THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

POSITION PAPER

ON

THE PHENOMENON OF

QUENCHING

adopted by the SCCNFP during the 11th plenary meeting
of 17 February 2000

1. Introduction

The concept that the skin sensitising activity of one chemical might be overcome by the presence of another chemical was introduced by the publication of Opdyke in 1976 (1). In this publication the term "quenching" was employed to describe the complete abrogation of the sensitising potential of 3 fragrance chemicals (cinnamaldehyde, citral and phenylacetaldehyde) by the presence of certain other fragrance chemicals, notably eugenol and limonene, at defined ratios to the sensitising agent. The conclusions were supported by a summary of human predictive test data.

The International Fragrance Research Association (IFRA) used the observations purportedly to limit the sensitising potential of fragrance compounds containing the three sensitising fragrance chemicals (2). Few publications have examined in detail the question of quenching; some supporting the original observations, others casting doubt upon them.

This position paper examines the evidence both for and against quenching, with particular relevance to the current understanding of the chemistry and biology of allergic contact dermatitis (ACD). The paper provides a reminder of the original observations and then reviews the 3 points at which quenching might operate, 1) at the chemical level, 2) during the induction of skin sensitisation or 3) during the elicitation of ACD.

2. Quenching : the origins

The induction of hypersensitivity to cinnamaldehyde in man was reported to be eliminated by the presence of an equal concentration of eugenol; citral's sensitising activity was 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 (1). Four years later these observations were brought into the IFRA guidelines as a possibility to reduce the sensitisation risk associated with cinnamaldehyde, citral and phenylacetaldehyde (2), acknowledged to be moderate to strong allergens.

3. Quenching : the chemistry

The pairs of chemical structures often discussed in quenching are in Figures 1-3. There is no evidence for an obvious chemical reaction/interaction. If such were to occur, the fragrance properties of the chemicals would be altered.

The common feature of the sensitising agents is that they organic aldehydes. It is very well know that other aldehydes are important contact allergens, eg formaldehyde and glutaraldehyde. Aldehydes can form Schiff's bases with amino groups, such as that of lysine. This is how they may haptenise skin proteins and so behave as a skin sensitiser (3). Such a proposition was made for cinnamaldehyde (4, 5). However, it has not been demonstrated that it is the aldehydic function of the sensitising fragrance chemicals involved in quenching that is the important in terms of their haptenic reactivity. From the summary in Table, it appears that the ability to form

Schiff's bases does not correlate with the relative potency of a range of sensitising aldehydes. Thus cinnamaldehyde might react via Michael addition across its aliphatic double bond rather than forming a Schiff's base. This mechanism would be more consistent with the observation that hexyl cinnamaldehyde is a weaker allergen - the alkyl side chain would have very little effect on Schiff's base activity, but would reduce the likelihood of the Michael addition reaction. Reaction mechanisms for citral and phenylacetaldehyde are open to speculation.

There has been a limited amount of work published which has searched for evidence of chemical interactions between the sensitising 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). Benezra and his group presented a number of studies on quenching (8, 9). 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. It was assumed that binding to insoluble protein would be associated with sensitisation. However, the mechanism was not understood, nor was there any indication of whether it would have any impact on sensitisation. In the presence of a fourfold higher concentration of limonene than normally would be required to block the sensitising effect in man there was about a 20% reduction as measured by binding to insoluble protein.

Nowadays it is believed that much of the binding of reactive chemicals to random skin proteins (soluble or insoluble) is unlikely to be relevant to sensitisation. Rather it is the specific interaction with cell surface proteins on Langerhans cells that seems likely to be the important step in the initiation of sensitisation (10).

The above suggests that there is no reasonably well substantiated (physico)chemical hypothesis with which to explain quenching.

4. Quenching : the induction of skin sensitisation

If quenching does not occur at the (physico)chemical level prior to skin contact, then it might occur in vivo during one or more stages of the processes involved in the induction of skin sensitisation. Details of this process are available (10, 11). However, there are a number of basic parameters: skin penetration, haptenisation by protein, skin metabolism, Langerhans cell activation and migration, and the stimulation of hapten specific T cell division in the draining lymph node. There is no available data demonstrating that the quenching agents reduce the skin penetration, or the bioavailability, of their sensitising partner. Nor is there data on any of the other specific components of the induction process.

In the work of Benezra using a guinea pig predictive test, the effect of limonene on the induction of skin sensitisation to citral unfortunately was not examined (8). This variable was investigated in an extensive series of investigations using a number of guinea pig models (12, 13). No quenching effect could be demonstrated.

These studies were conducted in accordance with OECD guidelines (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 cinnamaldehyde/eugenol (1:1) or citral/limonene (4:1). It should be noted that the extent of sensitisation in all of these tests was assessed via both the frequency and the intensity of the reactions elicited by challenge with unquenched material.

Guinea pig tests were carried out on quenching pair materials aged for up to 12 months (13). There was no change in the intensity of the induced sensitisation and no difference between the response to the quenched and unquenched material.

In contrast to the guinea pig methods, the murine local lymph node assay (LLNA) (15) measures directly the lymphocyte proliferative response in the lymph node draining the site of chemical application; the basic protocol provides dose response. The dose response data can be analysed to provide an estimate of the relative sensitising potency of the test substance applied (16). This is the estimated concentration required to generate a 3 fold stimulation of proliferation in the draining lymph node, the EC3 value. Using this approach, the impact of quenching agents on the sensitising potency of cinnamaldehyde and citral has been examined. The results have been published (17), but are summarised in Table 2. The clear outcome was that no quenching effect could be demonstrated in this mouse model.

Notwithstanding these results in animal models, from the original publication of Opdyke in 1976 (1) is clear that quenching had taken place in the human skin sensitisation tests that were conducted in the 1970s. The lack of any detailed information on these precludes further commentary however. More detailed summaries of the work done by the Fragrance Industry have become available (18). In the course of approximately 50 widely varied human sensitisation studies, quenching was investigated. An overview for citral/limonene is presented in Table 3, where the efficacy of quenching of citral by limonene, but not by eugenol (the cinnamaldehyde "partner") seems to be clearly demonstrated. Interestingly, eugenol may have increased the rate of reaction, but since it is also a sensitiser and was not challenged separately, it is not possible to conclude with absolute certainty that all of the reactivity is due to citral.

5. Quenching : the elicitation of skin sensitisation

Once skin sensitisation has been induced, then subsequent contact with the offending chemical above a threshold dose will result in an elicitation of ACD. That leads to the question 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 cinnamaldehyde/eugenol and citral/limonene. In this system, whether animals were highly sensitised or weakly sensitised, there was no difference in the abilities of quenched and unquenched material to elicit reactions. This was demonstrated when sensitisation had been induced either with quenched or with unquenched materials.

In addition to this work, investigations were also made on the effect of ageing the perfume mixture (13, and 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 sensitisation.

The efficacy of quenching agents during elicitation has been examined in man using a small panel of subjects with proven clinical allergy to cinnamaldehyde (12). Patch testing was carried out for 2 days with a range of doses of cinnamaldehyde in the presence and absence of eugenol. There was no difference at any of the test sites between the quenched and 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 minute patches were also applied so that non-immunologic contact urticaria to cinnamaldehyde 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-cinnamaldehyde allergic group (19). In several individuals, after 1-2 days, a delayed allergic reaction to this brief treatment with cinnamaldehyde occurred; remarkably there was an identical level of cinnamaldehyde induced allergic reaction at the site where there had been inhibition of the urticarial reaction by eugenol. This indicated that there was no functional relationship between the urticarial mechanism and the subsequent delayed allergic response.

6. Quenching : other considerations

If one fragrance ingredient can quench the sensitising action of another, the question is at what stage of the sensitisation process does this occur and by what mechanism? It must be assumed that any quenching effect must occur during the induction of skin sensitisation. The mechanism by which it would remains unknown.

Although interactions with the quenching agents, chemically all aldehydes, have been looked for, no specific mechanisms have been detected (6, 7). Suskind and Majeti (6) provided initial observations which suggested that competition for amino acid binding sites might be a part of a possible mechanism of quenching. However, it is hard to understand why the quenching agent does not then act as a good sensitiser; for example eugenol is an important contact allergen (20). If it blocks cinnamaldehyde allergy by competing for its binding sites (necessarily with greater efficacy, since quenching is apparently a complete inhibition), then one might reasonably expect to see more contact allergy to eugenol than cinnamaldehyde.

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 sensitisation, such as blocking migration of Langerhans cells to draining lymph nodes, or invoking general chemical options such as sacrifical oxidation of the quenching partner, are inadequate as an explanation. Eugenol quenches cinnamaldehyde; limonene either has no effect (guinea pig model) (12, 13) or may enhance the sensitising activity of cinnamaldehyde (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 some knowledge of the biochemistry that is involved in the induction of sensitisation to the chemicals concerned. As mentioned above, cinnamaldehyde probably reacts directly with skin protein via Michael addition across the aliphatic double bond. Being a prohapten, eugenol will only sensitise following metabolic activation. Consequently it is not easy to visualise how these would compete for protein sites (vide supra) in a way that would totally block the induction of sensitisation.

The question remains as to whether the requirement to quench the 3 sensitising materials (2) as recommended by the fragrance industry of 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 reaction rate. In contrast, cinnamaldehyde has been tested over many years because of its incorporation into the fragrance mix (21). Certain clinics have patch tested with the individuals allergens of this mix and have report the data over a period of 18 years (22). However, the frequency of positive diagnostic patch tests to any chemical depends to a great extent on population exposure to the substance in combination with dermatological referral and patch testing practice. Only where these matters are understood can further interpretation be applied to the data. The St. John's clinic data from London shows a steady decline in the incidence of positive reactions, down to a level which is currently close to zero. It cannot be concluded that this reflects the introduction of quenched cinnamaldehyde from the late 1970s onwards since, even in the St. John's data, referral patterns and patch test practice have not been constant over almost 20 years. Perhaps more importantly, there is no evidence that human skin exposure of the type that would lead to skin sensitisation is the same now as over these preceding 20 years. Other patch test data for cinnamaldehyde does not show the same declining trend (23, 24). Thus, convincing clinical evidence of the efficacy of quenching is lacking.

7. Summary

The evidence for existence of the **quenching phenomenon*** lies at present exclusively in the largely unpublished observations of the fragrance industry. The claim is that the skin sensitising activity of cinnamaldehyde, citral and phenylacetaldehyde can be completely blocked by the presence of a specific quenching agent during the induction, but not the elicitation of sensitisation. However, no subsequent evaluation of quenching has been able to reproduce the effect in animal models of sensitisation. These are models which have been well proven to be valuable in the prediction of human sensitisation (25, 26). In one case, the LLNA, quantitative measurements of the induction of sensitisation 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 physical, chemical, biochemical or immunobiological basis/hypothesis for a quenching activity can be readily evinced; particularly since it would appear only to operate 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 only.

* Quenching ***in this context means that the presence of a distinct chemical substances, also used as an ingredient of a fragrance compound, will inhibit the sensitising capacity of another substance.***

8. Tables

Table 1 Sensitisation potential versus ability to form Schiff's base

	Charge difference[1]	**Charge rank[2]**	**GPMT rank[3]**
Cinnamicaldehyde	0.5070	6	1
Phenylacetaldehyde	0.4661	9	2
Bourgeonal	0.4646	10	3
Phenylpropionaldehyde	0.4719	7	4
Cyclamen aldehyde	0.4623	12	5
Syringa aldehyde	0.4670	8	6
Lilial P	0.4623	11	7
Hexyl cinnamicaldehyde	0.5097	5	8
Vanillin	0.5210	3	9
Ethyl vanillin	0.5220	1	10
Anisic aldehyde	0.5212	2	11
Heliotropin	0.5121	4	12

[1]Charge difference across the aldehyde carbonyl group, the larger the number the greater the ability to form a Schiff's base

[2]Relative rank position in terms of ability to form a Schiff's base

[3]Relative skin sensitisation potency based on judgement of existing data

Table 2 Results of the investigation of quenching in the LLNA

Test Material	**LLNA EC3 value**
Cinnamicaldehyde	2.0%
Cinnamicaldehyde/eugenol (1: 1)	2.1%
Citral	13%
Citral/limonene (4: 1)	13%

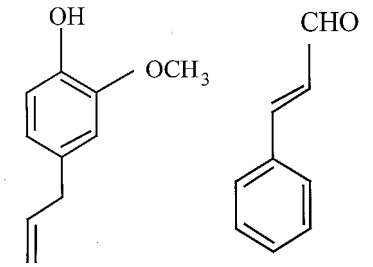
Table 3 Human data on quenching of citral sensitisation

Test Material	**Proportion of test subjects sensitised in human maximisation testing**[1]
Citral	20%
Citral + limonene	0%
Citral + eugenol	64%

[1]Results taken from RIFM compiled data (18)

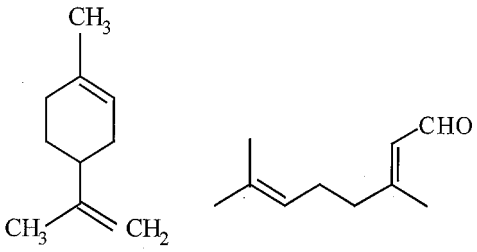
9. Figures

Figure 1 : the cinnamaldehyde and eugenol quenching pair



Eugenol Cinnamic aldehyde

Figure 2 : the citral and limonene quenching pair

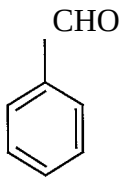


Limonene Citral

Figure 3 : the phenylacetaldehyde and dipropylene glycol quenching pair

CH_3 - CH(OH) - CH_2 – O - CH_2 - CH(OH) - CH_3

Dipropylene glycol



Phenylacetaldehyde

10. References

1. Opdyke D L J. Inhibition of sensitization reactions induced by certain aldehydes. Fd Cosmet Toxicol 1976:14: 197-198.
2. IFRA. Guideline. 1975.
3. Dupuis G, Benezra C. Allergic contact dermatitis to simple chemicals. New York. Marcel Dekker Inc. 1982.
4. Fisher A A, Dooms-Goossens A. The effect of perfume “ageing” on the allergenicity of individual perfume ingredients. Contact Dermatitis 1976: 2: 155-159.
5. Majeti V A, Suskind R R. Mechanism of cinnamaldehyde sensitization. Contact Dermatitis 1977: 3: 16-18.
6. Suskind R R, Majeti V A. Occupational and environmental allergic problems of the skin. J Dermatol 1976: 3: 3-12.
7. Pittz E P, Singer E J, Stankavich P, Drewnowski B. Mechanisms of quenching of contact dermatitis due to some aldehydes in perfumes. Abstract 399. Society of Toxicology Annual Meeting, Washington DC, 1980.
8. 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 Derm Venereol 1983: 63: 1-7.
9. Barbier P, Beneza C. The influence of limonene on induced delayed hypersensitivity to citral in guinea pigs. II. Label distribution in the skin of 14C-labellled citral. Acta Derm Venereol 1983: 63: 93-96.
10. Breit R. The Langerhans cell: the master key to contact dermatitis: a hypothesis. Arch Dermatol Res 1982: 272: 401-405.
11. Basketter D A, Gerberick G F, Kimber I, Willis C. Contact sensitisation mechanisms. In “Toxicology of Contact Dermatitis”, Wiley, Chichester 1999: 73-112.
12. Basketter D A, Allenby C F. The quenching of contact hypersensitivity reactions. Contact Dermatitis 1991: 25: 160-171.
13. Basketter D A, Allenby C F. The quenching of contact hypersensitivity reactions. In: Immunological and Pharmacological Aspects of Atopic and Contact Eczema. Vol 4, Eds Czernielewski J M, Karger, Basel, 1991, 224-228.
14. Organisation for Economic Cooperation and Development. Skin Sensitisation Test Guideline 406, Paris, 1992.
15. 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: in press.
16. 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.
17. Basketter D A. The value of animal assays and the quenching phenomenon. Proceedings of the Fragrance Symposium, Eds PJ Frosch, JD Johansen and I White, Springer-Verlag, Heidelberg, 1997: 166-174.
18. RIFM quenching summary 1999.
19. 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 cinnamaldehyde in humans and guinea pigs. Br J Derm 1990: 123: 595-606.

20. Rietschel R L, Fowler J F. Fisher's Contact Dermatitis, 4th edition, Williams and Wilkins, Baltimore 1995: 450-451.
21. Larsen W A. Perfume dermatitis. Arch Dermatol 1977: 113:623-626.
22. Buckley DA, Wakelin SH, Holloway D et al. The frequency of fragrance allergy in a patch test population over a seventeen-year period. Br. J Dermatol (2000) in press.
23. De Groot AC, Frosch PJ. Adverse reactions to fragrances. A clinical review. Contact Dermatitis 1997: 36: 57-86
24. Katsarou A, Armenaka M, Kalogeromitros D, Koufou V, Georgala S. Contact reactions to fragrances. Ann Allergy Asthma Immunol 1999: 82: 449-55
25. Andersen K E, Maibach H I. Guinea pig sensitization assays: An overview. In Contact allergy predictive tests in guinea pigs, Current Problems in Dermatology, Ed by K E Andersen and H I Maibach, Vol.14, New York: Karger 1985: 59-106.
26. Botham P A, Basketter D A, Maurer Th, Mueller D, Potokar M, Bontinck W J. Skin sensitization - a critical review of predictive test methods in animal and man. Food Chem Toxicol 1991: 29: 275-286.
27. 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: accepted.