diacryloxypropionate; III = glyceric acid; IV = acrylic acid.

The second sample of Merck Schuchardt acrylic acid, given to us with a gas chromatogram suggesting this sample was better than 99% pure, was subjected to vacuum evaporation and subsequent methylation of the residue. The amount of DAPA in this acrylic acid sample proved to be 7%. The BDH and Aldrich samples of acrylic acid, when treated in the same way, did not give any specific secondary product.

The results of the guinea pig experiments are summarized in Table 7 and show that both the Merck Schuchardt acrylic acid and DAPA, isolated from it, are strong sensitizers in the guinea pig. 5-Hexenoic acid was not a sensitizer. When using the same solvent (Aramek) and the same application method (open), the irritant potential of DAPA on the guinea pig skin was approximately equal to that of diacrylate esters, which are known irritants to the human skin (92). All animals, sensitized to the acrylic acid sample reacted to the 0.03 M solution of DAPA, but not to a 1 M solution of distilled acrylic acid. All animals, sensitized to DAPA reacted to a 1 M solution of Merck Schuchardt

TABLE 7: Sensitizing potential in the modified Freund's Complete Adjuvant Test.

Test substance	Induction	Challenge concentration*	Number of sensitized animals/ number of tested animals		
			day 21	day 35	day 49
Acrylic acid,	3·0.17 M	1 M	8/8	8/8	NT
from Merck Schuchardt	(=1.2%)	0.3 M	1/8	NT	7/8
α,β-Diacryloxypropionic acid	1·0.17 M**	0.03 M	8/8	NT	NT
	(=3.6%)	0.01 M	7/8	7/8	7/8
5-Hexenoic acid	3·0.17 M	3 M	0/8	0/8	0/8
$H_2C=CH-(CH_2)_3-COOH$	(=1.9%)	1 M	0/8	0/8	0/8
Controls	3·FCA/dist. water	all the above compounds	0/8	0/8	0/8

NT = not tested.

* In Aramek, 2 parts methyl ethyl ketone, one part peanut oil by volume.

** Different induction procedure (see Materials and Methods).

TABLE 8: Cross-reactions of sensitized animals to related compounds.

Test substance	Structure formula	Challenge concentration*	Animals sensitized to Merck Schuchardt acrylic acid	Animals sensitized to α,β-diacryloxy-propionic acid
Acrylic acid (distilled)	$H_2C=CH-COOH$	1 M (=7.2%)	0/7	0/7
Hexyl acrylate	$H_2C=CH-\overset{O}{\overset{\|\|}{C}}-O-(CH_2)_5-CH_3$	1 M (=15.6%)	0/7	0/7
Methacrylic acid	$H_2C=\underset{CH_3}{\underset{\|}{C}}-COOH$	1 M (=8.6%)	0/7	0/7
1,2-Ethanediol diacrylate	$H_2C=CH-\overset{O}{\overset{\|\|}{C}}-O-CH_2$ $H_2C=CH-\overset{O}{\overset{\|\|}{C}}-O-CH_2$	0.03 M (=0.5%)	0/7	0/7
Methyldiacryloxypropionate	$H_2C=CH-\overset{O}{\overset{\|\|}{C}}-O-CH_2$ $H_2C=CH-\overset{O}{\overset{\|\|}{C}}-O-\underset{COOCH_3}{\underset{\|}{CH}}$	0.03 M (=0.5%)	5/7	4/7

* In Aramek (2 parts methyl ethyl ketone, 1 part peanut oil by volume).

acrylic acid, but not to a 1 M solution of distilled acrylic acid or acrylic acid obtained from Aldrich or BDH. No reactions were observed to 4-methoxyphenol, except in one animal induced with impure acrylic acid. Results of the tests on cross-reactions are represented in Table 8: none of the sensitized animals reacted to hexyl acrylate, methacrylic acid or 1,2-ethanediol diacrylate. The methyl ester of the sensitizer gave positive reactions in the majority of animals sensitized to the parent α,β-diacryloxypropionic acid.

3.4.4. *Discussion*

The remarkable difference in the purity of acrylic acid, as specified by Merck Schuchardt and the purity observed by us may be ascribed to the use of different phases in analytical gas chromatography. In quality control by analytical g.c. mostly a phase is chosen which is good at separating the substance under investigation from its homologues or similar compounds. However, in the case of acrylic acid such a phase is not capable of detecting DAPA, due to retention and decomposition of the latter compound on the column. On our own columns retention and decomposition only occurred partially, so we could detect DAPA, although we could not make a reliable estimation of the purity of the acrylic acid samples by g.c.

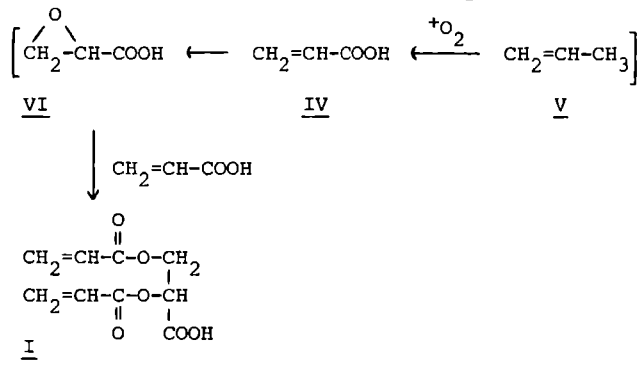
The presence of hexenoic acid may indicate the reaction from which the DAPA originates. A patent (12) describes the manufacture of 5-hexenoic acid from acrylic acid, propene, water, benzene and hydroquinone at 200°C. This suggests the technical synthesis of acrylic acid by catalytic oxidation of propene, a patented way of producing acrylic acid (115). A possible side-product of this oxidation is 2,3-epoxypropionic acid, that will react with the acrylic acid present in excess (Scheme 2).

The results of the guinea pig experiments demonstrate that DAPA is the cause of the allergic response to commercial grade acrylic acid manufactured by Schuchardt. It is rather unusual to discover an impurity in a substance by testing this substance in an animal sensitization test. However, this illustrates the detection sensitivity of the Freund's Complete Adjuvant Test. The observed scant cross-reaction pattern is in accordance with published results (157). We may draw 2 conclusions from this study:

1. Quality control by gas chromatography is not always a guarantee that a product does not contain considerable amounts of by-product(s).
2. Acrylic acid, in itself not allergenic, may contain a strongly sensitizing impurity, which can be removed by distillation. Unfortunately, not all

manufacturers perform this simple action. Purity of the product is important when acrylic acid is used as a copolymer in the production of acryl-based adhesives, lacquers and dental filling materials (96).

Scheme 2



Supposed origin of α,β-diacryloxypropionic acid in the production of acrylic acid. I and IV see Scheme 1; V = propene; VI = 2,3-epoxypropionic acid.

CHAPTER 4

PERMEABILITY OF SURGEONS' GLOVES TO METHYL METHACRYLATE, BUTYL ACRYLATE AND ACRYLAMIDE

4.1. *INTRODUCTION*

For the prevention of occupational skin disorders a choice can frequently be made out of an arsenal of possibilities (87). It is widely recognized that technical preventive measures (*e.g.* production in closed systems, mechanical ventilation etc.) are preferable to personal preventive measures (*e.g.* the wearing of masks, or gloves, application of barrier creams). Technical preventive measures can be better controlled and are less dependent on the cooperation of the workers involved. Technical prevention starts with the choice of the right material. Therefore newly introduced materials should be tested adequately for their toxicological behaviour in several biological test systems before they come into use. Examples of such investigations on acrylic monomers are the theses of Van der Walle (158) and Björkner (15).

Based on the results of such investigations technical measures can be taken and processing instructions can be given for every situation in which the new material is used. Unfortunately technical preventive measures cannot always be taken, sometimes because costs being too high, sometimes because of interference with the production process. In such cases personal preventive measures become necessary and inevitable.

The wearing of gloves is a personal preventive measure, which is meant to protect the skin against contact and subsequent absorption of noxious substances. This simple method is only adequate for short-term use, since the wearing of protective gloves during longer periods of the day is both impracticable and undesirable. Moreover the gloves should be clean, not damaged and they should be impermeable to the noxious substance. When the gloves do not come up to the latter requirement, a potentially dangerous situation exists. Then, the gloves will serve as an occlusive dressing and the absorption of the substance, penetrated through the gloves, will be enhanced, instead of prevented. The penetration of the given substance through the gloves also involves the risk that constituents of the gloves will be liberated with greater ease and will come into contact with the skin in greater amounts. In addition, the use of gloves may give a false sense of safety to the wearer and, possibly, a less clean way of working.

Therefore it is very important to test gloves for permeability to the substances against which they should offer protection. Several allergens are known to be able to penetrate through gloves (108). When an occupational exposure to an allergen occurs, adequate protection is even more urgently needed.

In the operating theatre the wearing of gloves, often during several hours, is unavoidable for reasons of asepsis. Only rubber gloves are employed usually. This means that if surgical personnel is exposed to a substance penetrating their gloves, these people run the risk of sensitization both to the penetrating substance and to the rubber auxiliary chemicals which are present in every rubber glove. Methyl methacrylate is used in orthopaedic surgery as a bone cement, particularly for the fixation of hip and knee endoprostheses. Contact sensitizations to methyl methacrylate used in the operating theatre have been described, even sensory neuritis, affecting those parts of the hands, most exposed to the acrylic cement. Recovery from the sensory neuritis takes several months (50) and an allergic contact sensitivity may stay on for years, if not for lifetime.

It is possible to distinguish several consecutive phases in the development of an allergic contact dermatitis, *viz.* a) induction, b) clinical manifestation, c) clinically silent sensitization with recurrences after unexpected exposure. Factors which play an important role in induction are: (physico) chemical and immunochemical properties of the contactant, concentration, vehicle, route of administration, duration, extension and the repetition of exposure, quality, health and protection of the exposed skin, proneness of the exposed organism to respond to stimulation with the formation of T-effector and T-repressor cells. In view of these facts, we may all be regarded as being on our way to become (contact) sensitized to one substance or another. Which substance will be "our" contact sensitizer depends on our pattern of life, resulting in a number of inevitable exposures. Whether such exposure has grave consequences depends on the possibility of replacing "our" contact allergen.

For an orthopaedic surgeon this means that he is on his way to develop contact sensitization to methyl methacrylate or other sensitizing components in the bone cement he uses, to rubber additives, etc. Nobody is able to detect how far he has already gone in the direction of sensitization, and how far he still has to go before sensitization becomes clinically apparent. The only way in which he may delay this disaster is by using a concentration of the

contact allergen as low as possible, to shorten each possible exposure, to protect the skin and keep it in good health.

The incidence of contact sensitizations among orthopaedic surgeons at present is not of great importance, but the expected incidence is to be feared. Due to the phenomenon of group-specificity it is to be expected that an individual, sensitized to methyl methacrylate will also react to quite a number of different acrylics (52), so that no allergen replacement is possible, and the surgeon must stop cementing endoprostheses. In addition, he will run a risk of recurrences of his dermatitis as a result of exposure to acrylics used for various purposes in everyday life (158).

The incidence of contact sensitization to rubber chemicals is so high in the general population that some 16 of them have been incorporated in the screening series of contact allergens of the International Contact Dermatitis Research Group (89). The frequency with which allergen replacement is demanded by surgeons indicates that contact sensitizations to these auxiliary substances are frequent among them. In addition it can be rather serious if replacement has become really impossible in certain cases, because the store of alternatives has been exhausted due to the development of contact sensitization to all rubber additives which are commonly used in the production of surgical gloves. To ease the urge for allergen replacement in cases of "glove" sensitization, it was Hjorth (67) who incited production of so-called "Elastyren" gloves, containing an auxiliary substance of low sensitizing capacity.

It has been demonstrated that methyl methacrylate easily penetrates the usual rubber gloves, whereas in thick vinyl gloves permeation of methyl methacrylate could not be detected (122). However, the use of thick gloves is inconvenient for surgeons. To quantify these findings, we designed a test system in which we examined the permeability to methyl methacrylate of a collection of surgeons' gloves from our hospital, and of some gloves made of alternative materials. In addition, the same test method was applied in preliminary studies on the penetration of butyl acrylate and acrylamide through some gloves. The latter 2 acrylic monomers were selected because of their industrial importance.

chemicals

Methyl methacrylate, butyl acrylate (Merck-Schuchardt), ethanol, methanol (Merck p.A.) and butanone-2 (B.D.H.) contained no impurities interfering with the analyses. Acrylamide (Aldrich) was recrystallized 3 times from methanol. Neopentyl alcohol (Aldrich), *n*-pentanol (B.D.H.) and octanol-2 (Merck) were fractionated before use.

samples

We collected 7 surgeons' gloves (nos. 1-7), 3 examination gloves (nos. 8-10) and a household glove (no. 11) (Table 1). To check the resistance of the glove materials to undiluted methyl methacrylate, samples, taken from the fingers of the gloves were soaked in undiluted methyl methacrylate for 1 h. Samples for permeation and density measurements were taken from the wrists of the gloves. In advance of the measurements, powder was removed by rinsing with ethanol and subsequent drying at ambient temperature.

diffusion chamber

The chamber consisted of two square blocks of glass in which cylindrical apertures had been made, closed on one side with glass. Holes drilled in the blocks made it possible to screw them together, using screws furnished with rubber O-rings. Samples were placed vertically between flat rubber rings (from which soluble material had been removed by soaking in butanone-2 for several days and drying), fitting to the flat areas surrounding the cylindrical apertures. Both compartments of the chamber could be reached through glass-stoppered connections to holes drilled in the walls of the cylinders. The compartments could contain a volume of 5.75 ml; the available glove sample area was 3.14 cm^2. The contents of the compartments were stirred magnetically. The chamber was placed in a double-walled beaker. Water was pumped from a thermostat through the space in the wall. The beaker was covered with a sheet of foam plastic with holes for the glass-stoppered connections, and a controlling thermometer to regulate the temperature in the beaker. Experiments were performed at 21.0±0.5°C, and 35.0±0.5°C. Liquids were heated to these temperatures before introduction.

analyses

For the experiments with methyl methacrylate analyses were performed with a Hewlett Packard 402 gas chromatograph with integrator 3373B, flame ionization detector and nitrogen carrier (2.5 $cm^3.min^{-1}$) on a 6' glass column containing 4.7% silicone OV 210 on Gaschrom Q. In an isotherm programme temperatures were: injection port 40°C, oven 43°C, detector 59°C. Mean retention times were: ethanol 50 s, neopentyl alcohol 125 s, methyl methacrylate 200 s, *n*-pentanol 285 s. In the solutions neopentyl alcohol served as an internal standard to correct pipetting errors; *n*-pentanol had been added to prevent tailing of the methyl methacrylate peak.

Of standard solutions of methyl methacrylate in ethanol, containing 22.7 mM neopentyl alcohol and 22.7 mM *n*-pentanol, 1 µl samples were analysed. The errors were: 1 mM: 10%,2-5 mM: 3.5%, 6-150 mM: 1%, 160-600 mM: 4%. A calibration curve was obtained that differed slightly from linear. In the diffusion chamber one compartment was filled with a blank solution: ethanol, containing 22.7 mM neopentyl alcohol, and 22.7 mM *n*-pentanol. The other was filled with 4.7 M (50% w/v) methyl methacrylate in ethanol with the same additions. At the moment of filling the latter compartment, a stopwatch and magnetic stirring were started. Eight to twelve 1 µl samples from the former compartment were analysed as a function of time within 90 min. Then the column was cleaned by heating to 250°C.

Each experiment was performed in duplicate. In addition three glove materials (nos. 2, 3, 5) were tested in double layers.

From gloves nos. 1, 2, 3, 4, 5 and 7 one used sample was rinsed with ethanol and dried. With these glove samples the analyses were repeated once. In glove no. 5 experiments were performed with material from both finger and wrist. For butyl acrylate the gas chromatograph analysis took place with a column identical to the one for methyl methacrylate but with the following isotherm programme temperatures: injection port 69°C, oven 70°C, detector 230°C.

For the analysis of acrylamide the glass column contained 5% silicone OV 101 on gaschrom Q and the isotherm programme temperatures were: injection port 90°C, oven 90°C and detector 240°C.

Both butyl acrylate and acrylamide were tested in the solvent methanol. Here *n*-pentanol served as an internal standard, while octanol-2 was added to prevent tailing of the butyl acrylate or acrylamide peak. Standard solutions of butyl acrylate and of acrylamide in methanol containing 20.4 mM *n*-pentanol and 7.7 mM octanol-2 were prepared. For the butyl acrylate solutions the error

was never above 2% in the range of 5-600 mM. The calibration curve differed slightly from linear in the concentration range above 400 mM. As for acrylamide, this could not be detected below a concentration of 60 mM. Between 60 and 75 mM the error was 6.5%, from 75 to 700 mM the error was 3%. The calibration curve was linear.

The analyses of butyl acrylate and acrylamide were performed once. One compartment of the diffusion chamber was filled with the blank solution: methanol, containing 20.4 mM *n*-pentanol and 7.7 mM octanol-2. The other chamber was filled with 3.1 M (40% w/v) butyl acrylate respectively 4.2 M (30% w/v) acrylamide in methanol containing the same additions. For butyl acrylate samples were taken every 10 minutes, while for acrylamide the intervals at which samples were taken varied from 6 to 30 hrs.

density and thickness

At the sampling site a square area (23x23 mm) was stamped and cut out from each glove. The material was weighed, and the density determined with a pycnometer. From the data obtained the mean thickness of the material was calculated. The error in the latter was 3%.

water permeability

We adapted our standard measurements of the water vapour loss from the skin (150) to estimate the water permeability of some of the materials before and after exposure to methyl methacrylate in the diffusion chamber. A measuring cup (area 1.875 cm) was pressed against the underside of the glove membrane. It was perfused with dry nitrogen as a carrier to take the penetrating water vapour to a modified Meeco moisture analyser. On top of the membrane an open glass cylinder was pressed, containing a 1.5 cm layer of water. The data were recorded after equilibrium was attained.

4.3. *RESULTS*

Undiluted methyl methacrylate dissolved the glove sample made of polystyrene-butadiene, while all six latex glove samples and the polychlorobutadiene sample showed expansion in the liquid, which reversed after removal from the methyl methacrylate. Yellow coloured material was released into the liquid methyl methacrylate from all latex and polychlorobutadiene glove samples. After drying, non-dyed samples were visibly bleached. Except for

Seamless Brown Milled Regular, the remaining five latex samples showed a loss of stickiness. The two vinyl glove samples firstly appeared to swell in the undiluted methyl methacrylate, but after 1 hr in the liquid they had shrunk. Obviously, material of these samples was left in the liquid, since the methyl methacrylate liquid foamed afterwards. Material from the polyethylene glove did not show any visible change; nor did the liquid in which it was soaked.

The diffusion of the 4.7 M solution methyl methacrylate appeared to be a linear function of time for all glove materials except vinyl. In the latter samples (nos. 9 and 10) a first rapid diffusion slowed down to 25% of its original rate after 90 min of exposure. After treatment with 4.7 M methyl methacrylate in ethanol, this material also appeared shrunken and harder at the end of the experiments. Values for diffusion rates were obtained from the straight lines or, for vinyl, from the first points of measurement. Extrapolation of the lines obtained to zero concentration showed time lags (Table 1), (Figure 1). Reproducibility of the results was good; deviations in duplicate experiments were less than 3%. Permeability at 35°C was substantially higher than that at 21°C; this difference was larger in synthetic polymers. Little permeability difference appeared in the various latex samples (Figure 1).

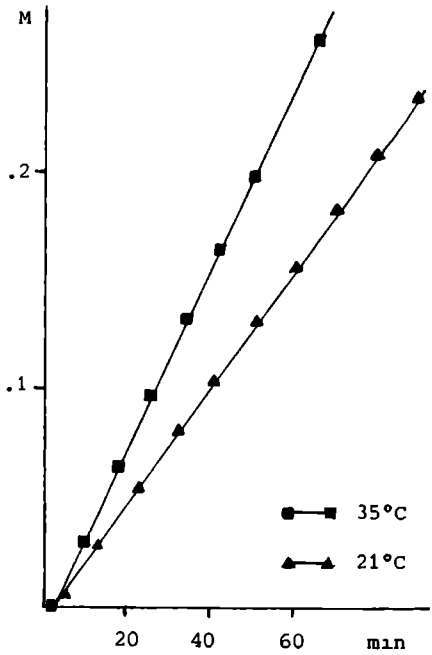


Figure 1: Permeability of surgeons' glove material (Seamless Brown Milled Regular) to methyl methacrylate in a diffusion chamber. Concentration in acceptor compartment is shown as a function of time. Start concentration in donor compartment was 4.7 M ethanol.

TABLE 1: Permeability of glove materials to 4.7 M methyl methacrylate (50% w/v) in ethanol.

Name and constitution of glove material such as designated by the manufacturer	Density $g \cdot cm^{-3}$	Thickness mm	21°C		35°C	
			Time lag min	$\mu mol \cdot min^{-1} \cdot cm^{-2}$	Time lag min	$\mu mol \cdot min^{-1} \cdot cm^{-2}$
1. Perry X-Am Tex* latex	0.939	0.125	2	6.7	1.5	11.3
2. Seamless Sensi Grip White* latex	0.908	0.175	3.5	4.9	1.5	7.9
double layer			12	2.23		
3. Seamless Sensi Grip Brown* latex, brown	0.923	0.178	3.5	4.7	1.5	7.6
double layer					6	4.4
4. Seamless Brown Milled Regular* latex, brown	0.935	0.157	3	4.9	2.5	7.3
5. Travenol Triflex* latex	0.938	0.175	3.5	4.7	2	7.3
double layer					6.5	3.4
6. Elastyren* polystyrene-butadiene	0.952	0.156	1.5	8.2	1	18.3
7. Pioneer Rollpruf* polychlorobutadiene, green	1.26	0.145	4	5.1	2	9.5
8. Gyno-Daktarin Dispos-a-glove polyethylene copolymer	0.935	0.026	1.75	1.74	1	6.1
9. Parke-Davis Ready-Wrap vinyl, blue	1.16	0.111	<1	12.2	not determined	

TABLE 1 (continued)

Name and constitution of glove material such as designated by the manufacturer	Density $g \cdot cm^{-3}$	Thickness mm	21°C		35°C	
			Time lag min	$\mu mol \cdot min^{-1} \cdot cm^{-2}$	Time lag min	$\mu mol \cdot min^{-1} \cdot cm^{-2}$
10. Tru-Touch vinyl	1.17	0.155	1.5	10.1	not determined	
11. Marigold household latex, red	not determined		4.5	3.5	not determined	

*Sterilized surgeons' glove.

The household glove (no. 11) had a rough surface, and measurement of its thickness would therefore not have been meaningful. Although this glove was made of considerably thicker latex than the surgeons' gloves, its permeability to methyl methacrylate was not much lower. The polystyrene-butadiene glove was ruptured during experiments in three out of four samples. In one experiment, performed with a sample from a finger of Travenol Triflex, an increase in thickness by 15% and a decrease in the diffusion rate by 25% appeared, as compared with the sample from the wrist.

Although repetition of the diffusion experiments with the same glove samples gave exactly the same results, much more sensitive measurements of the water permeability of the gloves usually revealed a decrease after exposure to methyl methacrylate in ethanol, except in two brands (Table 2): Seamless Sensi Grip White and Travenol Triflex.

TABLE 2: Permeability of surgeon's glove materials to water, before and after exposure to 4.7 M methyl methacrylate in ethanol, in $\mu g \cdot min^{-1} \cdot cm^{-2}$.

Glove material	Not exposed	exposed
1. Perry X-Am Tex latex	3.6	3.0
2. Seamless Sensi Grip White latex	1.7	1.8
3. Seamless Sensi Grip Brown latex	1.8	1.2
4. Seamless Brown Milled Regular latex	2.0	1.1
5. Travenol Triflex latex	1.9	1.9
6. Elastyren polystyrene-butadiene	3.2	not determined
7. Pioneer Rollpruf polychlorobutadiene	0.8	0.6

The diffusion of butyl acrylate appeared to be linear for the 2 latex glove materials. For the polystyrenebutadiene and the 2 vinyl gloves the diffusion decreased in time. To estimate the diffusion rate in the latter 3 gloves, the first points of measurement were used. Results are shown in Table 3.

TABLE 3: Diffusion rates of solutions of butyl acrylate and acrylamide in methanol through 5 surgical glove materials, as compared with the diffusion rates of a solution of methyl methacrylate in ethanol.

Nr.	Glove material	Diffusion rate* ($\mu mol \cdot min^{-1} \cdot cm^{-2}$)		
		Methyl methacrylate	butyl acrylate	acrylamide
1.	Perry X-Am Tex latex	6.7	3.9	0.10
2.	Travenol Triflex latex	4.7	2.3	**
6.	Elastyren polystyrene-butadiene	8.2	7.0	**
9.	Parke-Davis Ready-Wrap vinyl	12.2	11.1	0.37
10.	Tru-Touch vinyl	10.1	7.4	**

* Diffusion rate standardized for a concentration of 4.7 M in the donor compartment, assuming Fick's law of diffusion.

**Below limit of detection (0.08 $\mu mol \cdot min^{-1} \cdot cm^{-2}$)

No diffusion of acrylamide through Travenol Triflex, Elastyren and Tru-touch gloves could be detected after 30 hrs. Acrylamide appeared to permeate through the latex Perry X-Am Tex glove and the Parke-Davis glove. The diffusion rate of acrylamide through these 2 gloves was estimated by assuming a linear relationship in the diffusion versus time between 0 and 30 hrs after the start of the experiments. The results are summarized in Table 3.

4.4. *DISCUSSION*

In our diffusion experiments with methyl methacrylate, aimed at quantitative comparison of glove materials, a 4.7 M solution of methyl methacrylate in ethanol had to be used to minimize expansion of the membranes and extraction

of the auxiliary substances, which would have interfered with the analyses. Therefore we were not able to use the same conditions as encountered in the operating theatre, where a solution of poly(methyl methacrylate) in methyl methacrylate is employed. The concentration of methyl methacrylate monomer is declining in the course of the polymerization. This also means that the extrapolation of our results to the clinical situation is attended by uncertainties. However, we saw no way to bypass this problem.

While handling the so-called monomer-polymer adhesive mixture, the orthopaedic surgeon wears on top of his rubber gloves a pair of cotton gloves and on top of these another pair of rubber gloves. After insertion of the endoprosthesis, he removes the top rubber and cotton gloves and then continues operating the patient wearing the remaining gloves. The exact composition of the adhesive mixture is still unknown, but the greater part of the acrylic monomer it supposedly contains is still methyl methacrylate. In addition, there must be (96): a solvent, a starter (an amine or cobalt salt), a radical donor (benzoyl peroxide), and possibly an antibiotic. Benzoyl peroxide and hydroquinone are also known contact sensitizers (81, 155).

The time needed for insertion of the prosthesis is generally much longer than the time lag we found in our experiments, which is related to the time the methyl methacrylate needs to permeate the glove material. After removal of the outer gloves a considerable amount of monomer is left on and in the inner glove, as illustrated by our experiments with double layers. Taking into consideration the skin temperature of the surgeon, the measurements at 35°C are a better approximation of the real working circumstances than those at 21°C.

In contact with the cement, low molecular-weight ingredients of the gloves dissolve in the monomer, especially from expanded parts. Among those are oligomers and auxiliary substances such as rubber additives.

In the manufacturing process the glove, after being formed, stays on the mould with the fingers in downward direction. The final flow of the very viscous material results in thicker latex in the fingers than in the wrist, in accordance with our observation, but also results in thinner parts in the webs between the fingers. When the gloves are worn the contact is occlusive, which stimulates sensitization (85). Some materials showed a decrease in water permeability after exposure to methyl methacrylate. This means that the degree of occlusion increases after contact with the monomer.

Elastyren gloves were developed especially for surgeons who are contact-

allergic to most of the commonly used rubber additives. These gloves contain only one additive, which has a very low allergenic potential (67). In contact with methyl methacrylate, dissolution of this glove occurred in a short time. Pioneer Rollpruf is labelled "for those allergic to natural rubber" by the manufacturer. Natural rubber, however, does not induce sensitization (29). Probably the manufacturer of Pioneer means by this statement that these polychlorobutadiene gloves contain lesser amounts of accelerators and antioxidants than gloves made from natural rubber (53).

Considering the results of our investigation, there is no doubt that, when using these gloves, the surgeon is exposed to methacrylate under occlusion, but probably also to other contact-sensitizing chemicals such as benzoyl peroxide, hydroquinone, cobalt compounds, rubber additives, antibiotics, etc.

Vinyl appeared to be an inferior material, with the highest initial permeability and releasing plasticizer in the monomer. Polyethylene copolymer, although very thin, gave the best protection from methyl methacrylate diffusion. This material, however, is not sufficiently elastic, and is easily perforated by a sharp edge or a rough surface.

We must conclude that, during cementing of prostheses, protection is very unsatisfactory. Modern plastic technology should be able to produce a material that has the characteristics of polyethylene copolymer in the diffusion of methacrylate, but is thicker, mechanically stronger and somewhat more elastic, and does not contain sensitizing agents.

We tried to contact manufacturers of these materials but, even when the concern of occupational physicians in industries where similar problems exist and of dentists is added to ours, large scale demands are improbable, and production is consequently not very interesting today. Therefore other preventive measures should be developed to avoid contact with methyl methacrylate monomer while cementing endoprostheses. Fregert (56) recommended the following:

- packages of the liquid and powder should be of a disposable type
- mixing procedure should take place in a fume hood
- mixing should be done with utensils, without glove contact, until the material is no longer sticky
- the surgeon and other personnel with contact should use at least 2 layers and preferably 3 layers of gloves during handling of the cement, and then immediately remove all gloves
- utensils used for mixing, remnants of bone cement and gloves should be destroyed.

However, in the long run the development of a glove impermeable to methyl methacrylate and other acrylates will be necessary.

The results with the butyl acrylate and acrylamide experiments should be interpreted more qualitatively rather than quantitatively, because of the limited number of measurements and the use of different solvents. The observed differences in permeation rates of methyl methacrylate, butyl acrylate and acrylamide cannot apparently be explained by parameters like molecular weight, chemical reactivity or lipophilicity.

It does serve as an illustration for the need to test a glove for permeability to every substance, with which it comes into contact. It cannot be assumed on mere theoretical grounds that a glove will offer sufficient protection against a certain substance.

CHAPTER 5

KINETICS OF METHYL ACRYLATE AND METHYL METHACRYLATE AFTER INTRAVENOUS AND EPICUTANEOUS ADMINISTRATION IN THE GUINEA PIG

5.1. *INTRODUCTION*

Investigations on the metabolism of ^{14}C-methyl methacrylate in rats (21, 34) show an excretion of $^{14}CO_2$ up to 88% of a single dose after oral or intravenous administration. As for methyl acrylate, the binding to sulfhydryl groups appears to be a major detoxication pathway in addition to oxidation to CO_2 (137).

Despite the fact that under occupational circumstances skin exposure is likely to occur frequently, no quantitative data are available with respect to the percutaneous absorption of these compounds. Methyl methacrylate is known to penetrate through rubber gloves (122, 169), and may give rise to serious side effects in humans (49).

Therefore, one of the main goals of the experiments presented here is to quantify the transport process of acrylate molecules through the skin of guinea pigs. The characteristics of this process can be analyzed from acrylate concentration measurements after the epicutaneous dose. Classically, based on a 2 or more compartment model, the concentration *vs* time functions can then be fitted to a set of exponential functions (105). In contrast to this approach we used a model based on mathematical systems theory (98, 161) to calculate the acrylate transport process through the skin *vs* time. With the aid of deconvolution methods (25), we can calculate the absorption input function (AIF). This is to be interpreted as the number, or fraction of acrylate molecules entering the central bloodpool per time *vs* time. Information such as the total fraction transported, *i.e.* bioavailability, and the mean absorption time (MAT) can then be derived from this input function. With the aid of this method it is possible to study the role of the skin as a barrier in the kinetics of methyl methacrylate and methyl acrylate.

5.2. *MATERIALS AND METHODS*

chemicals

Methyl (2,3-^{14}C) acrylate, specific activity 0.73 mCi·mmol^{-1} and methyl (2-^{14}C) methacrylate, specific activity 0.347 mCi·mmol^{-1}, were obtained from

the Amersham Radiochemical Centre, England. (2,3-^{14}C) acrylic acid was prepared by hydrolysis of methyl (2,3-^{14}C) acrylate in 0.15 M NaOH, followed by neutralization with concentrated HCl. The NaH $^{14}CO_3$ was prepared by bubbling $^{14}CO_2$ through a 0.15 M NaOH solution.

Analytical grade ethanolamine and 2-propanol were from Merck (FRG) and hexanol, triethylamine and dodecanethiol came from Aldrich (Belgium). Soluene-350 and Instagel were obtained from Packard (The Netherlands).

animals

Female Dunkin Hartley guinea pigs, 5-7 months old, weighing 550-750 g were obtained from the Broekman Institute (The Netherlands). Under anesthesia polyethylene catheters (inner diameter 0.58 mm, outer diameter 0.96 mm) were placed into the femoral artery and the jugular vein and led subcutaneously to the nuchal area where they went out of the body. The guinea pig was used for the experiment after recovery from this operation, which was assumed when her weight was at the pre-operation level.

experimental design

During the experiments the animals were housed in all glass metabolism cages, designed to trap expired air and to separate urine from faeces. Food (Hope Farms, The Netherlands) and water were provided ad libitum. Air was drawn through the cage (volume 24 l) at a rate of 400 ml·min^{-1} by means of a Verder Uni V-200 peristaltic pump, so the pressure inside the cage was somewhat lower than outside. This air was led through a train of gas-washing bottles: the first one was empty and put on ice to trap expired water; the next two bottles contained 5% (v/v) dodecanethiol and 2.5% (v/v) triethylamine in hexanol to trap unmodified acrylate and the last two bottles contained 20% water in ethanolamine (v/v) to trap expired CO_2.

Samples from these bottles were taken frequently *via* syringe-connected catheters without interruption of the air-flow. The solutions in the bottles were exchanged for fresh ones at least 3 times in 24 hrs. Urine and faeces were collected at 24 hrs intervals. The top of the cage was closed by means of a tabletennisball, in which holes were drilled in order to let through the catheters of the guinea pig. Blood samples were taken by connecting the arterial catheter outside the cage with a syringe filled with heparinized saline and drawing the piston until the catheter was completely filled with

freshly obtained blood. Then the connection was interrupted and by means of the heart-pump the arterial blood dripped into a heparinized tube. When the required amount (0.1 ml) was collected, the catheter was again connected with the syringe and the content of the catheter and 0.1 ml saline was injected again into the animal.

administration of the compounds

For the intravenous application the radioactive compound, dissolved in 4 ml saline, was administered *via* an infusion of 15 or 30 minutes into the jugular vein catheter. The amount remaining in the catheter was washed down with 1.0 ml of saline. In these experiments the dose applied to each animal was for ME 0.092 mmol, for MMA 0.13 mmol, and for acrylic acid 0.15 mmol.

For the epicutaneous administration, the left flank of the guinea pig was shaved and a glass cup, inner diameter 16 mm, outer diameter 24 mm, height 10 mm was glued to the shaved skin with cyanoacrylate glue (Amycon's CY 2000, medium viscosity, Aldrich, Belgium) 18 hrs before the start of the experiment. The radioactive compound was injected undiluted in the cup, which was closed immediately afterwards with a glass-cover with cyanoacrylate glue. Applied doses were 0.11 mmol of ME and 0.19 mmol of MMA. Elastic adhesive bandage of 7.5 cm width (Tensoplast, Smith and Nephew Ltd., England) was wound around the animal in such a way as not to hinder its respiration.

In order to determine the residence time of $^{14}CO_2$ in the guinea pig body and in the metabolism cage, 3 additional experiments were carried out in which 4 ml of a 0.15 M $NaH^{14}CO_3$ solution was administrated intravenously in guinea pigs, not used before.

At the end of the experiments the animals were killed by an intravenously injected overdose of pentobarbital (Narcovet^R).

Preparation for liquid scintillation counting

Blood samples were fractionated in plasma and cells by centrifugation immediately after collection; they were stored at 4°C for less than 12 hrs.

Of the animals, exposed epicutaneously, the glass cup, when still on the animal just killed, was rinsed 3 times with methanol, then removed and again rinsed, once with methanol and twice with petroleum ether. Rinsing fluids were collected in glass-scintillation vials until counting. The remaining activity in the cup was determined by heating the cup in a solution of potassium bichromate (5% w/v) in concentrated sulphuric acid and trapping the

produced $^{14}CO_2$ in ethanolamine.

The exposed skin was widely excised, cut into small pieces with scissors and solubilized in Soluene-350.

Plasma, urine and washing-bottle samples were analyzed by direct scintillation counting of weighed samples in Instagel. The ethanolamine samples required addition of methanol to obtain clear, homogenous samples.

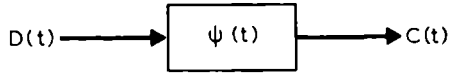
The faeces and the killed animals were stored at -20°C until counting. Of the exposed animals, lung, liver, kidney, spleen, heart and skin were assayed separately, brain and adrenals were also assayed in some animals. The remaining carcass and intestinal contents were homogenized. Faeces were also homogenized after addition of an equal volume of water. Quadruplicate weighed samples of the above-mentioned tissues and excreta were dissolved in 1 ml Soluene-350 and counted in 15 ml of an Instagel^R - 0.6 N HCl (9:1 v/v) mixture.

Erythrocytes (0.040 ml) were dissolved in 1 ml of Soluene-350 - 2-propanol (1:1 v/v), bleached by addition of 0.3 ml H_2O_2 35% (w/v) and counted in Instagel^R - 0.6 N HCl (9:1 v/v). All solutions were counted in a Packard Tri-Carb 3380 liquid scintillation counter.

5.3. *PHARMACODYNAMICAL ANALYSIS*

The tools used to calculate the acrylate transport process through the guinea pig skin have their roots in mathematical systems theory and gain popularity in pharmacological research. The line of thought can be explained with the aid of Figure 1, where a specific output function C(t) and dose input function D(t) can both be monitored as a function of time (*e.g.* plasma curves). The transport process passes molecules from input site to output site with a speed $\psi(t)$, according to the convolution integral equation (eq.1).

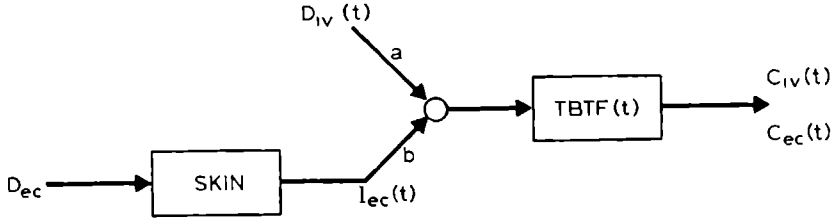
Figure 1: The "black box" notation, which input dosage flow D(t) and transport speed $\psi(t)$; the resulting concentration C(t) is analytically determined by the convolution equation.



The unknown $\psi(t)$ can be derived from this equation, by means of the mathematical process called deconvolution. Several deconvolution methods are known from literature (35, 36, 162, 163); in the one used in these investigations (25), the evaluation of the convolution integral equation is based on Fourier transformations.

The function of interest, *e.g.* the acrylate input flow into the central bloodpool, resulting from the skin absorption process, is derived from 2 different experiments (Fig. 2). Firstly, the total body transport function (TBTF) for the acrylates is determined (eq. 2), using an intravenous dose: the concentration profile is measured from a known input function, *e.g.* shot or infusion (Fig. 2, route a). Secondly, the unknown input function after epicutaneous application ($I_{ec}(t)$) is calculated from the resulting concentration profile (Fig. 2, route b), and the TBTF derived in the former step.

Figure 2: The system model used in the crossover study. Applying an intravenous dosage flow $D_{iv}(t)$ results in a concentration profile $C_{iv}(t)$ (route a), supplying information about the total body transport function TBTF(t). Application of an epicutaneous dose D_{ec} results in a concentration profile $C_{ec}(t)$ (route b), thus gaining information about the input dosage flow into the central bloodpool I(t), using the TBTF(t).



The overall formula is presented by equation 3 and the Area Under Curve (AUC) and MAT parameters by equations 4 and 5 respectively.

Because it is not only the acrylate activity, that is detected in the plasma, but also any appearing metabolite activity, the model of Figure 2 cannot be applied directly to this case. In fact, for all different types of metabolites a different TBTF is involved, which receives input either from the acrylate TBTF output site or from their precursors output site.

The new model is a composition of the various TBTF's of which the output refers to some ^{14}C-activity, and all the outputs are to be integrated to one pool to be able to describe the total plasma-^{14}C measurements (Fig. 3a). However, a combination of subsystems can be reduced to a single system which has the properties of all these subsystems (25). As can be seen from Figure 3a, the model can then be reduced to the configuration of Figure 2, whereby the TBTF is the function which describes the transport of ^{14}C-acrylate from the input reference point to ^{14}C-activity at the output reference point. So far for the plasma measurements; in case of the CO_2 measurements some

(1) $$C(t) = \int_0^t D(t-\theta) \cdot \psi(\theta) d\theta$$

(2) $$TBTF(\omega) = (V_B \cdot E) \cdot \frac{C_{iv}(\omega)}{D_{iv}(\omega)}$$

(3) $$I_{ec}(\omega) = \frac{(V_B \cdot E)\ \frac{C_{ec}(\omega)}{D_{ec}}}{TBTF(\omega)}$$

(4) $$AUC_{I_{ec}} = \int_0^{\sim} I_{ec}(t) dt$$

(5) $$MAT_{I_{ec}} = \int_0^{\sim} \frac{t \cdot I_{ec}(t) dt}{AUC_{I_{ec}}}$$

(6) a. $$C_{iv,CO_2}(\omega) = I_{iv}(\omega) \cdot \frac{1}{V_B} \cdot \frac{f_1 \cdot F_1(\omega)}{E_1} \cdot E_{01}$$

(6) b. $$C_{iv,pl}(\omega) = I_{iv}(\omega) \cdot \frac{1}{V_B} \cdot \{ \frac{f_1 \cdot F_1(\omega)}{E_1} + \frac{f_2 \cdot F_2(\omega)}{E_2} \}$$

(7) a. $$C_{ec,CO_2}(\omega) = D_{ec} \cdot \frac{F_s(\omega)}{V_B} \cdot \{ \frac{f_0 \cdot f_1\ F_1(\omega)}{E_1} \cdot E_{01} + \frac{f_4 \cdot F_4(\omega)}{E_4} \cdot E_{04} \}$$

(7) b. $$C_{ec,pl}(\omega) = D_{ec} \cdot \frac{F_s(\omega)}{V_B} \cdot \{ f_0 (\frac{f_1 \cdot F_1(\omega)}{E_1} + \frac{f_2 \cdot F_2(\omega)}{E_2}) + \frac{f_4 \cdot F_4(\omega)}{E_4} \}$$

(8) a. $$AIF_{CO_2}(\omega) = F_s(\omega) \cdot f_0 + F_s(\omega) \cdot \frac{\frac{f_4 \cdot F_4(\omega)}{E_4} \cdot E_{04}}{\frac{f_1 \cdot F_1(\omega)}{E_1} \cdot E_{01}}$$

(8) b. $$AIF_{pl}(\omega) = F_s(\omega) \cdot f_0 + F_s(\omega) \cdot \frac{\frac{f_4 \cdot F_4(\omega)}{E_4}}{\frac{f_1 \cdot F_1(\omega)}{E_1} + \frac{f_2 \cdot F_2(\omega)}{E_2}}$$

(9) a. $$AUC_{CO_2} = f_0 + \frac{f_4}{E_4} \cdot \frac{E_{04}}{\frac{f_1 \cdot E_{01}}{E_1}}$$

b. $$AUC_{pl} = f_0 + \frac{f_4}{E_4} \cdot \frac{1}{\frac{f_1}{E_1} + \frac{f_2}{E_2}}$$

(10) a. $$MAT_{CO_2} = MAT_s + \frac{1}{\frac{f_1}{(\frac{}{E_1})^2}} \cdot \frac{f_4}{E_4} \cdot \frac{f_1}{E_1} \cdot (MRT_4 - MRT_1) \cdot \frac{E_{04}}{E_{01}} \cdot \frac{1}{AUC_{CO_2}}$$

b. $$MAT_{pl} = MAT_s + \frac{1}{(\frac{f_1}{E_1} + \frac{f_2}{E_2})^2} \cdot \frac{f_4}{E_4} \cdot \frac{f_1}{E_1} \cdot \{(MRT_4 - MRT_1) + \frac{f_2}{E_2}(MRT_4 - MRT_2)\} \cdot \frac{1}{AUC_{pl}}$$

in which $f_1 + f_2 = 1$; $f_0 + f_4 = 1$

$C(t)$ = concentration output as a function of time.

$D(t)$ = dose input as a function of time.

$\psi(t)$ = transport as a function of time.

V_B = cardiac output.

$C_{iv}(\omega)$ = Fourier transformed concentration output function after intravenous dose.

I_{ec} = input function after epicutaneous dose.

$AUC_{I_{ec}}$ = area under curve of I_{ec}

$MAT_{I_{ec}}$ = mean absorption time of I_{ec}.

$C_{iv,CO_2}(\omega) = C_{iv}(\omega)$, of CO_2.

$C_{iv,pl}(\omega) = C_{iv}(\omega)$, of plasma.

F_i = component (molecule or group of molecules) with a Fourier transformed transport function $F_i(\omega)$.

E_i = extraction factor of component F_i.

E_{oi} = extraction factor of component F_i, which is transformed to CO_2.

f_0 = original acrylate fraction available from the skin absorption.

f_1 = fraction of original acrylate which does not bind to plasma.

f_2 = fraction of original acrylate which binds to plasma.

f_4 = metabolite fraction, formed in the skin

$F_s(\omega)$ = Fourier transformed transport function of the skin.

MAT_s = mean absorption time of the skin.

MRT = mean residence time.

Figure 3a: System model applying to plasma measurements.

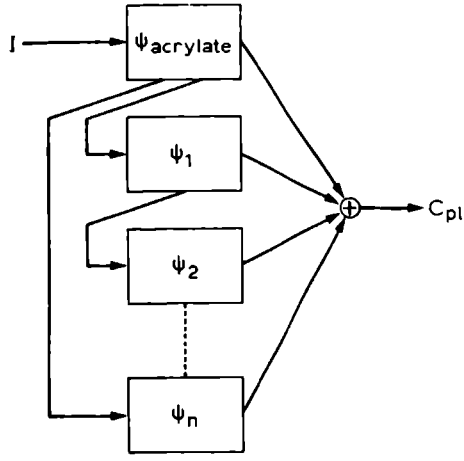
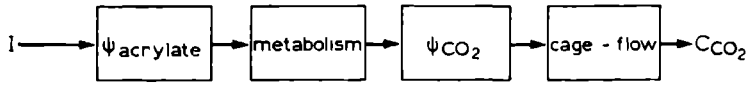


Figure 3b: System model for CO_2 measurement



extra subprocesses are involved too, like the metabolization step from acrylate to ultimately CO_2, the CO_2 expiration process, and finally the flow through the cage. The latter is considered to be a first order process. All these processes are sequential and, as it is, again the samples are not taken from the well-defined output reference site. Therefore, the model as depicted in Figure 2 has to be extended with those 3 subprocesses to be able to describe the output reference point in terms of CO_2 measurements (Fig. 3b). But because of the same reason that has just been mentioned for the plasma measurements, those subprocesses can be thought of as being part of the central subsystem, so the TBTF now stands for the transport of ^{14}C-acrylate from input reference to $^{14}CO_2$ at the cage output. As the flow through the cage is known, this subprocess will be eliminated from the $^{14}CO_2$ measurements using deconvolution, so the actual output reference will now be the expiration output site, and the model of Figure 3b also reduces to the one depicted in Figure 2.

5.4.1. *General*

For both acrylate esters and both ways of administration the excretion patterns are shown in Table 1, together with the results of the experiments with acrylic acid. As for faeces, in all experiments less than 1% of the dose was excreted *via* this pathway. The total amount of radioactivity which remained

TABLE 1: Excretion of radioactivity in guinea pigs after administration of methyl (^{14}C)acrylate (ME), methyl (^{14}C)methacrylate (MMA) and (^{14}C)acrylic acid (AA).

Substance	Administration route	Urine (% of dose)	$^{14}CO_2$ (% of dose	Exp. time (hours)
ME	intravenous[a]	47.8	36.5	24
ME	intravenous[a]	48.2	36.3	24
ME	intravenous[b]	46.5	36.7	28
ME	epicutaneous	32.6	39.8	28
ME	epicutaneous	41.8	36.9	29
ME	epicutaneous	31.6	46.0	39
ME	epicutaneous	40.3	38.7	52
MMA	intravenous[a]	9.7	69.7	24
MMA	intravenous[a]	10.1	68.9	28
MMA	intravenous[b]	11.3	66.5	24
MMA	epicutaneous	18.9	49.2	32
MMA	epicutaneous	15.9	55.4	60
MMA	epicutaneous	14.5	56.8	60
MMA	epicutaneous	19.5	57.6	60
AA	intravenous[a]	5.0	80	24
AA	intravenous[a]	5.1	78	24

[a]30 minutes and [b]15 minutes infusion.

in the body after termination of the experiment was measured in 10 animals and varied between 12% and 22% for both ME and MMA. Total recovery (mean±s.d.) was 93.5±2.3% (n = 6) for the epicutaneous experiments and 98.0±1.0% (n = 4) for the intravenous experiments without significant differences between the 2 tested acrylates.

Table 2 presents the data of the organ counts. In order to correct for differences in exposure time and dosage, the results are presented as relative to the activity ($dpm \cdot mg^{-1}$) of the homogenized carcass. Brains and adrenals which only were counted in 2 animals dosed with MMA, did not show a higher activity than other internal organs. Upon intravenous administration 1.5±0.2% of MMA and 2.3±0.2% of ME was excreted unmodified. With the epicutaneous experiments it was impossible to distinguish between leakage from the cup and excretion from the animal.

TABLE 2: Range of radioactivity of organs relative to the activity of the carcass after epicutaneous (ec) and intravenous (iv) administration.

Organ	Methylmethacrylate		Methylacrylate	
	ec (n = 3)	iv (n = 2)	ec (n = 3)	iv (n = 2)
Carcass	1.0	1.0	1.0	1.0
Skin	1.0-1.1	1.1-1.1	0.6-0.6	0.5-0.6
Liver	2.6-4.1	2.3-2.3	3.4-3.7	3.9-4.1
Lung	1.8-2.2	2.3-2.6	3.2-3.5	3.7-4.7
Spleen	2.2-3.1	2.7-4.2	2.7-5.4	2.9-4.1
Kidney	3.0-3.4	2.6-2.7	3.8-4.4	4.0-4.6
Heart	1.2-1.3	1.2-1.2	1.8-2.4	2.8-2.8

Since inhalation subsequent to leakage from the cup could interfere with the measured absorption, we only used the data from the epicutaneous experiments in which less than 5% was excreted as unmodified acrylate. The part of the dose remaining in and on the exposed skin was considered not to have entered the animal and thus not included in the given dose. Table 3 shows which part of the total dose remained in the skin and on the skin. The exposed skin of the animals dosed epicutaneously with ME always showed gross signs of inflammation (edema, reddening of the skin), while the animals treated with MMA did not show a visible change in the skin.

A significant amount of radioactivity was retained in the blood cells upon administration of ME. This cell-bound radioactivity did not decline up to 10 days after dosage. With intravenous administration the activity in the erythrocytes only rose during the infusion and not afterwards, while in the epicutaneous experiments a much longer time (10-20 hours) was needed to reach the maximum radioactivity. MMA nor acrylic acid gave rise to the

TABLE 3: Part of the dose of methyl acrylate (ME) and methyl methacrylate (MMA) that did not enter the systematic circulation in the epicutaneous experiments.

Substance	Skin (% of dose)	Exp. time (hours)
ME	12.3	28
ME	9.1	29
ME	25.8	39
ME	8.2	52
MMA	11.2	32
MMA	6.3	60
MMA	4.2	60
MMA	8.1	60

retaining of activity in the blood cells. Because of the apparent irreversibility of the binding of ME to blood cells we could compare the total amount of ^{14}C activity of these cells between intravenous and epicutaneous administration after correction for the total dose given. In this way we could determine the percentage of ME in the epicutaneous experiment, which reached the bloodstream in such a chemical form that it could bind to blood cells apparently irreversibly. For the intravenous experiments we found 5.6±0.3% of the dose in the blood cells (n = 3), for the epicutaneous experiments (n = 4) 4.2±0.4%, so it appeared that 75±11% of the dose passed the skin barrier unmodified.

5.4.2. *Pharmacokinetic results*

Primarily results will be summarized considering both CO_2 and plasma measurements (Table 4), because their discrepancy is responsible for the conclusions that have been drawn. Thereafter the calculated absorption input functions (AIF) are considered for CO_2 and plasma measurements (CO_2-AIF resp. PL-AIF) per acrylate type; the AIF's are truncated at that end-time which is the same as in the corresponding epicutaneous experiments. As total ^{14}C activities are determined, no differentiation can be made between the acrylate and any appearing metabolites in the plasma.

The CO_2 measurements of the bicarbonate experiments show a mean mean-transportation-time (MTT) for $^{14}CO_2$ of 67±2 min; the MTT through the cage is calculated to be 60±5 min, resulting in 7±5 min for the $^{14}CO_2$ elimination from the guinea

TABLE 4: Area under curve (AUC) and mean absorption time (MAT) parameters calculated from the CO_2 and plasma absorption input function (AIF), truncated (t)/extrapolated (∞), for methyl acrylate (ME) and methyl methacrylate (MMA). Also denoted is the time of the peak absorption of the corresponding AIF's. t_{max}.

Substance	CO_2-AIF		Plasma-AIF		t_{max}
	AUC_t/AUC_∞	MAT_t/MAT_∞ (minutes)	AUC_t/AUC_∞	MAT_t/MAT_∞ (minutes)	CO_2/PL-AIF (minutes)
ME	1.12/1.19	574/671	0.89/0.96	628/742	270/210
ME	0.97/1.15	389/658	0.83/0.93	510/774	200/ 30
ME	1.27/1.42	840/1096	a	a	350/ a
ME	0.88/1.08	730/1122	0.79/0.80	1303/1308	430/130
MMA	0.64/0.66	761/787	a	a	440/ a
MMA	0.56/0.77	906/1432	0.65/0.76	854/1147	570/910
MMA	0.74/0.79	1204/1427	a	a	480/ a
MMA	0.78/0.87	1231/1615	0.92/0.92	902/902	610/940

a: Could not be determined because of too little samples in the epicutaneous experiment.

pig. In fact the mean-transportation-time of CO_2 from the input is reference point to the expiration output-site.

Because of the homogenity of the group concerning the intravenous results (Table 1), the results are averaged for both the plasma and CO_2 measurements. Before averaging, all the i.v. concentration time functions were normalized to a unit-bolus input function, hereby making use of deconvolution with the infusion input function. Analogous, from all $^{14}CO_2$ measurements the cage-flow is eliminated by deconvolution of the original measurements with this first order process.

Both methyl acrylate (ME) and methyl methacrylate (MMA) intravenous experiments show the same difference between the CO_2 and plasma curves: the tail part of the latter decreases much more slowly compared with the former (Fig. 4). ME and MMA epicutaneous experimental results show the same phenomenon as the intravenous results do: the plasma ^{14}C activity decreases much more slowly compared with the CO_2 measurements (Fig. 5).

Focussing upon the AIF's for ME (Fig. 6) which are calculated according to the model presented in Fig. 2, the corresponding AUC's of the CO_2-AIF's vary from 0.88 to 1.27 (4 measurements), derived from the truncated AIF. The AUC's of the PL-AIF's are calculated from three experiments: 0.79 to 0.89.

Figure 4: The mean plasma and corresponding CO_2 total body transport functions determined after intravenous application of a) methyl acrylate (ME) and b) methyl methacrylate (MMA). These functions are the result of deconvoluting the concentration profiles with the infusion input function, thereafter the functions are normalized so that the area under curve (AUC) equals 1. The function values are expressed as the fraction of the total dose that is eliminated from the central bloodpool; the time axis is given in hours.

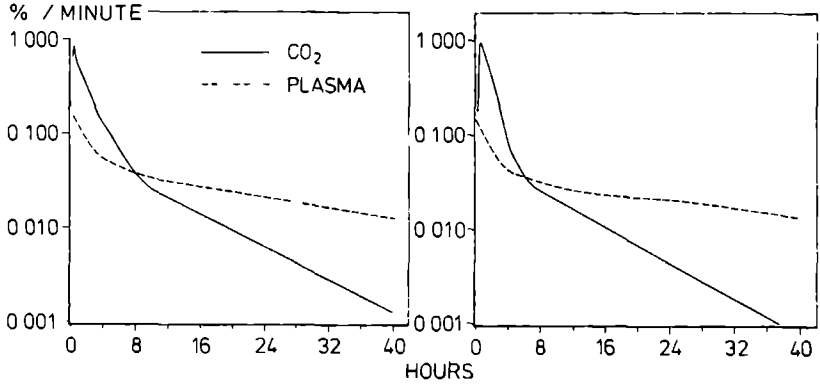


Figure 5: An example of the plasma and corresponding CO_2-^{14}C activity in 2 guinea pigs determined after epicutaneous application of a) methyl acrylate (ME) and b) methyl methacrylate (MMA). For the plasma data the dimension is DPM/50 µl plasma, and for the CO_2 data this is DPM exhaled per second.

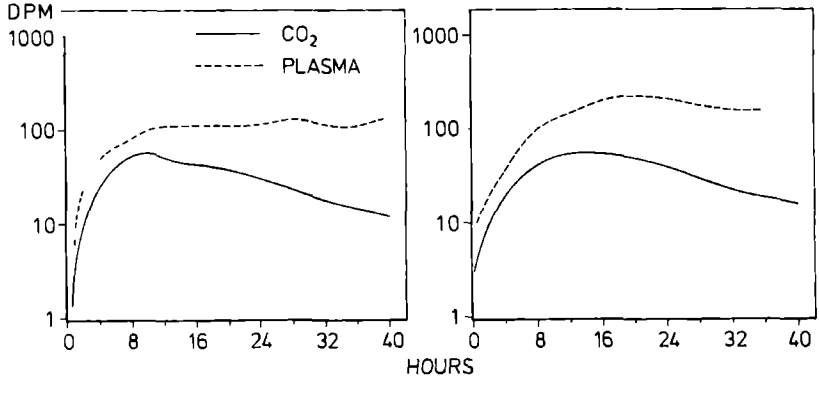


Figure 6: The absorption input functions (AIF) for methyl acrylate (ME) in 2 guinea pigs, calculated on basis of the CO_2 and plasma measurements. Note the remarkable difference between the 2 AIF's for each guinea pig. The function values are given as the fraction of the total dose applied epicutaneously that enters the central blood-pool. The time axis is given in hours.

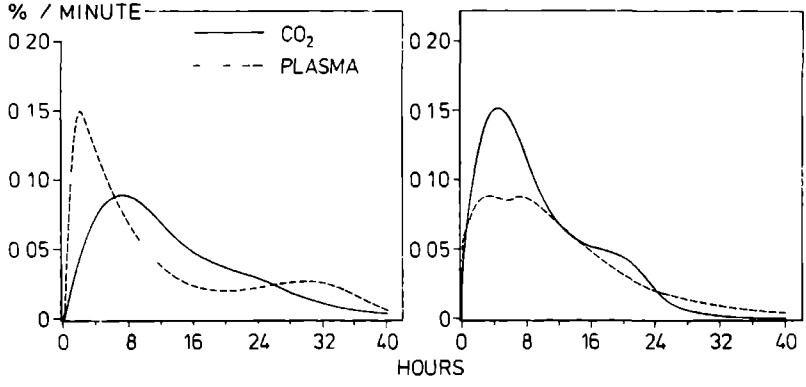
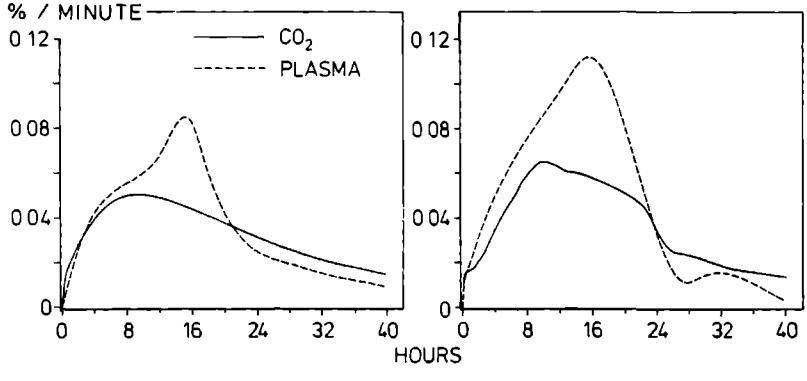


Figure 7: The absorption input functions (AIF) for methyl methacrylate (MMA) in 2 guinea pigs, calculated on basis of the CO_2 and plasma measurements. Note the remarkable difference between the 2 AIF's for each guinea pig. The function values are given as the fraction of the total dose applied epicutaneously that enters the central blood-pool. The time axis is given in hours.



After mono-exponential extrapolation the AUC's are resp. 1.08-1.42 and 0.80-0.96, the former resulting in a bioavailability of more than 100%. The MAT varies from 389-840 min (CO_2-AIF) and 510-1303 min (PL-AIF), after extrapolation resp. 658-1122 min and 742-1308 min.

In the case of MMA (Fig. 7), the AUC's of the CO_2-AIF's and PL-AIF's are 0.56-0.78 (n = 4) and 0.65-0.92 (n = 2) respectively; after extrapolating 0.66-0.87 and 0.76-0.92. The corresponding MAT's vary from 761-1231 min (CO_2-AIF) and 854-902 min (PL-AIF) and after extrapolation resp. 787-1615 min and 902-1147 min.

5.5. *DISCUSSION*

At the moment there is no "animal of choice" to study percutaneous absorption (22, 170). We chose the guinea pig for reasons of economy, easy handling, available experience on canulation of blood vessels and evidence that it is a reasonable animal model for this purpose (5).

The results of the intravenous administration of MMA to the guinea pig *i.e.* the rapid biotransformation to CO_2 are in good agreement with the results of Bratt and Hathway (21) and Crout *et al.* (34), who applied MMA to rats. Together with other experiments *in vivo* (39) and *in vitro* (43), the available results may suggest a rather low potency of MMA to yield reactive metabolites.

The excretion pattern of ME, with much more activity in the urine (Table 1), is markedly different from the one of MMA. There is also a considerable production of thioethers in the urine after administration of ME (137), as a consequence of the direct Michael addition of ME to glutathione (39). Another indication for the nucleophilic character of ME is the apparently irreversible binding of ME to erythrocytes, a phenomenon which is also encountered with acrylonitrile (1) and acrylamide (106). In spite of this alkylating potency of ME compared to MMA, we found no difference in the percentage of the dose which remained in the body after termination of the experiment.

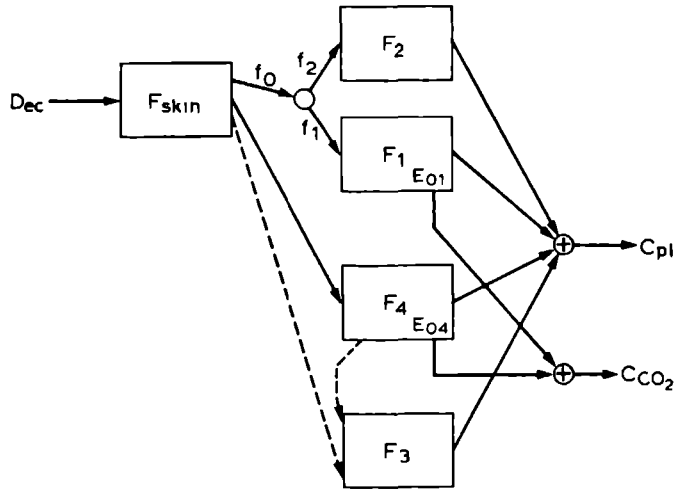
Hydrolysis appears to be the major detoxification pathway of ME (139). Since acrylic acid did not bind to erythrocytes, we could determine the upper boundary of the fraction of ME which was hydrolysed in the skin: 25±11%.

As shown in Figures 6 and 7 and in Table 4, there is an unexpected discrepancy between the acrylate absorption input functions calculated from the CO_2 data and on the other hand from the plasma data. This leads to the conclusion that the transport process of acrylate from guinea pig skin to

central bloodpool can not be modelled as it is in Figure 2 (route b). Moreover, the calculated functions will be an integration of a number of unknown processes among which is the acrylate skin transportation process. Although the number of experiments is limited especially in the case of MMA, conclusions can be drawn. First we will discuss the intravenous experimental outcomes (Fig. 4), then the AIF-ME results (Fig. 6) and afterwards the AIF-MMA results (Fig. 7).

On the basis of the proposed system model (Fig. 8), the conclusions will be discussed. At first the component F3 will not be taken into account

Figure 8: The system model for both methyl acrylate (ME) and methyl methacrylate (MMA). All boxes represent the transport processes of the molecules involved: input site and output site refer to the same kind of molecules or group of molecules. Formation of a (group of) metabolite(s) is depicted as the input flow into a new transport process and originating from the transport process of its precursor(s). Refer to the text for the meaning of the symbols.



(eqs. 6-10), but the results will show the necessity of its presence. All boxes depicted in the system model, represent the transport processes of the molecules involved: input site and output site always refer to the same kind of molecules or group of molecules. Formation of a (group of) metabolite(s) is depicted as the input flow into a new transport process and originating from the transport process of its precursor(s). The transport function for the acrylate will be called F1.

The difference between the plasma and CO_2 intravenous results in both acrylate experiments can be explained by assuming an extra component in the plasma kinetics, the transport function of which is called F2 in Figure 8. This component is additional to the plasma level and may be due to the very slow release from a binding site of the acrylate itself: from literature it is known that ME and MMA have approximately the same binding constants with bovine serum albumin (59).

The contribution of the $^{14}CO_2$ activity in the plasma is so small compared to all other components, that in all equations 6-10 its contribution has not been taken into account. Furthermore, the CO_2 clearance from the body is so fast (7±5 min) compared to the acrylate clearance (several hours), that the influence of the CO_2 clearance on the total results will be neglected. Referring to Table 4 the bioavailability resulting from the ME CO_2-AIF appears to be more than 100%. So, a larger amount of $^{14}CO_2$ has been formed in the epicutaneous experiment relative to the intravenous experiment. As shown in Table 1 the total $^{14}CO_2$ production seems independent of the acrylate infusion rate, thus a saturation effect of the metabolizing system is not very likely. Then the bioavailability over 100% can be explained by the fact that a metabolite of ME must have been formed in the guinea pig skin (F4). Then from equations 9a and 9b and Table 4 it follows that the partial CO_2 clearance of F1 (E_{01}) will be less than the partial CO_2 clearance of F4 (E_{04}).

From equations 8a and 8b it is evident that both AIF's are a sum of two different functions. The first function is the transport function of the skin itself and the second function is a convolution of this skin transport function with another complex function, which is composed of F1, F2 and F4 for the AIF_{pl} or F1 and F4 for the AIF_{CO_2}. Then the difference in the peak time absorption of these AIF's (t_{max}, Table 4) can be explained by the contribution of the very slow transport function in the denuminator of equation 8b compared to the one in 8a. These denuminators are directly related with the total body transport functions (eqs. 6a and 6b and Fig. 4).

As F2 is the transport function mainly responsible for the very slow decline in the plasma activity, its MRT will then be much larger than any MRT of the other components. For this reason it cannot be explained from equations 10a and 10b, why the MAT on basis of the plasma measurements is larger than the MAT on basis of the CO_2 measurements. Therefore, an extra component F3 is needed, which might result either from biotransformation in the guinea pig skin or from other components in the plasma. The one property that F3 must

have to explain the results, is a large MRT.

The conclusions from the MMA absorption input function are in essence comparable with those concerning the ME absorption input function, although derived differently. In all AIF cases (on the basis of the CO_2 measurements) the bioavailability appears to be substantially less than 100%. So, metabolite formation in the skin can then also be the conclusion, which implies an extra component F4, analogous to the case of ME.

The shift in the peak time absorption for the plasma AIF to the right from the CO_2-AIF (see t_{max}, Table 4) leads to a contradiction, when the same reasoning as in the ME case is applied here. So, the conclusion is that in the MMA system model another component F3 must be included too.

So, for both ME and MMA the equations 6-10 must be expanded with a component F3, which because of the uncertainty about its origin, will lead to a wide variety of possible system model equations. For reasons of the limited number of experiments, it is not possible to extract the one and only correct set of equations, and, with those, the proper system model. Then the conclusion is that for both acrylates at least one type of metabolite is formed in the skin.

The metabolite formation in the skin prohibited an exact determination of the permeation rate of the 2 tested acrylates through the guinea pig skin. On the other hand, it illustrates the need to take the metabolizing potential of the skin into account, when assessing the toxicological behaviour of a substance.

CHAPTER 6

ASSESSMENT OF TOPICAL TOXICITY OF 5 MONOMETHACRYLATE ESTERS ON HUMAN SKIN WITH WATER VAPOUR LOSS AND IMPEDANCE MEASUREMENTS

6.1. *INTRODUCTION*

Irritant contact dermatitis is believed to have a substantially higher prevalence than allergic contact dermatitis (74). This is not reflected in the amount of research, devoted to clarification of its pathogenesis. Reports on allergic contact dermatitis greatly outnumber those on irritant contact dermatitis. So, not surprisingly, there is a far greater agreement on the methods for predictive testing on allergenicity, than for tests on irritant capacities. The guinea pig is the animal of choice for determining the allergenic potential of substances. For the assessment of the irritant potential both albino rabbits and guinea pigs are used, without an exclusive preference for one of them (103).

An allergic contact dermatitis in man can be ultimately diagnosed by means of patch testing. Patch testing can be executed in the consulting room. It is a test with many pitfalls, but if done with care, it results in a straightforward answer: yes or no sensitization. A comparable diagnostic test for the detection of an irritant contact dermatitis, to be executed in the consulting room, is not available. Reasons for this may become apparent after the following explanatory thoughts.

An acute irritant contact dermatitis is likely to be the result of a single overwhelming exposure to a single causative factor. Diagnosis and treatment of such a condition is quite simple (94). On the other hand, a chronic irritant dermatitis is supposed to arise from a cumulation of a continuously changing variety of small causative factors. Each factor leads to a certain impairment of the skin function, thereby making the skin more vulnerable to an attack by a following causative factor. Diagnosis of this condition is difficult and is often made "per exclusionem". This is to say if in an eczematous patient "all" contact allergic investigations give negative results and reasons to suspect an atopy are absent.

However, Malten (97) tentatively formulated a number of clinical signs and symptoms by which it may be possible in certain uncomplicated cases, to recognize chronic irritant contact dermatitis at first sight. In case of such a dermatitis, the most important therapeutic measure is lowering the influence

of the most harmful causative factors. Therefore we need to know to which factors the patient's skin is especially vulnerable.

With predictive test methods, strong irritants will be readily recognized, but it is the detection of mild irritants which poses investigators for the greatest problems. Several animal and human test models have been proposed. Some investigators have tried to detect the mild irritants by intensifying the exposure, *e.g.* a closed patch procedure during 10 or even 21 days (74, 100, 123). Others have tried to enhance the sensitivity by damaging the skin before applying the test substance, *e.g.* by abrasion with hard particles (118), or scarification (58). More elegant methods are developed by Thiele and Malten (88, 90, 149, 150, 151). By means of measurements of 2 aspects of skin function, water vapour loss and impedance, an impairment of the health of the skin can be determined.

Some advantages of both methods are the facts that they result in numerical values, that combinations of exposures can be evaluated, that adaptation may be studied, that the dosages necessary are more closely related to exposure dosages encountered in everyday life, and that the effect of the experimental exposures ideally is barely visible clinically. Nevertheless the disadvantages are the facts that the instruments are very expensive, and that full investigation of a substance is time consuming, because a series of exposures on several volunteers may be necessary for a full evaluation.

In addition to the use of these methods to evaluate the irritant potential of a substance, these methods can also be valuable in the clinical setting. An increased vulnerability of a patient's skin to a factor in the patient's environment can be determined by exposure of the patient to a carefully chosen concentration of that factor, and measurement of water vapour loss and impedance of his skin. On the basis of the data thus obtained the patient can be advised.

We decided to use the water vapour loss and the impedance measurements on the human skin as test methods for the acrylic monomers under investigation. This study was intended to provide further data, leading to a dermatological risk assessment of these substances.

Previous investigations (158) had taught us that monoacrylates and dimethacrylates were strong sensitizers with guinea pig test methods, while diacrylates were severe irritants to the clipped guinea pig skin. In industrial workers, exposed to diacrylates, acute irritant effects on the skin are described (13), as well as delayed irritant effects (92) on the skin. The

latter may be regarded as a variation of the acute type.

Therefore, monoacrylates and dimethacrylates were excluded to avoid the risk of sensitizations in volunteers, and since we were primarily interested in the occurrence of mild irritant effects, neither the diacrylates were tested. So, the only group available for testing was the group of monomethacrylates.

6.2. *MATERIALS AND METHODS*

chemicals

The following methacrylates were used: ethyl, *n*-butyl, *t*-butyl, pentyl and 2-hydroxyethyl methacrylate. Purity, origin and structure formulas are described in Chapter 2.2. Analytical grade methyl ethyl ketone was obtained from Aldrich (Belgium).

water vapour loss measurements

The water vapour exuded by the skin is trapped in a metal skin cup, covering an area of 1.88 cm^2, and transported by a constant flow of dry nitrogen gas to a Meeco electrolytic water analyzer. With thermistors the skin temperature at each skin cup is also measured, since the normal water vapour loss is dependent on the skin temperature. The measurements of both water vapour loss and skin temperature are recorded on a computer (Mink 11, Digital Equipment). As soon as the temperature standardized water vapour loss remains constant (±1%) during 4 minutes, the measurement is stopped, and the result thus obtained, expressed in $\mu g \cdot cm^{-2} \cdot hr^{-1}$ is used.

impedance measurements

Four perspex cups, each covering a skin surface area of about 6 cm^2 are attached with Velcro strips to the volar site of each arm at the marked regions, and filled with a 0.015 M NaCl solution. After 60 minutes contact between this salt solution and skin (30 minutes in the measurement directly after exposure to the test solutions), platinum electrodes are inserted into the solution in each cup. An alternating current (25 Hz) is passed between all combinations of any 2 skin cups on one arm, and the impedance and phase angles are read with a Radiometer impedance meter GB 11^C (Copenhagen). The platinum electrodes consisted of a horizontal platinum plate (1 cm^2) with a

soldered wire, insulated by a hard PVC tube, passing through a rubber stopper, and connected to the impedance meter with insulated wires and plugs.

The rubber stopper fitted to the perspex cup. From the read impedance and phase angles, the skin resistance (kiloOhm) at each site was calculated (151).

experimental design

Volunteers are first allowed to come to rest for about half an hour prior to the measurements on a day. Six sites are marked on the volar site of both arms. The distance between wrist and elbow is divided in 5 equal parts. The site nearest to the wrist is marked as site no. 1, while the site in the cubital fossa is no. 5. One-fifth of the elbow-wrist distance, proximal from the elbow fold site no. 6 is marked.

The sites 2, 4 and 6 are exposed to the test solutions, site no. 3 is always used as an internal control site and remains unexposed to the test solutions or to the solvent of the test solutions. So, both arms have 3 test sites and 1 control site each. Site no. 1 is not used for physiological reasons (too high a variable sweat secretion), site no. 5 is not used for practical reasons (elbow flexion).

Several days before the experimental exposure, water vapour loss and impedance measurements were performed daily in all marked regions. The water vapour loss measurements always preceded the impedance measurements, since the exposure of all sites to a 0.015 M NaCl solution, necessary for the latter measurement, would make the water vapour loss measurement unreliable. Directly before exposure to the test solutions water vapour loss and impedance of the skin were measured. This also means that exposure to the test solutions occurred on a hydrated skin.

We used 3 concentrations of the methacrylates in methyl ethyl ketone: 2 M, 3 M and 4.5 M. Approximately 3 ml of a solution of the test substance was poured into metal skin cups (inner area about 6 cm^2), fixed to the above mentioned sites of the skin with Velcro strips. During exposure, the arms were frequently moved gently, to avoid concentration changes. Site 2 was always exposed to the 2 M solution, site 4 to the 3 M solution and site 6 to the 4.5 M solution. Duration of exposure of the sites on the right arm was twice as long as the exposure on the left arm, *e.g.* 5 minutes on the left arm and 10 minutes on the right arm. Each skin site was exposed only one time to the test solution. After exposure the skin sites 2, 4, and 6 were cleaned by 3 times wiping with tissues soaked in methyl ethyl ketone,

followed by rinsing with tap water and gently drying with a soft tissue.

Water vapour loss measurements were started again one day after the exposure. Impedance measurements were restarted directly after exposure, except in the experiments with ethyl methacrylate and in one experiment with *n*-butyl methacrylate (plotted in Figure 1), in which the impedance measurements were started again after one day.

Both parameters were recorded daily for at least 2 weeks, to collect data concerning the repair of the skin. Results are expressed as percentage increase or decrease to the simultaneously measured control values of site 3:

$$\left(\frac{\text{value of exposed site} - \text{value of control site}}{\text{value of control site}} \right) \times 100\%$$

The percentage thus obtained is compared with the percentages of that site before exposure.

Based on his experience, obtained during over 10 years of experimental research with these methods, both with volunteers and with patients, Malten (97) tentatively states that a rise in the water vapour loss or a decrease in the skin impedance of 50% or more, compared with the pre-exposure values, indicates a clinical risk. Analogous to this statement, we do not draw any conclusions, when the deviations of the water vapour loss and impedance measurements, compared to the pre-exposure values, are less than 50%.

In Table 1 data are shown on the duration of exposure and the volunteers of the experiments performed.

TABLE 1: Duration of exposure (in minutes) of the volunteers to the methacrylates, 2 M, 3 M and 4.5 M in methyl ethyl ketone.

Volunteer no.	1	2	3	4	5
Substance					
ethyl methacrylate	5',10' 20',40'				
n-butyl methacrylate	5',10' 20',40'		20',40'		
t-butyl methacrylate	5',10'				20',40'
pentyl methacrylate				30',60'	
2-hydroxyethyl methacrylate		5',10'	20',40'		

6.3. *RESULTS*

In none of the experiments performed (Table 1), deviations of the water vapour loss of the exposed skin of 50% or more could be observed. The results of the impedance measurements, carried out one or more days after exposure, neither showed deviations of 50% or more, compared with the pre-exposure values. As examples, results of one complete experiment with *t*-butyl methacrylate are given in Table 2a and b, and 2 independent experiments with *n*-butyl methacrylate are plotted in Figures 1 and 2.

TABLE 2a: Relative deviations in water vapour (%) before and after exposure to 2 M, 3 M or 4.5 M *t*-butyl methacrylate in methyl ethyl ketone.

Duration of exposure	20 minutes			40 minutes		
Test concentrations	2 M	3 M	4.5 M	2 M	3 M	4.5 M
Time before/after exposure						
1 day before exposure	138	92	97	102	94	74
directly before exposure	146	94	98	103	90	77
1 day after exposure	104	96	92	126	99	89
2 days after exposure	179	93	94	119	103	92
3 days after exposure	186	92	92	123	101	94
4 days after exposure	211	98	136	117	93	56
7 days after exposure	193	103	128	115	105	99
9 days after exposure	187	96	104	114	110	95
10 days after exposure	193	101	105	109	109	97
13 days after exposure	150	87	102	99	105	97
14 days after exposure	156	90	99	98	107	104
15 days after exposure	149	83	87	97	106	97
16 days after exposure	159	93	90	103	114	101
17 days after exposure	148	81	81	93	114	98
20 days after exposure	149	87	89	98	112	94

Figure 1

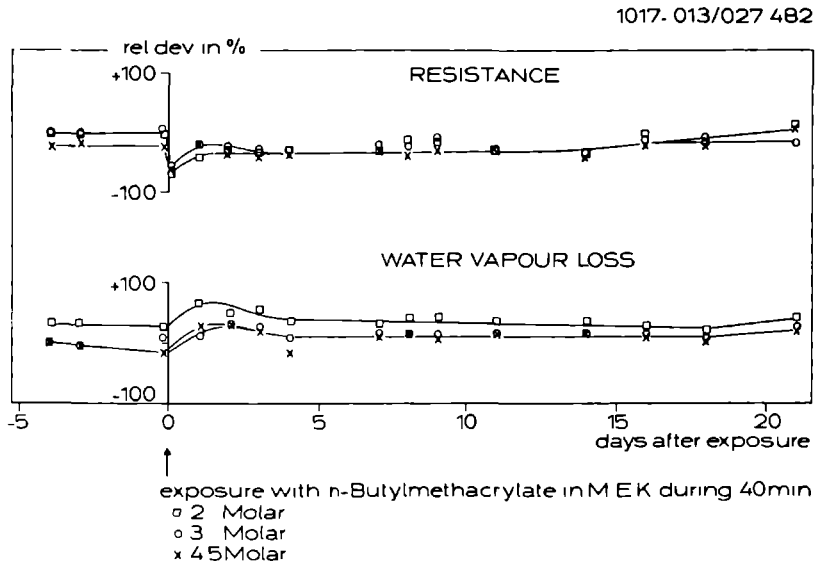


Figure 2

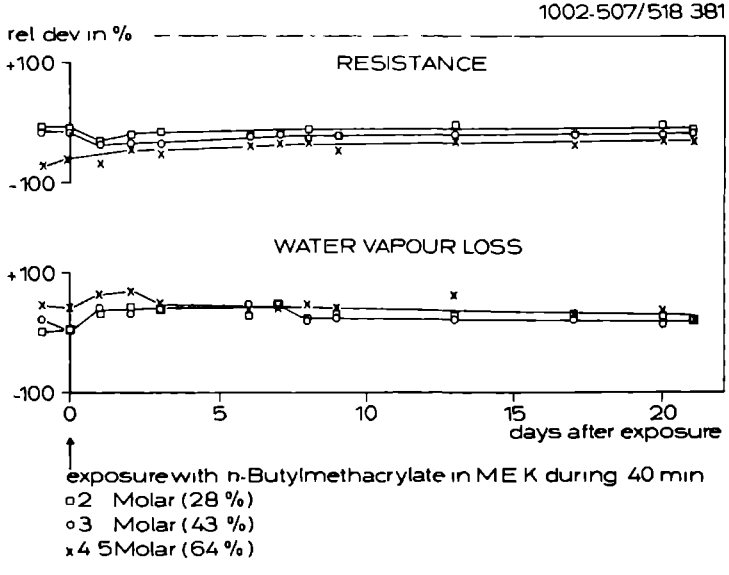


TABLE 2b: Relative deviations in skin impedance (%) before and after exposure to 2 M, 3 M or 4.5 M *t*-butyl methacrylate in methyl ethyl ketone.

Duration of exposure	20 minutes			40 minutes		
Test concentration	2 M	3 M	4.5 M	2 M	3 M	4.5 M
Time before/after exposure						
1 day before exposure	106	125	96	84	116	118
directly before exposure	80	122	69	80	128	96
directly after exposure	42	53	25	31	45	34
1 day after exposure	58	123	95	63	106	73
2 days after exposure	49	114	86	93	167	111
3 days after exposure	62	111	79	91	122	100
4 days after exposure	42	113	85	27	119	105
7 days after exposure	55	97	64	84	86	89
9 days after exposure	75	101	77	101	97	62
10 days after exposure	96	104	99	93	86	76
13 days after exposure	85	104	85	96	106	82
14 days after exposure	90	111	80	109	114	82
15 days after exposure	81	112	81	105	105	76
16 days after exposure	82	107	74	99	95	68
17 days after exposure	93	113	104	101	116	99
20 days after exposure	82	104	74	92	86	72

When the skin sites were exposed to the test solutions for 20 minutes or longer, the impedance measurements, performed directly after exposure, showed a decrease of 50% or more in some experiments. For all 4 methacrylates tested, the relative deviations in impedance directly before and directly after exposure of 20 minutes or longer, are shown in Table 3. The observed decrease in impedance appeared to be the most pronounced after the longest exposures.

In none of the experiments performed, visible changes of the exposed skin were noted.

6.4. *DISCUSSION*

An impairment of the skin, as seen after contact with an irritant factor, might lead to an enhancement of the permeability of the statum corneum for water. In addition, damage to the epithelial lining of the sweat gland

TABLE 3: Relative deviation in impedance (%) directly before and after exposure to solutions of *n*-butyl methacrylate, *t*-butyl methacrylate, 2-hydroxyethyl methacrylate and pentyl methacrylate.

Duration of exposure	20 minutes						40 minutes					
Test concentration	2 M		3 M		4.5 M		2 M		3 M		4.5 M	
	before exp	after exp	before exp	after exp	before exp	after exp	before exp	after exp	before exp	after exp	before exp	after exp
Substances												
n-butyl methacrylate	97	57	83	48	79	39*	97	36*	97	45*	76	31*
t-butyl methacrylate	80	42	122	53*	69	25*	80	31*	128	45*	96	34*
2-hydroxyethylmeth-acrylate	87	44	96	78	114	70	101	41*	133	55*	112	50*
pentyl methacrylate**	86	68	125	62*	126	54*	95	59	106	51*	105	45*

* Decrease of 50% or more compared to the measurement directly before exposure.

**Results after 30 resp. 60 minutes exposure.

duct might also result in an increase of the water vapour loss of the skin (151). A decrease in impedance of the skin is supposed to be due to a greater mobility of small ions such as Na^+, Cl^-, H_3O^+ and OH^- in the stratum corneum. This greater mobility is likely to be the result of changes in the protein configuration of the stratum corneum, as might occur after contact with an irritant factor.

The measured values of both parameters exhibit considerable differences between individuals. In order to reduce these spontaneous variations, the values are expressed as a percentage of a simultaneously measured value of a control site on the same arm. Still, also after this correction, the values may vary substantially. This is not surprising, since even in guinea pigs of the same strain, maintained under identical circumstances, a considerable difference in reactivity to irritants exists. This goes even more for human volunteers, with a much greater variety in both inner and outer influences on the skin, like "genetic make-up", hormonal status, psychological condition, possible internal medication, and outside the experimental situation exposure to for instance climatological conditions, soaps, cosmetics, fat solvents and irradiation. Therefore the normal, Gaussian curve for resistance to a certain factor is expected to be flatter in humans than in laboratory animals.

In one individual man too, the measured values vary in time. In some individuals the highest value before exposure to a test substance is twice the lowest value before this exposure. Other individuals exhibit less variation in the measured values. It is in our opinion not allowed to test only individuals with such a low spontaneous variation. This would mean a selection of volunteers, which makes an extrapolation of the results unreliable. So, although this means a loss of sensitivity of the methods, we feel that only deviations in the measured values, greater than 50% compared to pre-exposure values, indicate an impairment of the skin of the tested volunteers.

In addition, we have to keep in mind that with water vapour loss and impedance measurements we do not measure <u>direct</u> effects of a substance on the skin. In the skin itself, a variety of reactions may occur as a response to contact with a substance. Some of these reactions will lead, for instance, to a decrease of the permeability of the skin to water; other reactions will have the opposite effect. A change in the exposure conditions of a substance to the skin may lead to another set of reactions. We only "see" the summation of the effects of all these reactions on, for instance, the permeability of the skin to water, but we do not see the contribution of all these different

reactions, or homoiostatic mechanisms, of the skin.

A simple dose-effect relationship is not very likely under such circumstances. The possibility exists that a higher dose will have less effect on one or both parameters, measured by us.

The observed decrease in impedance of the skin directly after exposure to the tested methacrylates during 20 minutes or longer (Table 3), may indicate a risk for the health of the skin. This applies to persons, who come into contact with these methacrylates under comparable circumstances. This conclusion, however, needs to be drawn very cautiously. We cannot exclude the possibility that the observed decrease in impedance is due to an effect of the solvent (methyl ethyl ketone) on the skin. The control sites were exposed neither to the methacrylate under investigation, nor to the solvent during the experiments.

Another investigation, in which one of the two control sites is indeed exposed to the solvent, will be necessary for a further support of the conclusion. It is not sufficient to state that a 20 minute exposure to methyl ethyl ketone hardly affected the measured parameters in other volunteers. It cannot be excluded that the volunteers, exposed to the methacrylates in the experiments reported in this chapter, might, however, have a lowered natural resistance against this solvent.

The greatly expanded number of applications of acrylic monomers has led to a considerable exposure of the human skin to these compounds.

This may lead to irritant or allergic reactions of the skin, or to systemic effects, when the acrylic monomer penetrates through the skin. Special attention is given to the use of acrylic monomers in adhesive materials, since this application is likely to result in intimate contact with the skin.

In Chapter 1 four subject-matters are described:

1. the basic principles of polymerization reactions, leading to the production of plastic materials;
2. the applications of acrylic monomers in the manufacturing of a great variety of polymers;
3. the basic principles of adhesive bonding;
4. a review of the toxicological literature on acrylate esters.

Chapter 2 deals with the assessment of the mutagenic activity of 27 acrylate esters and 5 acrylamides. To this purpose the Salmonella microsome assay was selected as the test method, in view of its high sensitivity and extensive validation.

No mutagenic effects are observed with this method. The addition of an external metabolizing system (liver homogenate) had no influence on this result. The conclusion is that this result diminishes the probability of a carcinogenic effect of the tested acrylic monomers.

In Chapter 3 acrylic constituents of adhesive tapes are investigated for their sensitizing potential with guinea pig test methods.

2-Ethylhexyl acrylate proved to be a strong sensitizer in the Freund's Complete Adjuvant Test. However, an extract from an adhesive tape, containing 2-ethylhexyl acrylate, did not provoke any allergic reactions in the guinea pigs, sensitized to 2-ethylhexyl acrylate.

Acrylamide, N-hydroxymethyl acrylamide and N,N'-methylene bis acrylamide appeared to be moderate sensitizers in the Split Adjuvant Test, while methacrylamide, *t*-butyl acrylamide, N-dodecyl maleamic acid and N-*t*-butyl maleamic acid appeared to be weak or non-sensitizers with the Split Adjuvant Test. Acrylamide, methacrylamide and N-hydroxymethyl acrylamide were also investigated with the Freund's Complete Adjuvant Test. A good correlation between the two test methods is found.

When it is allowed to use the data, obtained in guinea pig experiments, for the human situation, then the difference in sensitizing potential of the above mentioned substances may be used as a guideline in allergen replacement.

A strongly sensitizing impurity in acrylic acid is identified as α,β-diacryloxy propionic acid with the aid of physico-chemical analysis and the Freund's Complete Adjuvant Test. This finding demonstrates the necessity of using pure products as starting materials in the synthesis of adhesives and other end products.

The rapid diffusion of methyl methacrylate through surgeons' gloves is described in Chapter 4. Furthermore, methyl methacrylate is able to act as a solvent for auxiliary substances in the rubber glove. Both properties of methyl methacrylate mean a risk of sensitization for the personnel in the operating theatre. These people come into contact with the methyl methacrylate, present in bone cement used to fix endoprostheses to bone. Preliminary studies indicate that butyl acrylate and acrylamide are also able to penetrate through surgeons' gloves.

Chapter 5 deals with the permeation *in vivo* of methyl methacrylate and methyl acrylate through the guinea pig skin. The comparison of the excretion of radioactive labelled metabolites of the 2 acrylate esters after intravenous or epicutaneous dosage, leads to an estimation of the permeation *in vivo* with the aid of the mathematical process called deconvolution.

Methyl acrylate penetrates faster through the guinea pig skin than methyl methacrylate. Methyl acrylate appears to bind irreversibly to the erythrocytes; this in contrast with acrylic acid or methyl methacrylate. The latter result demonstrates the importance of hydrolysis in the detoxification of acrylate esters, and of the importance of the methyl group at the α-carbon atom for diminishing the toxicity of acrylate esters.

Biotransformation of both acrylate esters in the skin could be demonstrated, but this local biotransformation made an exact determination of the permeation rate with the method used impossible.

In Chapter 6 the measurement of the irritant potential of 5 monomethacrylate esters on the living human skin is described. As parameters for damage to the skin deviations of water vapour loss and impedance of the skin, as compared to control sites on the skin, are taken. An exposure of the volunteers of 20 minutes or longer to a solution of one of the methacrylate esters (2 M, 3 M, 4.5 M) in methyl ethyl ketone caused a decrease in impedance of 50% or more directly after exposure.

This result is suggestive for a health risk of the skin, exposed to comparable concentrations during a comparable time. However, an effect of the solvent on the skin was not excluded with certainty.

Further experiments will be necessary to clarify this.

Het aantal toepassingen van acrylverbindingen is sinds de 2e wereldoorlog sterk toegenomen. Terwijl vroeger vooral de nadruk lag op bulk-productie in gesloten systemen (m.n. perspex productie), zijn er geleidelijk aan ook meer ambachtelijke toepassingen gevonden voor het gebruik van deze stoffen. Tandprothesemateriaal, drukinkten, verven en lijmen bevatten vaak acrylverbindingen. Deze nieuwe toepassingen gaan gepaard met een ander risico en een gewijzigd patroon van blootstelling. Derhalve hoort naast de aandacht voor de gevolgen van acute kortdurende blootstelling aan hoge concentraties, zoals die voorkomen na een ongeluk in een gesloten productiesysteem, nu ook de invloed van frequentere, langdurige blootstelling aan lage concentraties onderzocht te worden. Met name de blootstelling van de huid kan hierbij aanzienlijk zijn. Hierbij kan de huid doelwit-orgaan zijn, met als gevolg b.v. een dermatitis op allergische of orthoergische basis. Daarnaast kan een stof door de huid heen penetreren en een heel scala aan systemische effecten veroorzaken.

Teneinde een bijdrage te leveren tot een zo verantwoord mogelijke risicoevaluatie van het gebruik van acrylverbindingen, staan in dit proefschrift een aantal experimentele onderzoeken beschreven naar het dermatotoxicologisch gedrag van acrylverbindingen. Met name werd aandacht besteed aan het gebruik van acrylverbindingen in lijmen, aangezien hierbij intensief contact met de huid regelmatig voorkomt.

In hoofdstuk 1 worden in een algemene inleiding de volgende onderwerpen uiteengezet:

1. De basisprincipes van de productie van kunststoffen door polymerisatiereacties.
2. De toepassingen van de diverse acrylverbindingen.
3. De fysich-chemische achtergrond van de aard van lijmverbindingen.
4. Een overzicht van de toxicologische literatuur over acrylesters.

In het tweede hoofdstuk wordt een onderzoek beschreven naar de mutagene eigenschappen van 27 acrylesters en 5 acrylamides. Omdat uit eerdere onderzoeken naar voren was gekomen dat acrylverbindingen een covalente binding met eiwitten kunnen aangaan, werd besloten om na te gaan of deze monomeren ook een structurele verandering van DNA, een mutatie, kunnen bewerkstelligen.

De Salmonella-microsoom test of Ames-test is hiervoor gebruikt, aangezien deze mutageniteitstest uitgebreid gevalideerd en zeer sensitief is. Als een stof mutageen is, dan is de kans dat deze stof carcinogeen is sterk verhoogd.

Met de gebruikte test-methode kon evenwel geen mutagene activiteit van de onderzochte verbindingen gedetecteerd worden.

Het derde hoofdstuk behandelt de sensibiliserende eigenschappen van acrylaten, welke voorkomen in zelfklevende pleisters. Twee dierexperimentele methoden werden gebruikt om de sensibiliserende potentie vast te leggen, nl. de Freund's Complete Adjuvant Test (FCAT) en de Split Adjuvant Test (SAT). 2-Ethylhexylacrylaat bleek een sterke sensibilisator in de FCAT, maar een extract van een pleister, welke 2-ethylhexylacrylaat bevatte, gaf geen allergische reactie in de voor 2-ethylhexylacrylaat gesensibiliseerde dieren. Blijkbaar was de concentratie van vrij 2-ethylhexylacrylaat in de pleister te laag om nog een allergische reactie te bewerkstelligen.

Acrylamide, N-hydroxymethylacrylamide en N,N'-methyleenbisacrylamide bleken matige sensibilisatoren te zijn in de SAT, terwijl methacrylamide, *t*-butylacrylamide, N-dodecylmaleamide zuur en N-*t*-butylmaleamide zuur zwak of niet sensibiliseerden in de SAT. Acrylamide, N-hydroxymethylacrylamide en methacrylamide werden ook met de FCAT onderzocht. De resultaten van beide onderzoeksmethoden bleken goed met elkaar overeen te stemmen. De keuze tussen beide testmethoden hangt af van de fysisch-chemische eigenschappen van de stof. Om de FCAT te kunnen gebruiken - deze is eenvoudiger uit te voeren - dient de te onderzoeken stof oplosbaar te zijn in water of olie.

Het waargenomen verschil in sensibiliserende potentie tussen de hierboven genoemde stoffen kan gebruikt worden als een richtlijn bij eventueel noodzakelijke vervanging. Zo zou het matig sensibiliserende acrylamide bij een hiervoor opgetreden sensibilisatie vervangen kunnen worden door methacrylamide.

Dat men ook bij monomeren zonder allergene eigenschappen op zijn hoede hoort te zijn voor het risico van sensibilisatie, wordt geïllustreerd door het vinden van een sterk allergene verontreiniging in monsters van acrylzuur (hoofdstuk 3.4.). Deze verontreiniging kon geïdentificeerd worden als α,β-diacryloxypropionzuur. Deze stof ontstaat vermoedelijk als bijproduct van een bepaalde industriële synthese van acrylzuur. Indien dit verontreinigde acrylzuur gebruikt wordt voor de fabricage van producten als lijmen en verven, waarbij huidcontact frequent voorkomt, dan betekent dat een duidelijk risico van sensibilisatie. Het gebruik van zo zuiver mogelijke grondstoffen bij dit soort productieprocessen lijkt dan ook noodzakelijk.

Methylmethacrylaat is het voornaamste bestanddeel van "botcement", dat gebruikt wordt om metalen heup- en knieprotheses te bevestigen aan bot.

Een orthopeed komt aldus tijdens b.v. een heupprothese-operatie in contact met methylmethacrylaat, een bekende contact-sensibilisator. De mate van diffusie van methylmethacrylaat door een aantal chirurgische handschoenen dient men te kennen, alvorens een advies te kunnen geven welke handschoen voor de orthopedische praktijk het meest geschikt is. Een onderzoek naar deze doorlaatbaarheid staat beschreven in hoofdstuk 4. Geen van de onderzochte 11 merken handschoenen bleek in staat langer dan 5 minuten het methylmethacrylaat tegen te houden. Bovendien bleek dat de rubber-hulpstoffen uit de handschoenen wegdiffundeerden onder invloed van het methylmethacrylaat. Aangezien rubberhulpstoffen tot de meest frequente veroorzakers van contact-allergieën behoren, betekent dit nog een additief risico voor de geëxponeerde personen. Een oriënterend onderzoek toonde voorts aan dat butylacrylaat en acrylamide ook door deze rubber handschoenen heen penetreerden.

Hoofdstuk 5 gaat over de doorlaatbaarheid *in vivo* van methylacrylaat en methylmethacrylaat door de huid van de cavia. De vergelijking van het verloop van de uitscheiding van radioactief gemerkte acrylaten tussen intraveneuze en epicutane toediening, geeft hierbij met behulp van het wiskundige proces van deconvolutie een schatting van de doorlaatbaarheid *in vivo*. Methylacrylaat gaat sneller door de caviahuid dan methylmethacrylaat. Methylacrylaat gaat een irreversibele binding aan met de rode bloedcellen, dit in tegenstelling tot methylmethacrylaat of acrylzuur. Dit laatste resultaat toont het belang aan van de methylgroep aan het α-koolstofatoom voor de vermindering van de toxiciteit en het belang van de hydrolyse bij de ontgifting van de acrylesters.

Biotransformatie van beide acrylesters in de huid kon aangetoond worden. Deze biotransformatie verhinderde wel een exactere bepaling van de doorlaatbaarheid van de huid met deze methode.

In hoofdstuk 6 wordt de meting van de irriterende eigenschappen van 5 monomethacrylaten op de menselijke huid beschreven. Hierbij zijn als parameters voor beschadiging van de huid de waterdampafgifte en de electrische weerstand van de huid genomen, vergeleken met de waarden hiervan op controleplaatsen op de huid. Als de huid van de binnenzijde van de onderarm van vrijwilligers blootgesteld werd gedurende 20 minuten of langer aan een oplossing van een van de methacrylaten in methylethylketon in concentraties van 2 M of hoger, dan werd een daling in de electrische weerstand van de huid gezien van 50% of meer vergeleken met de controlewaarden. Deze daling van de huidweerstand werd alleen gezien onmiddellijk na de blootstelling. Hoewel een

effect van het gebruikte oplosmiddel op de huid niet volledig uitgesloten kan worden, is het verkregen resultaat toch suggestief voor een gezondheidsrisico van de huid, die blootgesteld wordt aan dergelijke concentraties gedurende vergelijkbare tijden.

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C U R R I C U L U M V I T A E

Theo Waegemaekers is op 27 september 1954 geboren te Roosendaal. In deze plaats bezocht hij de lagere school en het Norbertus Lyceum, alwaar hij in 1972 het diploma gymnasium-β behaalde. Daarna heeft hij Geneeskunde gestudeerd aan de Katholieke Universiteit te Nijmegen. Het doktoraal-examen werd behaald in 1977. Hierna heeft hij als wetenschappelijke stage een voedingsonderzoek verricht in Coimbatore, India. Het artsexamen werd behaald in februari 1980.

In 1980 heeft hij een half jaar gewerkt als verzekeringsgeneeskundige op het GAK te Eindhoven. Van januari 1981 tot december 1983 is hij werkzaam geweest als wetenschappelijk medewerker op de afdeling Dermatologie, sectie Arbeidsdermatologie van de Nijmeegse Universiteit.

Sinds december 1983 werkt hij als bedrijfsarts bij de GG&GD te Apeldoorn. Hij is gehuwd met Els van den Dries en heeft sinds oktober 1984 een zoon.

S T E L L I N G E N

1. Het gebruik van handschoenen ter bescherming tegen contact met een bepaalde stof dient voorafgegaan te worden door een onderzoek naar de doorlaatbaarheid van deze handschoenen voor deze bepaalde stof.

2. Het ongereglementeerd toestaan van de aanbeveling "hypo-allergeen" op een gebruiksartikel werkt kwakzalverij in de hand.

3. Gezien de promotor-werking van benzoyl peroxide is de toepassing van deze stof in locale anti-acné preparaten niet aan te raden.
   Slaga, TJ, Klein-Szanto AJP, Triplett LL, Yotti LP, Trosko JE, Science 1982; 231: 1023-1025.

4. Het postuleren van de afwezigheid van een no-effect level bij genotoxische stoffen is celbiologisch gezien terecht, maar vanuit een epidemiologisch standpunt uit bekeken onjuist.

5. Elke arbeidssituatie fungeert in wezen ten dele als een proeflaboratorium van de volksgezondheid en de milieuhygiëne. Daarom dienen zowel de arbeiders als de arbeidsomstandigheden adequaat begeleid en geobserveerd te worden vanuit de arbeidsgeneeskunde.

6. Documentatie- en archiveringstechnieken horen thuis in het basisprogramma van een wetenschappelijke opleiding.

7. Bij de bemesting van landbouwgrond dient zuiveringsslib, gezien de daarin voorkomende concentraties zware metalen, niet gebruikt te worden.

8. De sinds Plato verkondigde hypothese, dat een excellent onderzoeker ook een excellent bestuurder zal zijn en vice versa, wordt niet bevestigd in de praktijk van de universiteitssituatie.
   Plato, Politeia, 473d.

9. De overheersende positie van de LD_{50} bij wettelijke veiligheidsvoorschriften toont aan dat de adsorptie van relevante toxicologische onderzoeksmethoden aan de wetgever een uiterst traag proces is.

10. "Beschaafde" landen als de V.S. en Groot-Brittanië, die meer dan de helft van het beschikbare overheidsbudget voor onderzoek besteden aan militaire research, bevorderen op deze manier de onveiligheid in de wereld en remmen tegelijkertijd de vooruitgang van de wetenschap.

New Scientist, 20/27 December 1984.

Theo Waegemaekers
6 juni 1985