

Review Article

Quenching: fact or fiction?

DAVID BASKETTER

SEAC Toxicology Unit, Unilever Research Colworth, Colworth House, Sharnbrook, Bedford UK

Fragrance chemicals are a frequently reported cause of allergic contact dermatitis (ACD), a matter which has recently come into considerable prominence, to the point that legislation in Europe is under serious consideration. Certain skin-sensitizing fragrance chemicals have been reported by the producing industry to be rendered safe (quenched), at least in terms of ACD, when they are used in the presence of a specific quenching agent. Accordingly, it seemed timely to review this apparent quenching phenomenon, considering the available data and potential mechanistic hypotheses that might be used to explain it. If it is correct, it should be a phenomenon of potentially enormous value in the elimination of the allergenic properties of at least a proportion of common skin sensitizers. Whilst there is some evidence in man for the occurrence of quenching during the induction of skin sensitization, a much more substantial body of work has failed to find supportive evidence in various animals models, at a chemical level or at elicitation in human subjects with existing allergy. On balance, it is concluded that quenching of fragrance allergens is a phenomenon still awaiting positive evidence of existence.

Key words: quenching; fragrance; cinnamal; citral; phenylacetaldehyde; eugenol; *d*-limonene; α -pinene; phenylethyl alcohol; dipropylene glycol; contact allergy; allergic contact dermatitis; chemical basis; immunological basis. © Munksgaard, 2000.

Accepted for publication 5 May 2000

The concept that the skin sensitising activity of one chemical might be overcome by the presence of another chemical was given life by the publication of Opdyke (1) a quarter of a century ago. In this all too brief publication, the term “quenching” was employed to describe the complete abrogation of the sensitizing potential of 3 fragrance chemicals (cinnamal, citral and phenylacetaldehyde) by the presence of certain other fragrance chemicals (notably eugenol and limonene) at defined ratios to the sensitizing agent. The conclusions were supported by a summary of human predictive test data. Unfortunately, the absence of any details (numbers tested, reproducibility, etc.) have tended to compromise the credibility of the report. Subsequently, the International Fragrance Research Association (IFRA) used the observations as a means to limit the sensitizing activity of perfumes containing the 3 sensitizing fragrance chemicals (2). Since that time, only a modest number of publications have examined in further detail the question of quenching, some lending credence to the original observations, others casting substantial doubt upon it. It is the purpose of this paper to

examine the evidence both for and against quenching, particularly in the light of our current knowledge of the chemistry and biology of allergic contact dermatitis (ACD). The review is structured to provide a reminder of the original observations and then considers the 3 points at which quenching might operate, at the chemical level, during the induction of skin sensitization or during the elicitation of ACD. Finally, some concluding thoughts are given.

Quenching: the Origins

In the 1st paper on quenching, Opdyke (1) reported that the investigations had been prompted by the difference between the sensitizing activity of certain fragrances compared to the non-sensitizing activity of the essential oils from which they were derived. The original experimental observations have been summarized in Table 1. As can be seen, the information is somewhat limited. The sensitizing activity of cinnamal in man was reported to be eliminated by the presence of an equal concentration of eugenol; citral's sensitizing activity was

Table 1. Original quenching observations*

Sensitizing agent	Quenching agent	Ratio	Total fragrance concentration (%)	Result of human maximization test
cinnamal	none		2	+
cinnamal	eugenol	1:1	6	—
cinnamal	limonene	1:1	6	+
citral	none		4	+
citral	limonene	4:1	5	—
lemongrass oil ^{a)}	(none)		4	—
phenylacetaldehyde	none		2	+
phenylacetaldehyde	dipropylene glycol	1:1	4	—

*Adapted from ref. (1) with permission.

^{a)}Contains 85% citral, arguably with natural quenching agents present.

eliminated by the presence of *d*-limonene or α -pinene at a ratio of 4:1; phenylacetaldehyde allergy was blocked by equal parts of either phenylethyl alcohol or dipropylene glycol. 4 years later these details were brought into the IFRA guidelines as a

way of reducing the sensitization risk associated with cinnamal, citral and phenylacetaldehyde (2). It is not appropriate here to regurgitate much historic data from both guinea pig and human predictive models, which clearly had demonstrated that these (unquenched) materials were all moderate to strong allergens.

At the time of writing this review, no further data to support the original observations had been placed into the peer-reviewed literature by the fragrance industry, although it is known that work is in progress.

Quenching: the Chemistry

The pairs of chemical structures normally involved in the quenching story are presented in Figs. 1–3. It is not clear from these structures that there would be an obvious simple chemical reaction/interaction, a neutralization, such as occurs with an acid and a base. Indeed, if such were to occur, the fragrance properties of the chemicals would be changed and/or destroyed. There is no aromatic evidence for such an event – no significant change in odour.

The common feature of the sensitizing agents is that they are all organic aldehydes. It is very well known that other such aldehydes are important contact allergens, e.g., formaldehyde and glutaraldehyde. Aldehydes can form Schiff bases with amino groups, such as that of lysine. This is how they may haptenize skin proteins and so behave as skin sensitizers (3). Such a proposition was made for cinnamal (4, 5). However, it has not been demonstrated that it is the aldehydic function of the sensitizing fragrance chemicals involved in the quenching story that is the important in terms of their haptenic reactivity. In work to be reported in more detail elsewhere, but summarized in Table 2, it appears that the ability to form Schiff bases does not correlate well with the relative potency of a range of sensitizing aldehydes. In reality, cinnamal



Cinnamal

Eugenol

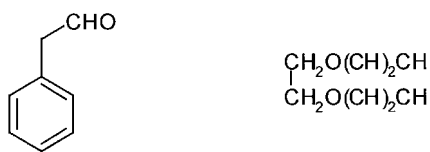
Fig. 1. The cinnamal and eugenol quenching pair.



Citral

d-Limonene

Fig. 2. The citral and limonene quenching pair.



Phenylacetaldehyde

Dipropylene glycol

Fig. 3. The phenylacetaldehyde and dipropylene glycol quenching pair.

Table 2. Sensitization potential versus ability to form a Schiff base

	Class ^{a)}	Charge diff. C=O ^{b)}
cinnamal	S	0.4957
phenylacetaldehyde	S	0.4768
4-tert-butylhydrocinnamal	S	0.4677
phenylpropionaldehyde	S	0.4787
α -methyl-4-isopropylphenylpropionaldehyde	S	0.4799
4-methylphenylacetaldehyde	S	0.4787
methyl cinnamal	M	0.5167
amyl cinnamal	M	0.5167
hexyl cinnamal	M	0.5059
butyl cinnamal	M	0.5073
4-tert-butyl- α -methyl hydrocinnamal	M	0.4797
4-methyl hydrocinnamal	W	0.4736
vanillin	W	0.5173
ethyl vanillin	N	0.5204
4-methoxybenzaldehyde	N	0.5242
3,4-methylene dioxybenzaldehyde	N	0.5185
benzaldehyde	N	0.5171

^{a)} Relative skin sensitization class based on judgement of existing predictive test data. S=strong, M=moderate; W=weak; N=non-sensitizer

^{b)} Charge difference across the aldehyde carbonyl group, the larger the number, the greater the propensity to form a stable Schiff base.

might react via Michael addition across its aliphatic double bond, rather than forming a Schiff base. This mechanism would be more consistent with the observation that hexyl cinnamal is a weaker allergen – the alkyl side chain would have very little effect on Schiff base activity, but would reduce the likelihood of the Michael addition reaction. Potential reaction mechanisms for citral and phenylacetaldehyde are open to speculation, although formation of a Schiff base seems likely at least for the latter.

There has been a limited amount of work published which has searched for evidence of chemical interactions between the sensitizing and the quenching agents. Majeti and Suskind attempted to uncover evidence of either physical or chemical interactions, but without any success (6). Pittz and co-workers undertook similar investigations, but again were unable to identify a chemical mechanism for quenching (7). In France, Benezra (8, 9) worked with his colleagues on a number of studies of quenching. In particular, they used radiolabelled citral to investigate protein binding. The results showed that limonene caused an increase in overall binding to soluble compared to insoluble, protein. There is an implicit assumption that binding to insoluble protein would be associated with sensitization. In reality, the mechanism was not understood, nor was there any indication of whether it would

have any impact on sensitization. Interestingly, the data can be expressed in terms of the reduction in binding to insoluble protein, in which case the effect is of the order of only 20% – and this was in the presence of a 4 $\times$ higher concentration of limonene than normally would be required to block totally the sensitizing effect in man. It is reasonable to suggest now that much of the binding of reactive chemicals to random skin proteins (soluble or insoluble) is unlikely to be relevant to sensitization. Rather, it is the specific interaction with proteins, and notably cell-surface proteins on Langerhans cells, that seems likely to be the important step in the initiation of sensitization (10).

So, the initial conclusion from the above must be that there is no reasonably well substantiated (physico) chemical hypothesis with which to explain quenching phenomena.

Quenching: the Induction of Skin Sensitization

If quenching does not occur at the (physico)chemical level prior to skin contact, then the next conceivable point at which it might occur is during 1 or more stages of the processes involved in the induction of skin sensitization. The details of this complex immunobiological process can be found elsewhere (10, 11). However, we can consider a number of basic elements: skin penetration, haptenization of protein, skin metabolism, Langerhans cell activation and migration, and the stimulation of hapten-specific T-cell division in the draining lymph node. As far as this author is aware, there is no data, published or unpublished, which demonstrates that the quenching agents reduce the skin penetration, or more importantly bioavailability, of their sensitizing partner. Nor is there data on any of the other specific elements of the induction process.

Clearly, 1 way around this shortage of detailed mechanistic information is to examine the process as a whole. Here, there is a relative wealth of information from the examination of possible quenching in predictive models. In the work of the Strasbourg group, using a guinea pig predictive test, the effect of limonene on the induction of skin sensitization to citral, unfortunately, was not examined (8). This variable was investigated thoroughly, however, in an extensive series of investigations using a variety of guinea pig models (12, 13). The outcome of these studies can be summarized quite simply – no quenching effect could be demonstrated.

Let us consider the outcome of the published guinea pig work (12, 13) in more detail. Studies were conducted in OECD standard assays (14), which encompass both adjuvant and non-adjuvant

tests, as well as in non-standard methods. This meant that all the variables of dose level, dosing route (intradermal injection, epicutaneous application) and use of Freund's complete adjuvant were explored in relation to quenching. In no case, in more than 20 individual tests, was evidence of quenching demonstrated with combinations of cinnamal/eugenol (1:1) or citral/limonene (4:1). It should be noted that the extent of sensitization in all of these tests was assessed via both the frequency and the intensity of the reactions elicited by challenge with unquenched material. Hence, it was specifically quenching during induction that was examined.

As maturation of the quenching pair was thought to be important, guinea pig tests were carried out on material aged for up to 12 months (13). There was no change in the intensity of the induced sensitization and no difference between the response to the quenched and unquenched material.

More recently, a new predictive sensitization test has been developed, the murine local lymph node assay (LLNA). This assay has been fully evaluated as an alternative to the standard guinea pig tests and is regarded as a fully validated alternative (15). In contrast to the guinea pig methods, the LLNA measures directly the lymphocyte proliferative response in the lymph node draining the site of chemical application; the basic protocol provides dose response. Furthermore, recent work has shown that the dose-response data can be interrogated to provide an estimate of the relative sensitizing potency of the test substance applied (16). This is the estimated concentration required to generate a $3\times$ stimulation of proliferation in the draining lymph node, the EC3 value. Using this approach, the impact of quenching agents on the sensitizing potency of cinnamal and citral was examined. The results have been published (17), but are summarized in Table 3. The clear outcome was that no quenching effect could be demonstrated in this mouse model.

Notwithstanding these results in animal models, the original publication of Opdyke (1) is quite clear that the quenching phenomenon had taken place in

Table 4. Human data on quenching of citral sensitization

Test material	Proportion of test subjects sensitized in human maximization testing ^{a)} (%)
citral	20
citral + limonene	0
citral + eugenol	64

^{a)} Results taken from RIFM compiled data (18).

the human skin sensitization tests that were conducted in the 1970s. The lack of any detailed information on these precludes further commentary, however. More recently, the author has had access to more detailed summaries of the work done by the fragrance industry (18). In the course of approximately 50 widely varied human sensitization studies, quenching was investigated. An overview for citral/limonene is presented in Table 4, where the efficacy of quenching of citral by limonene, but not by eugenol (the cinnamal "partner"), seems to be clearly demonstrated. Interestingly, eugenol may have increased the rate of reaction, but since it is also a sensitizer and was not challenged separately, it is not possible to conclude with absolute certainty that all of the reactivity is due to citral.

Quenching: the Elicitation of Skin Sensitization

Once skin sensitization has been induced, then subsequent contact with the offending chemical above a threshold dose will result in the elicitation of ACD. So, can quenching be shown to operate at this level? As with induction, a substantial body of work has been carried out in the guinea pig model (12, 13). Studies were conducted with cinnamal/eugenol and citral/limonene. In this system, whether animals were highly sensitized or whether relatively weakly sensitized, there was no difference in the abilities of quenched and unquenched material to elicit reactions. This was demonstrated when sensitization had been induced either with quenched or with unquenched material.

In addition to this work, investigations were also made on the effect of ageing the perfume mixture (13, unpublished Unilever data). Whether material was tested (in the guinea pig) immediately after mixing or after up to 12 months ageing, there was no quenching effect demonstrable at the elicitation phase of skin sensitization.

The efficacy of quenching agents during elicitation has been examined in man using a small panel of subjects with proven clinical allergy to cinnamal (12). Patch testing was carried out for 48 h with a range of doses of cinnamal in the presence and absence of eugenol. There was no difference at any of the test sites between the quenched and

Table 3. Results of the investigation of quenching in the LLNA

Test material	LLNA EC3 ^{a)} value (%)
cinnamal	2.0
cinnamal/eugenol (1:1)	2.1
citral	13
citral/limonene (4:1)	13

^{a)} EC3=the estimated concentration necessary to cause a $3\times$ stimulation of proliferation (compared to vehicle treated controls) in lymph nodes draining the site of chemical application.

the unquenched material. This included sites where the elicitation reaction was very weak, suggesting that there was not even a minor inhibitory effect produced by eugenol. In this panel, 20 min patches were also applied so that non-immunologic contact urticaria from cinnamal and its known inhibition by eugenol could be examined. The panel reacted as expected and eugenol inhibited the urticaria to the same extent as in a non-cinnamal-allergic group (19). In several individuals, after 1–2 days, a delayed allergic reaction to this brief treatment with cinnamal occurred; remarkably there was an identical level of cinnamal-induced allergic reaction at the site where there had been inhibition of the urticarial reaction by eugenol. This indicated quite clearly that there was no functional relationship between the urticarial mechanism and the subsequent delayed allergic response.

Quenching: other Considerations

Consideration of the information presented above leads to the inevitable question: if one fragrance ingredient can quench the sensitising action of another, at what stage of the sensitization process does this occur and by what mechanism? It is clear from the experimental evidence that quenching does not occur during the elicitation process. Thus the response must be that any quenching occurs during the induction of skin sensitization. The mechanism by which it might occur is difficult to imagine. All the quenched materials are aldehydes – might this be of significance? Although interactions with the quenching agents have been looked for, no specific mechanisms have been found (6, 7). However, since there is no certainty regarding the reaction mechanism for protein derivation by cinnamal, citral or phenylacetaldehyde, it is possible that the necessary types of interaction have been missed. Suskind & Majeti (6) provided initial observations which suggested that competition for amino acid binding sites might be at least a part of the mechanism of quenching. However, it is hard to understand why the quenching agent does not then act as a good sensitizer; for example, eugenol is well known as a contact allergen (e.g., 20). If it blocks cinnamal allergy by competing for its binding sites (necessarily with great efficacy, since quenching is apparently a complete inhibition), then one might reasonably expect to see more contact allergy to eugenol than cinnamal.

The specificity of the putative quenching effect brings its own consequences – it means that mechanisms involving inhibition of general biologic events during the induction of sensitization, such as blocking migration of Langerhans cells to draining lymph nodes, or invoking general chemical options

such as sacrificial oxidation of the quenching partner, are inadequate as an explanation. Eugenol quenches cinnamal; limonene either has no effect (guinea pig model) (12, 13) or may enhance the sensitizing activity of cinnamal (man) (18). This suggests either a specific chemical interaction, or a specific immunobiological event, such as haptenic competition. The latter possibility is hard to comment upon without at least some knowledge of the chemistry that is actually involved in the induction of sensitization to the chemicals involved. As mentioned above, cinnamal probably reacts directly with skin protein via Michael addition across the aliphatic double bond. Being a prohaptens, eugenol will only sensitize following metabolic activation. Consequently, it is not easy to visualize how these would compete for protein sites (*vide supra*) in a way that would totally block the induction of sensitization.

Another question that can be posed is whether the introduction by the fragrance industry of the requirement to quench the 3 sensitizing materials (2) has had any impact on the incidence of ACD. Unfortunately, neither citral nor phenylacetaldehyde are commonly investigated allergens clinically, so it is not possible to detect any alteration to the frequency of their detection. In relative contrast, cinnamal has been tested over many years due to its incorporation into the fragrance mix (21). Certain clinics have taken the trouble to patch test with the individuals allergens of this mix and report the data. However, before making any comments on that information, it is important to sound a serious note of caution. The frequency of positive diagnostic patch tests to any chemical depends to a very great extent on human exposure to the substance, in combination with dermatologic referral and patch testing practice. Only where these matters are well understood can further interpretation be applied to the data. So, what information exists? The St John's Institute of Dermatology contact dermatitis clinic in London has published data for cinnamal patch testing over a period of some 18 years (22). This shows a steady decline in the incidence of positive reactions, down to a level which is currently close to zero. Might this reflect the introduction of quenched cinnamal from the late 1970s onwards? It cannot be concluded that it does since, even in the St John's clinic, referral patterns and patch test practice have not been completely constant over almost 2 decades. Perhaps more importantly, there is no evidence that human skin exposure of the type that would lead to skin sensitization is the same now as over the preceding 20 years. Other patch test data for cinnamal does not show the same declining trend (23, 24). Thus, convincing clinical evidence of the efficacy of quenching is lacking.

Conclusions

The evidence for existence of the quenching phenomenon lies at present exclusively in the largely unpublished observations of the fragrance industry, with that data arising from the conduct of a number of human sensitization tests. The claim is that the skin sensitizing activity of cinnamal, citral and phenylacetaldehyde can be completely blocked by the presence of a specific quenching agent during the induction, but not the elicitation, of sensitization. However, no subsequent evaluation of quenching has been able to reproduce the effect in animal models of sensitization. These are models which have been well proven to be valuable in the prediction of human sensitization (25, 26). In 1 case, the LLNA, quantitative measurements of the induction of sensitization in the mouse have demonstrated the absence of any quenching effect. This is made all the more significant in view of the recent demonstration that this model may even provide an indication of the relative potency of allergens for man (27). Furthermore, no satisfactory (physico)-chemical or immunobiological basis/hypothesis for a quenching activity can readily be evinced, particularly since it would appear to be required to operate only during induction in man. Thus, on the balance of the evidence presently available, the existence of quenching of certain fragrance allergens by other specific fragrance components should be regarded as a hypothesis still lacking substantive proof. The reliable use of the quenching phenomenon to reduce sensitizing activity by design (28) remains elusive.

References

- Opdyke D L J. Inhibition of sensitization reactions induced by certain aldehydes. *Fd Cosmet Toxicol* 1976; 14: 197–198.
- International Fragrance Research Association. *Code of Practice*. IFRA, Geneva, 1980.
- Dupuis G, Benezra C. *Allergic contact dermatitis to simple chemicals*. New York: Marcel Dekker, Inc: 1982.
- Fisher A A, Dooms-Goossens A. The effect of perfume "ageing" on the allergenicity of individual perfume ingredients. *Contact Dermatitis* 1976; 2: 155–159.
- Majeti V A, Suskind R R. Mechanism of cinnamaldehyde sensitization. *Contact Dermatitis* 1977; 3: 16–18.
- Suskind R R, Majeti V A. Occupational and environmental allergic problems of the skin. *J Dermatol* 1976; 3: 3–12.
- Pittz E P, Singer E J, Stankavich P, Drewnowski B. *Mechanisms of quenching of contact dermatitis due to some aldehydes in perfumes*. Society of Toxicology Annual Meeting, Washington DC, 1980, abstr. #399.
- Hanau D, Grosshans E, Barbier P, Benezra C. The influence of limonene on induced delayed hypersensitivity to citral in guinea pigs (I). Histological study. *Acta Dermatovenereologica* 1983; 63: 1–7.
- Barbier P, Benezra C. The influence of limonene on induced delayed hypersensitivity to citral in guinea pigs (II). Label distribution in the skin of ^{14}C -labelled citral. *Acta Dermatovenereologica* 1983; 63: 93–96.
- Breit R. The Langerhans cell: the master key to contact dermatitis: a hypothesis. *Arch Dermatol Res* 1982; 272: 401–405.
- Basketter D A, Gerberick G F, Kimber I, Willis C. Contact sensitisation mechanisms. In: *Toxicology of contact dermatitis*. Chichester: Wiley, 1999: 73–112.
- Basketter D A, Allenby C F. The quenching of contact hypersensitivity reactions. *Contact Dermatitis* 1991; 25: 160–171.
- Basketter D A, Allenby C F. The quenching of contact hypersensitivity reactions. In: Czernielewski J M (ed): *Immunological and pharmacological aspects of atopic and contact eczema*, vol 4. Basel: Karger, 1991: 224–228.
- Organisation for Economic Cooperation and Development. *Skin Sensitisation Test Guideline 406*. Paris, 1992.
- Gerberick G F, Ryan C A, Kimber I, Dearman R J, Lea L J, Basketter D A. Local lymph node assay validation assessment for regulatory purposes. *Am J Cont Derm* 2000; 11: 13–18.
- Basketter D A, Lea L, Cooper K, Dickens A, Briggs D, Pate I, Dearman R J, Kimber I. A comparison of statistical approaches to derivation of EC3 values from local lymph node assay dose responses. *J Appl Toxicol* 1999; 19: 261–266.
- Basketter D A. The value of animal assays and the quenching phenomenon. In: Frosch P J, Johansen J D, White I (ed): *Proceedings of the Fragrance Symposium*. Heidelberg: Springer-Verlag, 1997: 166–174.
- RIFM quenching summary 1999.
- Safford R J, Allenby C F, Basketter D A, Goodwin B F J. Immediate reactions to fragrance chemicals and a study of factors influencing contact urticaria to cinnamal in humans and guinea pigs. *Br J Derm* 1990; 123: 595–606.
- Rietschel R L, Fowler J F. *Fisher's Contact Dermatitis*, 4th edition. Baltimore: Williams and Wilkins, 1995: 450–451.
- Larsen W A. Perfume dermatitis. *Arch Dermatol* 1977; 113: 623–626.
- Buckley D A, Wakelin S H, Seed P T, Holloway D, Rycroft R J G, White I R, McFadden J P. The frequency of fragrance allergy in a patch test population over a 17 year period. *Brit J Dermatol* 2000; 142: 279–283.
- De Groot A C, Frosch P J. Adverse reactions to fragrances. A clinical review. *Contact Dermatitis* 1997; 36: 57–86.
- Katsarou A, Armenaka M, Kalogeromitros D, Koufou V, Georgala S. Contact reactions to fragrances. *Ann Allergy Asthma Immunol* 1999; 82: 449–455.
- Andersen K E, Maibach H I. Guinea pig sensitization assays: an overview. In: Andersen K E, Maibach H I (eds): *Contact allergy predictive tests in guinea pigs. Current Problems in Dermatology*, New York: Karger, 1985: 14: 59–106.
- Botham P A, Basketter D A, Maurer Th, Mueller D, Potozkar M, Bontinck W J. Skin sensitization – a critical review of predictive test methods in animal and man. *Food Chem Toxicol* 1991; 29: 275–286.
- Basketter D A, Blaikie L, Dearman R J, Kimber I, Ryan C A, Gerberick G F, Harvey P, Evans P, White I R, Rycroft R J G. Use of the local lymph node assay for the estimation of relative contact allergenic potency. *Contact Dermatitis* 2000; 42: 344–348.
- Dooms-Goossens A. Reducing sensitizing potential by pharmaceutical and cosmetic design. *J Amer Acad Dermatol* 1984; 10: 547–553.

Address:

D. A. Basketter
SEAC Toxicology Unit
Unilever Research Colworth
Sharnbrook, Bedford, MK44 1LQ
UK