

SENSITIZATION CAPACITY OF EPOXY RESIN OLIGOMERS IN THE GUINEA PIG

Arnar Thorgeirsson, Sigfrid Fregert and Olle Ramnäs

Department of Dermatology, Section of Occupational Dermatology, University of Lund, Lund, Sweden
and Department of Engineering Chemistry, Chalmers University of Technology,
Gothenburg, Sweden

Abstract. Low molecular weight oligomers of an epoxy resin of bisphenol A diglycidyl ether type were isolated by gel permeation chromatography. The sensitizing capacity of these oligomers was assessed with the "guinea pig maximization test". The oligomer with the molecular weight of 340 sensitized 80% to 100% of the animals, but produced no cross reactions to the other oligomers. The MW 624 oligomer sensitized 56-60% of the animals and 30% of these showed cross reactions to the MW 340 oligomer. The MW 908 and MW 1192 oligomers elicited no reactions. It was shown that sensitization with epoxy resin mixtures of high average molecular weight depends mainly on the content of the MW 340 oligomer.

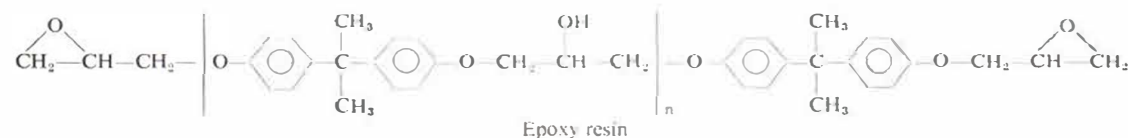
Key words: Guinea pig maximization test; Sensitization capacity; Gel permeation chromatography; Epoxy resin oligomers

In a previous investigation (7) the sensitization capacity of epoxy resin mixtures with differing average molecular weights (MW) was established with the "guinea pig maximization test" (GPM-test).

To ascertain the correlation, if any, between molecular weight and sensitization capacity, guinea pigs were sensitized with low molecular weight oligomers of epoxy resins. As far as the authors are aware, no such investigations have hitherto been published. The oligomers used were isolated from samples of commercial epoxy resins by means of gel permeation chromatography (GPC).

MATERIAL AND METHODS

Chemicals. The epoxy resins used were of diglycidyl ether bisphenol A type supplied by the Swedish Plastics Federation (Formula 1).



The repeated part has a MW of 284. Thus, the MWs of the oligomers are 340 ($n=0$), 624 ($n=1$), 908 ($n=2$), 1192 ($n=3$), etc. (Fig. 1).

Sensitization

Three sensitization techniques were used: (I) The guinea pig maximization test (3, 4, 6, 7). (II) Topical exposure with and without sodium lauryl sulphate (MW 340 oligomer). (III) Single intradermal injection (5) (MW 340 oligomer).

Sensitization by the guinea pig maximization test

The animals were sensitized by a two-stage procedure: intradermal injections and topical application.

Animals were sensitized with each isolated oligomer dissolved in acetone. The same concentration was used for intradermal injection as for topical application (Table I). In preliminary studies a 5% concentration of the MW 340 resin was found to sensitize 100% of the animals.

Five series (altogether 61 animals) were sensitized with 5% (w/v) MW 340 oligomer as in previous work (7). One additional series of 10 animals was sensitized with 0.5% MW 340 oligomer.

An equimolar concentration was used for the other oligomers, as follows: (i) two series 10 and 18 animals respectively, were sensitized with 9.2% (w/v) MW 624 oligomer; (ii) three series of 10, 15 and 5 animals, respectively, were sensitized with 13.5% (w/v) MW 908

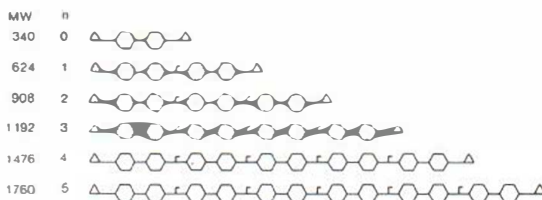


Fig. 1. MWs of epoxy resin oligomers.

Table I. Concentrations of epoxy resin oligomers for sensitization and challenge (GPM-test)

Oligomers (MW)	Vehicle	Sensitization, intradermal and topical (% w/v)	Challenge (% w/v)
340	Acetone	5	1 & 5
340	Acetone	0.5	1 & 5
624	Acetone	9.2	1.8 & 9.2
908	Acetone	13.5	2.7 & 13.5
1 192	Acetone	17.5	3.5 & 17.5

oligomer; (iii) one series of 15 animals was sensitized with 17.5% (w/v) MW 1 192 oligomer.

Challenge. Two weeks after the second stage of sensitization a 24-hour occluded patch test (Al-test, Imeco, Astra Agency) was performed on the flank. In preliminary studies a concentration of 1% of MW 340 was found to be suitable. An equimolar patch test concentration was used for each of the other oligomers as well as with a fivefold concentration. The test concentrations are given in Table I.

The animals' flanks were clipped and shaved with an electric razor. Challenge was not preceded by chemical depilation.

Cross patch testing. Simultaneous with the challenge with the oligomer used in the induction, the animals were patch tested with all the other oligomers.

Reading of challenge. The challenge site was evaluated 24-hours after removal of patches. Three hours prior to reading, the test site was shaved with an electric razor. Only evident redness and swelling was regarded as an allergic reaction. The reactions were judged independently by two persons.

Controls. The control animals were of the same age and weight and were exposed to complete Freund's adjuvant and vehicle intradermally as the animals in the experimental groups. When the sensitized animals in each series were challenged the control animals were patch tested with the epoxy resin oligomers in corresponding concentrations.

Sensitization by topical exposure

In one series of 10 animals the flank was clipped and shaved. A 2 by 4 cm Whatman 3MM paper was saturated (about 0.2 ml) with 20% (w/v) MW 340 oligomer and

Table II. Challenge reactions in animals sensitized with epoxy resin oligomers (GPM-test)

Oligomers (MW)	No. of animals in each series	Reacting animals	
		No.	%
340 ^a	20	20	100
340	15	15	100
340	8	8	100
340	10	10	100
340	18	15	83
340	10	8	80
340 ^b	15	10	67
624	10	6	60
624	18	10	56
908	10	0	0
908	15	0	0
908	5	0	0
1 192	15	0	0

^a Series from previous work (7).

^b Sensitization with 0.5% (Table I).

applied as a 24-hour closed patch test. This procedure was repeated after 3 days. After 2 weeks a patch test was performed on the opposite flank as in the maximization test.

In another series of 17 animals the flank was clipped and shaved, after which 10% sodium lauryl sulphate in vaseline was rubbed into the skin. After 24-hours a 2 by 4 cm Whatman 3MM paper saturated with 20% (w/v) MW 340 oligomer was left under occlusion on the slightly inflamed skin for 48-hours. After 2 weeks the animals were challenged as in the maximization test.

Sensitization by one intradermal injection

One series of 15 animals was sensitized with only one intradermal injection (5). 0.1 ml of a 20% (w/v) mixture MW 340 oligomer dissolved in acetone and blended with an equal amount of complete Freund's adjuvant was injected intradermally in the shoulder region. After 2 weeks the animals were challenged as in the maximization test.

Gel Permeation Chromatography of Epoxy resins (2). The low molecular weight oligomers of epoxy resin with average MW about 900 were separated in preparative scale. Two columns in series (185×2.54 cm and 40×2.54 cm) filled with polystyrene-divinylbenzene beads (Bio-Bead SX-2 and SX-1, respectively, from Bio Rad Laboratories) were used. The eluent was distilled tetrahydrofuran. Flow rate 1 cm³ min⁻¹. The columns

Table III. Cross reactions in animals sensitized with epoxy resin oligomers

Sensitizing oligomers (MW)	No. of animals tested	Cross reactions to oligomers (%)			
		MW 340	MW 624	MW 908	MW 1 192
340	61		0	0	0
624	10	30		0	0
624	18	28		0	0
908	30	0	3		0
1 192	15	0	0	0	

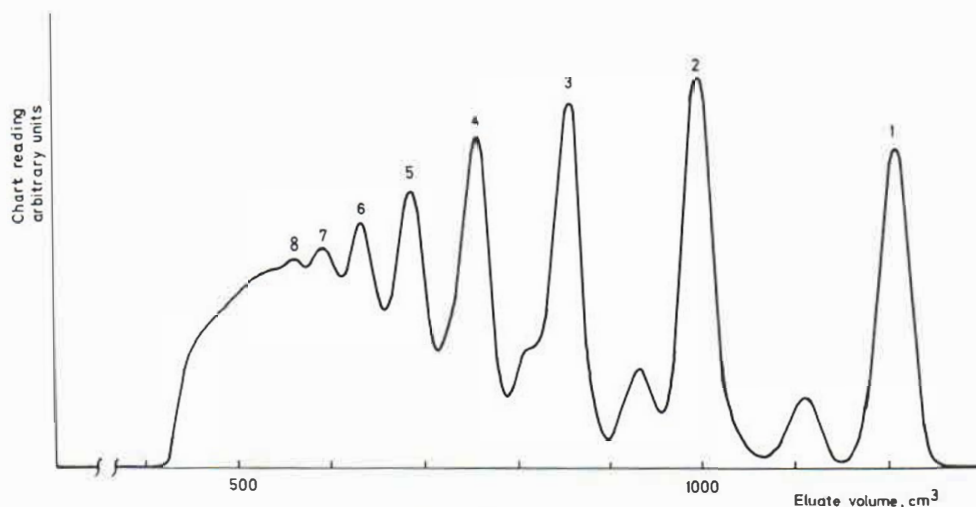


Fig. 2. Gel permeation chromatogram (GPC) of epoxy resin, average MW 900. Peak 1: oligomer MW 340, peak 2: oligomer MW 624, etc. (Fig. 1).

were kept at room temperature. A differential refractometer (LDC Refracto Monitor) was used as detector. About 1 g epoxy resin was fractionated in each of ten runs. A typical chromatogram is illustrated in Fig. 2. The eluate was collected in an automatic fraction collector. Oligomers with more than 5 bisphenol A units were collected in one fraction and very narrow fractions were taken of the 5 other main epoxy compounds. The fractions were evaporated under reduced pressure at 30°C in a thin film evaporator.

The purity of the fractions was checked by re-chromatography on a small diameter column (0.6 cm). Only one peak was seen on each chromatogram.

The starting material of the epoxy resin, bisphenol A and epichlorhydrin, was eluted after the last peak. This peak exhibited the same elution volume as the diglycidyl ether of bisphenol A with MW 340 (Ciba-Geigy). A linear relationship was found between the logarithm of MW and the elution volume of all the oligomers on a small diameter column filled with Bio-Bead SX-2. The peaks can thus be assumed to correspond to oligomers with a MW of $340 + n \times 284$.

GPC on the small diameter column was carried out on commercial resin mixtures with average MWs of 900, 1 280 and 1 850, respectively, in order to determine the content of the oligomers with MW 340, 624 and 908 (Table IV).

In addition some series of animals were challenged with the MW 340, 624 and 908 epoxy oligomers isolated by adsorption column chromatography (to be published).

Because of a low sensitization rate the same induction procedure was repeated with the same animals immediately after the challenge reactions had been read. After a further week the animals were rechallenged.

RESULTS

Topical irritancy. In 25% concentration none of the oligomers produced patch test reactions. Nor did

even the strongest concentration studied, 80%, of the MW 340 oligomer.

The guinea pig maximization test. The test reactions are summarized in Table II and cross test reactions in Table III.

The MW 340 oligomer produced reactions in 80–100% of the animals when sensitized with 5% concentration. Three series were challenged with 1% and 5% MW 340 oligomer, but there was no difference in the number of reactions to these two concentrations. When sensitized with 0.5% (one-tenth of ordinary) concentration, 67% (10/15) of the animals reacted.

The MW 624 oligomer elicited a reaction in 56% and 60% of the animals. A 9.2% patch test concentration did not produce more reactions than did the 1.8% concentration.

Of the animals sensitized with the MW 624 oligomer, 30% showed cross reactions to the MW 340 oligomer (Table III). It was always the same

Table IV. Content of oligomers in epoxy resin mixtures determined by GPC

Epoxy resin (average MW)	Content of oligomers (%)		
	MW 340	MW 624	MW 908
900	10	13	12
1 280	10	3	17
1 850	5	Trace	10

animal that reacted to both the MW 340 and MW 624 oligomers. The MW 624 oligomer sensitized animals produced no cross reactions to the other oligomers.

Animals sensitized with the MW 908 oligomer were unresponsive to this oligomer, but one animal (1/30) displayed a cross reaction to the MW 624 oligomer in fivefold (9.2%) equimolar concentration. No cross reactions to the other oligomers were noted.

Animals induced with the MW 1192 oligomer were unresponsive and displayed no cross reactions to the other oligomers.

The oligomers of MWs 340, 624 and 908 isolated by adsorption column chromatography produced the same reactions as those isolated by GPC.

No reactions to the oligomers were seen in the control animals.

Sensitization by topical exposure. Topical sensitization with 20% MW 340 oligomer without sodium lauryl sulphate produced no reactions in the animals.

Sensitization with the MW 340 oligomer by pretreatment with sodium lauryl sulphate produced reactions in 18% (3/17) of the animals. When the same sensitization procedure was repeated in the same animals, 47% (8/17) reacted.

Sensitization by one intradermal injection. After sensitization with only one intradermal injection of MW 340 oligomer, 30% (3/10) of the animals responded when challenged.

DISCUSSION

Gel permeation chromatography showed that resin mixtures of higher average MW, viz. 900, 1280 and 1850, also contained MW 340 oligomer (Table IV). This explains the challenge reactions to MW 340 oligomer found in a previous investigation (7), in which animals were sensitized with resin mixtures of higher average MW. The average MW 900 and MW 1280 resins contained MW 624 oligomer too. In the previous study, challenge reactions to high MW resin mixtures were probably a consequence of simultaneous sensitization to MW 340 and MW 624 oligomers.

The high sensitization capacity of MW 340 oligomer was established in a previous work (7). In the present series 80–100% of the animals became sensitized. A comparatively high sensitization rate (67%) was also noted when animals were sensitized

with only one-tenth the concentration ordinarily used in the maximization test.

The MW 340 oligomer proved not to be irritant in 80%, the strongest concentration studied. This may explain why topical sensitization was unsuccessful without pretreatment with sodium lauryl sulphate, while almost 50% of the animals became sensitized when so pretreated.

Of the animals sensitized with only one intradermal injection of MW 340 oligomer, 30% became sensitized—which further demonstrates that this oligomer is a potent sensitizer.

The MW 624 oligomer also seems to be a sensitizer, though it is probably less antigenic than the MW 340 oligomer, since it does not possess reciprocal cross reactivity and its sensitization rate is somewhat lower than that of the MW 340 oligomer.

Animals sensitized with MW 340 oligomer showed no reaction to the other three oligomers of higher molecular weights, while 30% of animals sensitized with MW 624 oligomer exhibited reversed cross reactions to the MW 340 oligomer. These animals did not cross react to the other two higher MW oligomers.

The pattern of cross reactions between MW 340 and MW 624 oligomers cannot be attributed to contamination of MW 340 by MW 624 or to lack of penetration of the MW 624 oligomer. It probably indicates that the MW 340 oligomer is the dominant antigenic determinant and hence the most potent sensitizer among oligomers of this type. Absence of reciprocal cross reactivity is known in the case of other allergens (1).

The MW 908 oligomer produced no reactions, but one animal (1/30) showed a reaction to MW 624 oligomer, though only in a fivefold (9.2%) equimolar concentration. Since contamination between MW 624 and MW 908 oligomers was excluded, the absence of challenge reaction to MW 908 oligomer may be explained as an insufficient percutaneous absorption of this oligomer. Absence of cross reactivity to the MW 340 oligomer may be due to too great a difference in molecular size.

The MW 1192 oligomer in one series did not show reactions to this or cross reactions to the other oligomers.

It is obvious that MW 340 oligomer is a potent sensitizer and that the sensitization capacity of epoxy resin oligomers diminishes with increase in molecular weight.

Investigations are in progress to ascertain whether persons sensitized with commercial epoxy resin mixtures react in the same manner as do the animals.

ACKNOWLEDGEMENTS

This investigation was carried out in cooperation with the Swedish Plastics Federation. The work has been supported by the Swedish Work Environment Fund, project no. ASF 74/327.

REFERENCES

1. Baer, H., Dawson, C. H. & Kurtz, A. P.: Delayed contact sensitivity to catechols. *J Immun* 101: 1243, 1968.
2. Batzer, H. & Zahir, S. A.: Studies in the molecular weight distribution of epoxide resin. I. Gel permeation chromatography of epoxide resins. *J Appl Polym Sci* 19: 585, 1975.
3. Magnusson, B. & Kligman, A. M.: The identification of contact allergens by animal assay. The guinea pig maximization test. *J Invest Dermatol* 52: 268, 1969.
4. — Allergic Contact Dermatitis in the Guinea Pig. Identification of Contact Allergens. Ch. C. Thomas Publisher, Springfield, Ill., 1970.
5. Ritz, H. L., Connor, D. S. & Sauter, E. D.: Contact sensitization of guinea-pigs with unsaturated and halogenated sultones. *Contact Dermatitis* 1: 349, 1975.
6. Thorgeirsson, A., Fregert, S. & Magnusson, B.: Allergenicity of epoxy-reactive diluents in the guinea pig. *Berufsdermatosen* 23: 178, 1975.
7. Thorgeirsson, A. & Fregert, S.: Allergenicity of epoxy resins in the guinea pig. *Acta Dermatovener (Stockholm)* 57: 253, 1977.

Received May 9, 1977

S. Fregert, M.D.
Section of Occupational Dermatology
University Hospital
S-221 85 Lund
Sweden