

IS THERE EVIDENCE THAT METHYL HEPTINE CARBONATE CAUSES ALLERGIC CONTACT DERMATITIS?

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The fragrance material methyl heptine carbonate has been cited as a moderately frequent cause of cosmetics-related allergic contact dermatitis. A review of the literature shows that when the underlying clinical data are analyzed, there is only one published case where a possible causal link to a cosmetic product has been established. Predictive tests in a computational model, in animal systems, and in volunteers have demonstrated a significant sensitization potential for this substance but reports of clinical cases of allergy are rare with no new reports appearing in the last two decades. The infrequency of reported cases of allergy may be linked to risk management procedures put in place by the International Fragrance Association (IFRA) more than 20 years ago.

Keywords: Allergic contact dermatitis; Methyl heptine carbonate

INTRODUCTION

Methyl heptine carbonate (Fig. 1) has been cited as a moderately frequent cause of allergic contact dermatitis (1). The following reviews the published evidence relating to the sensitization potential of methyl heptine carbonate (2-octynoic acid, methyl ester, CASRN 111-12-6, EINECS 203-836-6). A semi-quantitative evaluation of the different reports cited below has been made in accordance with the rules outlined by Hostynek and Maibach (2) which are based on those made by Benezra and coworkers (3) in 1985. This approach has been used to examine six other substances that have also been cited as frequent causes of contact dermatitis: alpha-iso-methylionone (4), anisyl alcohol (5), linalool (6), amylcinnamic aldehyde (7), citronellol (8), and geraniol (9).

METHODS

The medical literature was searched using the electronic databases. Biosis, Caplu, Embase, RTECS, Toxlit, Medline/HealthStar, Toxnet, Science Citation Index (from 1960 to September 2003), and ISI Current contents (1997 through

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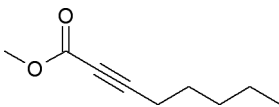


Figure 1 Methyl heptine carbonate.

January 2006). Search terms included “methyl heptine carbonate,” “allergic contact dermatitis,” “sensitization,” and “patch tests.” Standard textbooks on dermatology in English, French, and German were screened via their indices. Copies of all cited publications were obtained. The Research Institute for Fragrance Materials Inc. kindly made available copies of unpublished studies performed by its members or performed under its commission.

The semi-quantitative evaluation of the different reports obtained by these means was based on the method outlined in the study by Hostynek and Maibach (2). This is briefly outlined in the Appendix. Different criteria are applied to predictive studies and clinical patch tests, but both relate to the amount of detail provided. For clinical patch tests, top scores are attributed to those studies that eliminate false-positive reactions and attribute these unambiguously to the presence of the allergen in a specific product to which the patient has reacted.

RESULTS

Analysis of the Methyl Heptine Carbonate Structure for Contact Sensitization Potential by a Structure-Activity Relationship (QSAR) Model Predictive of Human Allergic Contact Dermatitis

A comprehensive quantitative structure-activity relationship model combining both structural alerts with barrier-diffusion criteria was developed by BIOSAR to predict cell-mediated immunity (allergic contact dermatitis) in humans (10). Trained and validated on 50 fragrance chemicals of common use, that model performed with 88% concordance, with 100% specificity in predicting nonallergens and 83% sensitivity for allergens. The BIOSAR model classified methyl heptine carbonate as an allergen.

Data from Predictive Tests on Animals

Several nonadjuvant tests have been performed on methyl heptine carbonate that indicate a significant sensitization potential (Table 1). Two reports of open epicutaneous tests carried out at 2%, and at the “maximum non irritant concentration” gave positive effects, which were not quantified (11,12) and which, due to the absence of experimental detail, are ascribed a degree of confidence of only 2. Another open epicutaneous test of higher reliability (confidence score of 3), in which induction was carried out at 3%, gave sensitization reactions at 0.3% (13,14). In a similar study by the same authors no reactions were observed when induction was carried out at 30% (14), but the absence of experimental details confers only low reliability (score of 2) to this study.

Table 1 Predictive testing of methyl heptine carbonate

Ref.	Test material identified	Type of test	Test conditions provided?	Was test fully maximized?	Were there adequate controls?	Was number of subjects sufficient?	Were results presented in adequate detail?	Final rating
11	No data	OET	No	No, at 2%	No data	No data	No	2
12	No data	OET	No	Max. non-irrit. concentration	No data	No data	No	2
13	No data	OET	Yes	10%	No data	No data	No	3
14	No data	OET	No	30%	No data	No data	No	2
15	No data	Draize	No	0.1%	No data	No data	No	1
16	“Commercial”	Draize (single induction)	Yes	Max. non-irritant dose	No data	10	No	3
17	No data	Draize (single induction)	Yes	Max. non-irritant dose	No data	10	No	3
18	No data	Draize (double induction)	Yes	Max. non-irritant dose	No data	10	No	3
19	No data	CET	Yes	Up to 3%	No data	20/dose	Yes	4
20	No data	CET	Yes	Up to 3%	No data	14–10/dose	Yes	4
21	No data	BT	Yes	Max. non-irrit. concentration	10 positive & 10 negative	20/dose	Yes	5
22	No data	Maguire	Yes	Max. non-irritant dose	Concurrent tests	8	Yes	3
23	No data	FCAT	Yes	“sub-irritant”	No data	No data	No	1
24	No data	GPMT	Yes	“sub-irritant”	No data	No data	No	1
25	No data	GPMT	Yes	Max. non-irritant dose	20 challenge & 20 vehicle	20/dose	Yes	5
26	No data	LLNA	Yes, OECD	Max. non-irritant dose	Zero dose group	6 groups of 4 animals (OECD)	Yes	5
27	No data	HMT	Yes	2% & SLS	Concurrent tests	25	Yes	2
28	No data	HMT	Yes	2% & SLS	Concurrent tests	25	Yes	2
29	No data	HMT	Yes	2% & SLS	Concurrent tests	25	Yes	2
30	No data	HMT(2×)	Yes	2% & SLS	Concurrent tests	25	Yes	2
31	No data	HMT(7×)	Yes	2% & SLS	Concurrent tests	19–25	Yes	2
32	No data	HMT(2×)	Yes	2% & SLS	Concurrent tests	25–26	Yes	2
33	No data	HMT	Yes	2% & SLS	Concurrent tests	27	Yes	2
34	No data	HMT(2×)	Yes	2% & SLS	Concurrent tests	25	Yes	2

(Continued)

Table 1 Continued

Ref.	Test material identified	Type of test	Test conditions provided?	Was test fully maximized?	Were there adequate controls?	Was number of subjects sufficient?	Were results presented in adequate detail?	Final rating
31	No data	Single patch	Yes	5%	No data	45	Yes	2
31	No data	Single patch	Yes	20%	No data	37	Yes	2
31	No data	Single patch	Yes	30%	No data	10	Yes	2
32	No data	HRIPT	Yes	0.25%	Historical controls only	95	Yes	2
33	No data	HRIPT	Yes	Only 0.1%	Historical controls only	82	Yes	2
34	No data	HRIPT	Yes	Only 0.1%	Concurrent tests	36	Yes	2
35	No data	HRIPT	Yes	Only 0.1%	Concurrent tests	33	Yes	2
36	No data	HRIPT	Yes	Only 0.1%	Concurrent tests	71	Yes	2
34	No data	HRIPT	Yes	Only 0.05% with 0.05% methyl octane carbonate	Concurrent tests	37	Yes	2
37	No data	HRIPT	Yes	Only 0.05% with 0.05% methyl octane carbonate	Concurrent tests	64	Yes	2

Note: OET: Open Epicutaneous Test, Draize Guinea Pig Sensitization Test; CET: Closed Epicutaneous Test; BT: Buehler Test, Maguire Test; FCAT: Freund's Complete Adjuvant Test; GPMT: Guinea Pig Maximization Test; LLNA: Local Lymph Node Assay; OECD: According to OECD Guidelines; HMT: Human Maximization Test; SLS: sodium lauryl sulfate; HRIPT: Human Repeated Insult Patch Test.

An incompletely reported Draize test (score of 1) carried out on guinea pigs gave no sensitization at intradermal induction and challenge doses of 0.1% (13). A modified Draize test (only one intradermal injection) on guinea pigs at a concentration of 0.05% for induction and 10% for topical challenge gave an unspecified proportion of sensitization reactions (15). This was ascribed a degree of confidence of 3. Another similarly modified Draize test, in which the induction concentration was the “minimally irritant dose” with challenge at the maximum nonirritant dose, gave 4 reactions in 10 guinea pigs (16) but could not be ascribed a higher confidence level than 3 due to the incompleteness of experimental details. When the same workers modified the above procedure to include two induction doses, the proportion of animals reacting rose to 8 of 10 (16).

A closed epicutaneous test (Buehler method) of high quality (score of 4) using an induction concentration of 3% gave 14 of 20 reactions on challenge at 0.3%, 15 of 20 at 0.9%, and 17 of 20 at 3% (17). A similarly high quality closed epicutaneous tests (score of 4) produced 13 of 13 reactions on initial challenge at 0.3, 0.9, and 3.0%, but rechallenge after 7 days gave 1 of 10 reactions at 0.3%, 4 of 10 at 0.9%, and 1 of 10 at 3% (18).

A good quality Buehler test (score of 5) in which 20 guinea pigs were treated with an induction dose of 2.5% gave 14 of 20 reactions to a challenge dose of 5%, 12 of 20 to a challenge dose of 1.5%, and 9 of 20 reactions at 0.5% (19).

Predictive tests using Freund’s complete adjuvant showed more consistently positive results, although a good quality (score of 3) Maguire hyposensitivity test using doses of 2% topical methyl heptine carbonate for induction and challenge gave no reactions (20). An incompletely reported Freund’s complete adjuvant test (FCAT) was reported to give sensitization (13); but no details of the conditions or the results were reported, and hence the level of confidence ascribable to this study was low (score of 1).

In the guinea pig maximization test (again incompletely reported and hence rated only with a score of 1) an unreported percentage of animals reacted when methyl heptine carbonate was administered at 5% (intradermally) and 25% topically with challenge at a “subirritant concentration” (13). In another guinea pig maximization test reported in better detail (ranked as 5) three separate induction/challenge regimens (intradermal induction: 0.625%, 5%, and 10%; topical induction: 1%, 3%, and 30%; challenge: 0.3%, 0.9%, and 3%) gave 18 of 20 at the least severe and middle regimens and 20 of 20 at the most severe (21).

A local lymph node assay of high quality (ranking of 5) in ethanol/diethyl phthalate (1:3) gave stimulation indexes of 5.7 at 0.5%, 7.3 at 1%, 9.0 at 2.5%, 12.3 at 5%, and 14.7 at 10%. This indicated that methyl heptine carbonate has an EC₃ value below 0.5% and hence is considered as a strong allergen (22).

Data from Predictive Tests on Human Volunteers

Human maximization tests have been performed on methyl heptine carbonate. These have been attributed a degree of confidence of 2 due to the low doses employed and incomplete reporting. Despite this, they nonetheless demonstrate the significant sensitization potential of this substance in humans.

Maximization tests performed at 2% gave 7 of 25 reactions (23), 0 of 25 reactions (24), 3 of 25 reactions (25), 10 of 25 reactions (26), 7 of 25 reactions (26), 4 of 24 reactions (27), 5 of 22 reactions (27), 1 of 24 reactions (27), 4 of 24 reactions (27), 7 of 25 reactions (28), and 5 of 26 reactions (28).

A study in which a freshly prepared sample of methyl heptine carbonate (at 2%) was used in conjunction with the antioxidant BHA (Butylated Hydroxyanisole) gave 0 of 21 reactions, which may implicate the ultimate role of autoxidation products of this substance in sensitization (27). This hypothesis is perhaps strengthened by the observation that a 14-month air-exposed sample of methyl heptine carbonate at 2% gave 8 of 25 reactions (29). Attempts to demonstrate antagonism failed to show reduced levels of sensitization when methyl heptine carbonate was applied at 2% but in the presence of other fragrance substances. For instance when mixed with an equal quantity of geraniol, 9 of 25 reactions were observed (30). When mixed with an equal quantity of d-limonene, 2 of 19 reactions were observed (27) and 4 of 23 reactions were observed when the concentration of d-limonene was increased 9 times (27). When mixed with 9 times its weight of phenylethyl alcohol, 4 of 27 reactions were observed (30).

Studies using single induction patches followed by a single challenge patch aimed at developing diagnostic methods for immunodeficiency diseases were performed on patients suffering from chest diseases (31). With an induction dose of 5% followed by challenge at 1%, 6 of 45 reactions were observed, induction at 20% followed by challenge at 10% produced 17 of 37 reactions, and induction at 30% (challenge dose not indicated) produced 10 of 10 reactions. As these tests were not specifically aimed at determining the allergenic potency of methyl heptine carbonate, a score of 2 was ascribed.

Human repeat insult patch tests have been carried out on methyl heptine carbonate, but their degree of confidence is only rated as 2 because of the limited number of subjects, low concentrations, and the absence of controls. Studies in ethanol gave mixed results. At 0.25%, 2 reactions were observed in 41 subjects (30), whereas others carried out at 0.1% gave no reactions in 40 or in 42 subjects (33). More recent studies were carried out in a mixture of ethanol and diethyl phthalate (3:1). At 0.1% methyl heptine carbonate, there were 1 of 36 reactions in one study (34), 0 of 33 in another (35), and 0 of 71 in another study (36). When the test material was 0.1% of a 1:1 mixture of methyl heptine carbonate and its homologue, methyl octine carbonate, 3 of 37 and 1 of 64 reactions were observed (34,37).

Clinical Diagnostic Tests on Patients

Methyl heptine carbonate has been applied to patients' skin in routine patch testing (Table 2). The following reports indicate positive elicitation reactions to methyl heptine carbonate when this was one of the patch test substances. In some cases, other substances were patch tested simultaneously to methyl heptine carbonate and as a result it is not possible to attribute causality due to possible cross-reactivity. All of these reports are examined here in light of the criteria elaborated in Table 3 (in the Appendix) to determine clinical relevance (i.e., whether methyl heptine carbonate had caused the allergic dermatitis from which these patients were suffering).

Only two of the studies reviewed relate the cause of the patient's allergy to a specific source. In one case (38), a patient who was sensitive to an after-shave product

reacted to three constituents of that aftershave: methyl heptine carbonate (when patch tested at 0.5%), hydroxycitronellal (at 10%), and cinnamic alcohol (at 5%). Although this study did not single out any of these as the possible causative allergen, we have attributed it with a level of confidence of 4. Another case relates to possible workplace exposure of a laboratory assistant in a fragrance manufacturing company (39) who reacted when patch tested to high concentrations (1%) of methyl heptine carbonate and methyl octane carbonate but not to other International Contact Dermatitis Research Group (ICDRG) allergens. Although there was no clear direct link to exposure, a level of confidence of 4 was attributed in this case.

In other reports, there is no clear link to an exposure scenario. In one case, 1 of 4 bakers who may have been exposed to substances such as methyl heptine carbonate when used in flavorings, reacted to this substance at 0.5% (40). The patient reacted also to 4 unrelated fragrance/flavoring ingredients, and there was no clear direct link to exposure to any particular product containing methyl heptine carbonate. A level of confidence of 2 was attributed to the causality of this substance. In a multicenter study carried out in North America between 1979 and 1980, 1% of 278 patients patch tested reacted to 1% methyl heptine carbonate (41). On the basis of the results seen in human predictive testing, it is possible that 1% may be too high for patch testing. This study also showed a high degree of polyreactivity. The reacting patients reacted to an average of 4.5 of the test materials. A score of 1 was given.

A pilot multicenter study gave a reaction in a patient tested alone at 8%, in another patient among 26 tested at 2%, and in another 1 of 34 tested at 0.5% (42). It was reported that irritant reactions may occur at concentrations of 2% or higher. There was no linkage of these reactions to any particular product and the study was accorded a score of 2. A larger scale multicenter study reported by the same authors (42) indicated that 2 of 182 patients reacted to a concentration of 0.5% and was accorded the same rating. Two Japanese studies presented as reviews have been rated as 2. In one, the patch testing of patients with 0.5% methyl heptine carbonate in a cosmetic cream base gave 43 + reactions and 25 +/- reactions of 1994 (43). Another indicates that 1 patient of 65 tested with 5% methyl heptine carbonate gave a strong reaction within 24 hours and another reacted after 1 week (44,45).

DISCUSSION

Unlike the subjects of previous articles in this series (alpha-iso-methylionone [4], anisyl alcohol [5], linalool [6], amlycinnamic aldehyde [7], citronellol [8] and geraniol [9]), methyl heptine carbonate is a strong skin sensitizer in most predictive tests on animals. The local lymph node assay, a test method particularly designed to determine the allergenic potential of substances, indicates that methyl heptine carbonate is indeed a strong sensitizer, placing it in the second most potent category of 4 classes of allergens (46). This substance also shows a marked potential for sensitization when tested on volunteers at sufficiently high levels.

Yet there is an apparent paradox. Methyl heptine carbonate is not a frequent sensitizer in dermatological patients. A likely causal relationship has only been established for one case: a patient's use of an after-shave product containing this substance. There was also a probable link to workplace exposure in the fragrance industry and maybe in the bakery industry, but the cause of allergies does not result

Table 2 Diagnostic patch tests implicating methyl heptine carbonate

Ref.	Primary report	Number & condition of patients	Conditions of patch testing given	Patients reacting & scores	Has irritancy been excluded?
38	Primary	1: ecz	No 0.5%: Pet	1: score?	Yes
39	Primary	1: ecz	No 1%: MEK	1: strong	Yes
40	Primary	1: ecz	No 0.5%: Pet	1: not given	Yes
41	Multi. Cent.	278: ecz	No 1%: Pet	3: not given	No
42	Multi. Cent.	61: ecz	No 8%, 2% & 0.5%: Pet	1/1 at 8% 1/26 at 2% 1/34 at 0.5%	8% and 2% considered irritant
43	Review	1994 of which 10% ecz	No 0.5% in cream base	25 with +/- 43 with +	No
44, 45	Review	65	No 5%: Pet	One with ++ to +++ for days 2-8	No

Note: Primary = Primary report of cases; Multi. Cent. = multicenter study, may be reported separately; Review = primary reports cited; ecz. = eczematic; Pet. = petrolatum; MEK = Methyl Ethyl Ketone; acet. = acetone; score? = intensity of reaction not recorded; +/- = doubtful; + = mild; erythema only; ++ to +++ = severe; unknown = reactions to other substances possible but cannot be determined from the report, or cross reactions are possible but cannot be determined from the report; phased testing = procedures to avoid "excited skin" producing false-positive results.

Reaction to other materials?	Cross reactions likely?	Excited skin excluded?	ROAT or PUT used	Linkage to specific product	Final score
Reacted to hydroxyl- citronellal and cinnamic alcohol	No	No	No	Yes. All three substances in aftershave	4
Analogous methyl octane carbonate	Yes	No	No	No but linkage to workplace exposure	4
4 other substances	No	No	No	No	2
1.5 reactions/patient	Unknown	No	No	No	1
Reactions to 10 other substances	Unknown	No	No	No	2
No data	Unknown	No	No	No	2
No data	Unknown	No	No	No	2

from the use of cosmetics or other consumer products. A limited number of other studies describe reactions to methyl heptine carbonate in multiple-substance patch testing on previously sensitized patients. However, these studies have been carried out under severe exposure conditions and do not go as far as establishing clinical relevance according to current procedures (47–49); and no new reports have been published for more than 20 years.

One explanation for the very low frequency of reactions to methyl heptine carbonate may be the low frequency of its use in patch testing. However, if the true prevalence of allergy to this substance, such as this with a longstanding use in fragrances, was high, it would certainly have found its way into the patch testing trays of dermatologists around the world. It is therefore enticing to invoke an alternative explanation. Could this be due to its restricted use? The International Fragrance Association first imposed a limit on the use of methyl heptine carbonate in 1976. Methyl heptine carbonate is limited to a maximum concentration of 0.01% in consumer products such as cosmetics whether used alone or in combination with methyl octine carbonate (50). Limiting the magnitude of exposure but not its frequency, may be an effective form of risk management.

CONCLUSION

Predictive tests in animals and humans indicate with a good degree of confidence that methyl heptine carbonate has a significant sensitization potential. Yet it is not a frequently observed cosmetic allergen, with only one published case where a possible causal link has been established with the use of a product. Other clinical data indicate possible levels of allergy among patients, but no new reports have appeared for more than 20 years. While its inclusion in cosmetic labeling declarations (51) and routine patch testing materials may provide further information on its possible causal role in allergic contact dermatitis, the evidence currently available does not indicate that methyl heptine carbonate is a frequent fragrance allergen. A possible explanation could be that risk management procedures in place for more than 20 years have reduced exposure and hence the acquisition of clinical allergy to this substance. Because of its markedly restricted use, we may never clarify how potent an allergen it may be in clinical use. With the other fragrance allergens previously reviewed, the current ready availability of fragrance allergens for patch testing may provide clarification (52).

Appendix Assignment of degree of confidence

Test qualification degree of confidence

Meets all criteria = 5

Number of cases is marginal = 4

Some parameters questionable = 3

Lack of controls; no corroborating results = 2

Unreliable results = 1

Fails all criteria = 0

Note: Criteria: vehicle-treated and untreated controls; test concentration sufficient for response; use of appropriate vehicle; adequate compound purity; significant number of cases used.

REFERENCES

1. The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP). Opinion concerning fragrance allergy in consumers. A review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens. Adopted by the SCCNFP during the plenary session of 8 December 1999 (SCCNFP/0017/98 Final, December 1999).
2. Hostynek JJ, Maibach HI. Operational definition of a causative contact allergen—a study with six fragrance allergens. *Exog Dermatol* 2003; 2:279–285.
3. Benezra C, Sigman CC, Perry LR, Helmes CT, Maibach HI. A systemic search for structure-activity relationships of skin contact sensitizers: methodology. *J Invest Dermatol* 1985; 85:351–356.
4. Hostynek JJ, Maibach HI. Is there evidence that alpha-iso-methylionone causes allergic dermatitis? *Exog Dermatol* 2004; 3:121–125.
5. Hostynek JJ, Maibach HI. Is there evidence that anisyl alcohol causes allergic dermatitis? *Exog Dermatol* 2003; 2:230–233.
6. Hostynek JJ, Maibach HI. Is there evidence that linalool causes allergic dermatitis? *Exog Dermatol* 2003; 2:223–229.
7. Hostynek JJ. Is there evidence that amylcinnamic aldehyde causes allergic dermatitis? *Exog Dermatol* 2004; 3:35–46.
8. Hostynek JJ, Maibach HI. Sensitization potential of citronellol. *Dermatology* 2004; 3:307–312.
9. Hostynek JJ, Maibach HI. Is there evidence that geraniol causes allergic dermatitis? *Exog Dermatol* 2003; 3:318–331.
10. Hostynek JJ, Magee PS. Performance of an SARQSAR model predictive of human ACD. *In Vitro Mol Toxicol* 1999; 12:203–211.
11. Klecak G. The open epicutaneous test (OET), a predictive test procedure in the guinea pig for estimation of allergenic properties of simple chemical compounds, their mixtures and of finished cosmetic preparations. Presented at the International Federation Societies Cosmetic Chemists, Basel, Switzerland, September 18, 1979.
12. Klecak G. The Freund's complete adjuvant test and the open epicutaneous test. *Curr Prob Dermatol* 1985; 14:152–171.
13. Klecak G, Geleick H, Frey JR: Screening of fragrance materials for allergenicity in the guinea pig. I. Comparison of four testing methods. *J Soc Cosmetic Chem Jpn* 1977; 28:53–64.
14. Givaudan Corporation for the Research Institute for Fragrance Materials Inc. Open and Closed Epicutaneous and Maximization Tests of Fragrance Materials in Guinea Pigs. 1985.
15. Sharp DW. The sensitization potential of some perfume ingredients tested using a modified Draize procedure. *Toxicology* 1978; 9:261–271.
16. Johnson AW, Goodwin BFJ. The Draize test and modifications. *Curr Prob Dermatol* 1985; 14:31–38.
17. Buehler E, Kreuzmann J, Fischer J, for the Research Institute for Fragrance Materials, Inc. Closed epicutaneous test of methyl-2-octynoate, methyl-2-nonynoate, benzyl acetate, trans,trans-2,4-hexadienal, 2-hexylidene cyclopentanone, hexen-2-al, trans-2-hexenal diethyl acetal and isoeugenol in guinea pigs. 1985.
18. Buehler E, Kreuzmann J, Fischer J, for the Research Institute for Fragrance Materials, Inc. Closed epicutaneous test of 2-hexylidenecyclopentanone, methyl 2-nonynoate and methyl 2-octynoate in guinea pigs. 1985.
19. Buehler E, Kreuzmann J, Garling B, for the Research Institute for Fragrance Materials, Inc. Delayed contact hypersensitivity study of methyl heptine carbonate in guinea pigs. 1986.

20. Prince HN, for the Research Institute for Fragrance Materials, Inc. Maguire hypersensitivity test of fragrance materials in the guinea pig. 1973.
21. Buehler E, Kreuzmann JJ, Doyle RL, for the Research Institute for Fragrance Materials, Inc. Guinea pig maximization test. Report to RIFM. 1985.
22. Betts CJ, Dearman RJ, for the Research Institute for Fragrance Materials, Inc. Methyl 2-octynoate diluted with vehicle 1:3 EtOH:DEP: Local Lymph Node Assay. Report to RIFM. 2005.
23. Kligman AM, for the Research Institute for Fragrance Materials, Inc. The contact-sensitization potential of fragrance materials by maximization testing in humans. 1972.
24. Epstein WL, for the Research Institute for Fragrance Materials, Inc. Report on human maximization studies. 1974.
25. Kligman AM, for the Research Institute for Fragrance Materials, Inc. Report on human maximization studies. 1974.
26. Kligman AM, for the Research Institute for Fragrance Materials, Inc. Report on human maximization studies. 1975.
27. Epstein WL, for the Research Institute for Fragrance Materials, Inc. Report on human maximization studies. 1975.
28. Epstein WL, for the Research Institute for Fragrance Materials, Inc. Report on human maximization studies. 1976.
29. Kligman AM, for the Research Institute for Fragrance Materials, Inc. Report on human maximization studies. 1977.
30. Kligman AM, for the Research Institute for Fragrance Materials, Inc. Report on human maximization studies. 1973.
31. Takanami I. Newly recognized primary sensitizers, methyl heptine carbonate and costus root oil to evaluate cutaneous delayed hypersensitivity on various chest diseases. *Nippon Kyobu Shikkan Gako* 1981; 19:478–786.
32. IFF Incorporated. Human repeated patch test on methyl-2-nonynoate and isoeugenol. Unpublished report made available to the Research Institute for Fragrance Materials, Inc. 1964.
33. IFF Incorporated. Repeated insult patch test of cinnamyl nitrile, methyl 2-octynoate and ethyl alcohol in human subjects. Unpublished report made available to the Research Institute for Fragrance Materials, Inc. 1965.
34. Harrison LB, Yoskowitz BL, Stolman LP, for the Research Institute for Fragrance Materials, Inc. Human repeated insult patch test of methyl 2-octynoate and methyl 2-nonynoate. Unpublished report No. 12367. 1989.
35. Harrison LB, Yoskowitz BL, Stolman LP, for the Research Institute for Fragrance Materials, Inc. Human repeated insult patch test of methyl 2-octynoate. Unpublished report No. 12368. 1989.
36. Harrison LB, Zigarelli B, Stolman LP, for the Research Institute for Fragrance Materials, Inc. Repeated insult patch test of methyl 2-octynoate in human subjects. Unpublished report No. 12452. 1990.
37. Harrison LB, Zigarelli B, Stolman LP, for the Research Institute for Fragrance Materials, Inc. Repeated insult patch test of methyl 2-octynoate and methyl 2-nonynoate in human subjects. Unpublished report No. 12456. 1990.
38. VanKetel WG. Dermatitis from an aftershave. *Contact Dermatitis* 1978; 4:117.
39. English JSC, Rycroft RJG. Allergic contact dermatitis from methyl heptine and methyl octine carbonates. *Contact Dermatitis* 1988; 18:174–175.
40. Malten KE. Four bakers showing positive patch-tests to a number of fragrance materials, which can also be used as flavors. *Acta Derm Venereol Suppl (Stockh)* 1979; 85:117–121.

41. Mitchell JC, Adams RM, Glendenning WE, Fisher A, Kanof N, Larsen W, Maibach HI, Rudner EJ, Schnorr W, Storrs F, Taylor JS. Results of standard patch tests with substances abandoned. *Contact Dermatitis* 1982; 8:336–337.
42. Malten KE, Van Ketel WG, Nater JP, Leim DH. Reactions in selected patients to 22 fragrance materials. *Contact Dermatitis* 1984; 11:1–10.
43. International Fragrance Association. Closed patch test of fragrance materials on the epidermis. Unpublished report. 1974.
44. Ishihara M. Problems of closed patch tests with ingredients of cosmetic products. *J Jpn Cosmetic Sci Soci* 1977; 1:87–102.
45. Ishihara M. The environment and the skin. *J Med Soc Toho Univ* 1978; 25:750–766.
46. Basketter DA, Blaikie L, Dearman RJ, Kimber I, Ryan CA, Gerberick GF, Harvey P, Evans P, White IR, Rycroft RJG. Use of the local lymph node assay for the estimation of relative contact allergenic potency. *Contact Dermatitis* 2000; 42:344–348.
47. Ale SI, Maibach HI. Clinical relevance in allergic contact dermatitis. *Dermatosen* 1995; 43:119–121.
48. Lachapelle J-M. A proposed relevance scoring system for positive allergic patch test reactions: practical implications and limitations. *Contact Dermatitis* 1997; 36:39–43.
49. de Groot AC. Clinical relevance of positive patch test reactions to preservatives and fragrances. *Contact Dermatitis* 1999; 41:224–227.
50. International Fragrance Association (IFRA). Code & Standards. <http://www.ifraorg.org>, accessed April 6, 2006.
51. European Commission. Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the member states relating to cosmetic products. *Official Journal of the European Union* 2003; L66:26–35.
52. Vey M. Procedures for supplying fragrance information promptly to dermatologists. *Contact Dermatitis* 2003; 48:56–58.