www.cropwatch.org

THE FIRST TRULY INDEPENDENT WATCHDOG FOR THOSE WORKING WITH NATURAL AROMATIC MATERIALS

E: info@cropwatch.org T: ++44 (0)7771 872 521

Quenching: "A Cage in search of a Bird[1]."

[Slightly modified from a feature first published in *Cropwatch Newsletter* 6, June 2007].

[1. With sincere apologies to Frank Kafka].

Background.

The term 'quenching' referred to a phenomenon where it was considered that the induction of (skin) sensitizing potential of one fragrance ingredient could be nullified by the presence of another, thus rendering it safe. Opdyke (1976) had originally given examples of this process for three individual fragrance chemicals, triggered by observations with citral-containing essential oils which allegedly demonstrated lack of sensitization potential, compared with the weak to moderate sensitiser, citral.

Chemical:	Naturally occurs in:	Quenched by:
Cinnamaldehyde	Cassia, cinnamon oils etc	Eugenol 1:1
Citral (= mixture of neral + geranial)	Lemongrass, litsea cubeba & citrus oils etc	d-limonene or alpha-pinene 1:4 with citral
Phenylacetaldehyde	Minor component of many essential oils & fruits.	β-phenylethyl alcohol or dipropylene glycol 1:1

Table 1 – Examples of sensitizing chemicals which could be appropriately quenched, according to Opdyke (1976).

IFRA subsequently re-issued Standards for these ingredients (e.g. for citral: IFRA 1980) reflecting the fact that inhibition of the induction of sensitization could be prevented by addition of appropriate materials. In the case of citral, 25% of the concentration of d-limonene would be needed case of citral.

Many of us concerned with natural products have strongly and persistently maintained that the toxicological properties of individual substances within natural complex biological materials (such as essential oils) cannot necessarily be determined by toxicological studies conducted on isolated synthetic versions of the same chemical. One of the several arguments against this way of working is because matrix effects – the effects of co-occurring components in natural materials – are not considered. To some therefore, including many of those in the aromatherapy profession such as Robert Tisserand (Tisserand 2003), quenching

was seized upon as 'proof' of the fact that matrix effects could be shown to operate in complex biological materials. In fact more robust proof is now available, but we digress….

Some of us were less than convinced by the validity of the quenching hypothesis, however. Firstly, and foremost, no robust physiological biochemical / immunological mechanism to support the hypothesis had ever been advanced (N.B. we use the word 'robust', because those mechanistic suggestions which have been advanced - chemical interaction, altered absorption, anti-inflammatory activity, and competitive inhibition (Guin *et al.* 1984), are shot through with holes!). Secondly, the phenomenon was not reproduced satisfactorily, by either the original workers (who failed to properly document their original experimental conditions) or by subsequent investigators. Basketter (2000) wrote an excellent review paper on the subject entitled "Quenching: fact of fiction?", - the broad conclusion of which is that the quenching phenomena is 'a hypothesis without proof'. Other toxicologists have previously distanced themselves in print on this issue also. However some credence for the existence of quenching was thought to be gained from the work of Api & Isola (2000), who apparently showed that citral in conjunction with certain terpene/alcohol mixtures, inhibited the induction of skin sensitisation in humans, mimicking the conditions found in dermal exposure to certain essential oils.

In spite of Basketter's article mentioned above, Tisserand (2003) subsequently wrote a paper in support of the quenching hypothesis, citing the work of Hanau *et al*. (1983), Guin *et al* (1984) & Allenby *et al*. (1984). Basketter (2000) had previously argued that Hanau article, at least, related to the binding of chemicals to random skin protein (soluble or insoluble), and was therefore largely irrelevant, since it was only the specific binding, (probably to surface proteins of Langerhans cells), which was important in the initiation of sensitization, after the work of Breit (1982). The earlier work of Guin *et al.* (1984) & Allenby *et al*. (1984) cited by Tisserand, has to be viewed in context with Basketter's subsequent work with citral-limonene and cinnamaldehyde-eugenol mixtures using the guinea-pig maximization test, where no indication of quenching was found (Basketter 1991a, Basketter 1991b). Similarly more modern LLNA studies in mice seemed to indicate a similar conclusion to Basketter's in 2000.

In the same year (2000) the SCCP adopted a Position Paper on quenching (SCCNFP/0294/00) which concluded that "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" – Basketter's opinions & words more or less exactly (Basketter 2000).

Four years on, Lalko & Api (2004) of RIFM published a paper entitled "The potency of citral in the Local Lymph Node Assay" which considered the sensitizing potential of citral plus two citral-rich essential oils (Lemongrass oil & Litsea cubeba oil) applied in a 1:3 ethanol: DEP (!) vehicle (*Cropwatch comments*: deployment of DEP in this testing regime is surely a curious choice

given the strong anti-phthalate feelings amongst 'green' cosmetics consumers worldwide).

The authors found EC3 values (EC3 values are said to represent relative potency according to the methodology of Basketter *et al* 2000b) of 6.5% and 8.4% for Lemongrass & Litsea cubeba oils respectively, which was not significantly different from that for citral (6.3%). They further found little significant difference between citral quenched with limonene & straight citral (EC values of 0.8% & 1.2% respectively). Further, the authors found that in humans a NOEL of ~0.5% or 1400µg/cm^2 exists for the induction of sensitization to citral. In conclusion, Lalkol & Api conceded that Basketter (2000) had shown that quenching could not be demonstrated in conventional animal models such as the guinea-pig maximisation test, & concluded that the quenching effect of citral could not be demonstrated in the LLNA assay either.

Where this puts the credibility of the previous Opdyke (1976) & Api & Isola (2000) findings, where the induction of sensitization of citral-containing essential oils or citral-terpene or terpene alcohol mixtures was shown to be obviated, is not clear to us at present – presumably either these findings are to be ignored, or the interpretation of the results from earlier animal models & LLNA test has shortcomings which we need to know about. Either way, we don't seem to have been offered an explanation, and with so much credence apparently resting on the LLNA testing procedure in corporate toxicologist-led sensitization studies, Cropwatch feels that this matter should be cleared up.

Further, this incomplete study only seems to beg further questions – citral is the term describing a chemical mixture of structural isomers of neral & geranial in various ratios according to origin. Any effects due to isomer distribution were seemingly not evaluated or discounted. The purity of citral used was only 99.5% - effects of impurities were not mentioned The species & geographic origins of the Lemongrass oil were not stipulated (Lemongrass oils from *C. flexuosus* Nees ex Stued.*, C. citratus* (DC) Stapf. and *C. pendulus* Nees ex Stued.are all easily commercially available). Likewise, in practice, commercial 'Litsea cubeba oil' is distilled from several species apart from *Litsea cubeba* including *Litsea enosma* & *L. mollifolia.* Further, commercial Lemongrass & *Litsea cubeba* oils frequently contain adulterants like synthetic citral, and blind trust on industry to provide authentic oils 100% derived from the named botanical species is simply not professionally acceptable. Best practice dictates that the essential oil plant-source has to be identified at source by a suitably experienced botanist, and the derived oil batch subsequently lot-tracked to guard against interference (adulteration), or, that the investigators personally distill their own test material, as of course, many workers do. Finally, we have seen criticisms supporting the contention that the LLNA method is unreliable with weak sensitizers (presumably such as citral) and the results in this area can be open to interpretation. For industry to have any faith in this methodology, we need to understand these issues more completely.

IFRA Ducks Out.

In June 2004, according to the IFRA Hazards Working Group opinion "quenching phenomena" effects can still be taken into account (according to Section 3.3 of the EU Dangerous Preparations Directive 88/379/EEC); however quenching phenomena effects between eugenol and cinnamic aldehyde are now unsupported according to the Notification No 4. of 38th Amendment to the IFRA Standard."

On to more immediate times, the end-note of the revised citral standard under the IFRA's 40th Amendment 2007 quietly says (not of course, any apology to industry for the fact that their previous guidance may have been wrong!) but:

"This Standard cancels and replaces the existing one on citral, which was based on the no longer supported 'quenching' phenomenon."

So, in spite of all the previous confused messages, in 2007 (apparently) IFRA is indicating that it no longer supports quenching, period. You still may find this news is a little surprising, revealed as it is, buried at the bottom of a document. After all, it is only 3 years ago since Lalko & Api clearly stated "The quenching of sensitization to citral by the addition of d-limonene has been demonstrated in humans" (Lalko & Api 2004), and It is only 1 year ago (actually March 2006) since Api and her aroma-industry toxicology pals (including Basketter), reproduced the fact that under the existing IFRA Standard, citral has no concentration limit wrt their given example (hydroalcoholic products for unshaven skin, and in solid antiperspirant product types) (Api *et al.* 2006).

30 Years of Argument Ended over the Existence of a Quenching Effect.

So there you have it. Apparently we have to write off the findings a lot of previous published dermatological work (Nilsson *et al.* 2004, Safford *et al*. 1990, Guin *et al.* 1984, Allenby *et al.* 1984, Hanau *et al.* 1983, Api & Isola 2000 etc.) which seemingly helped to confirm the existence of a quenching effect. If we now accept this work as being flawed, it must beg many questions about the professional standing of much published research work in dermatology.

N.B. Cropwatch does not support animal testing of cosmetic ingredients in any shape or from, including the LLNA assay procedure, but merely reports above on data publicly available in the scientific literature.

References.

Allenby C F, Goodwin B F J, Safford R J (1984) "Diminution of immediate reactions to cinnamic aldehyde by eugenol." *Contact Dermatitis* **11**, 322-323

Api, A.M., Basketter D.A., Cadby P.A., Cano M.-F., Ellis G., Gerberik F. Griem P. McNamee P.M., Ryan C.A. & Saffor B. (2006) "Dermal sensitisation quantitative risk assessment (QRA) for fragrance assessment" - Paper presented at the *5th Annual Meeting & ToxExpo, San Diego, California Mar 5-9th 2006* – see http://www.rifm.org/doc/amapi%20poster%20sot%202006%20v2.pdf

Api, A.M. & Isola, D.A. (2000) "Quenching of citral sensitization demonstrated in a human repeated insult patch test." *The Toxicologist,* **54**(1), 154.

Basketter D.A. & Allenby C F. (1991a) “The quenching of contact hypersensitivity reactions.” *Contact Dermatitis* **25**, 160–171.

Basketter D.A. & Allenby C.F. (1991b) “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.

Basketter D. (2000) "Quenching: fact or fiction?" *Contact Dermatitis* **43**, 253–258.

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. (2000b) “Use of the local lymph node assay for estimation of relative contact allergenic potential.” *Contact Dermatitis*, **42**, 344-348.

Briet R. (1982) “The Langerhans cell: the master key to contact dermatitis: a hypothesis.” *Arch Dermatol Res* **272**, 401–405.

Guin J D, Meyer B N, Drake R D & Haffley P. (1984) “The effect of quenching agents on contact urticaria caused by cinnamic aldehyde.” *Journal of the American Academy of Dermatology* **10**(1), 45-51

Hanau D, Grosshans E, Barbier P, Benezra C. (1983) “The influence of limonene on induced delayed hypersensitivity to citral in guinea pigs. I. Histological study.” *Acta Derm Venereol* **63**,: 1-7..

IFRA (1980) Code of Practice - Citral Standard, Brussels, Belgium.

Lalko J. & Api A.M. (2004) “Citral in the local lymph node assay". Paper presented at the *American College of Toxicology (ACT) - November 7-10, 2004 - Palm Springs, CA*

Nilsson A-M., Jonsson C..; Luthman K.., J. Lars G., Nilsson J.L.G., Ann-Therese Karlberg A-T. (2004) "Inhibition of the Sensitizing Effect of Carvone by the Addition of Non-Allergenic Compounds." *Acta Dermato-Venereologica* **84**(2),99-105(7)

Opdyke D L J. (1976) Inhibition of sensitization reactions induced by certain aldehydes. *Fd Cosmet Toxicol* **14**, 197–198.

Safford R.J., Basketter D.A., Allenby C.F., Goodwin B.F.. (1990) "Immediate contact reactions to chemicals in the fragrance mix and a study of the quenching action of eugenol." *Br J Dermatol.* **123**(5), 595-606

.

Tisserand R (2003) “Skin sensitivity quenching in essential oils.”