Isoeugenol

	97-54-1,
	5932-68-3 (trans-),
	5912-86-7 (cis-)
Sensitizing effects (2003)	**Sh**

In concentrations of up to 6% isoeugenol (2-methoxy-4-(1-propenyl)phenol) is found as a component in some plants and in essential oils from these plants, such as clove oil or Ylang-Ylang oil (Opdyke 1975; Thompson et al. 1983), usually accompanied by eugenol. Isoeugenol may be prepared synthetically by base-catalyzed isomerization of eugenol. Commercial isoeugenol contains mainly the trans-isomer and is frequently used as a fragrance component. Furthermore, isoeugenol is also an intermediate product in the synthesis of vanillin.

Allergenic effects

Effects in humans

In 50 volunteers a 32% solution of isoeugenol in acetone applied occlusively for a period of 48 hours was found to be moderately irritating (Motoyoshi et al. 1979). In a 48-hour patch test 8% isoeugenol in petrolatum was found to be mildly irritating in volunteers (no further details). A patch test with preparations of 5% and 2% induced erythematous irritations in 3 of 35 and 1 of 30 volunteers with healthy skin, while a preparation of 0.1% in 99% ethanol induced an erythematous irritation in 1 of 54 “persons with dermatitis" (no further details) (Opdyke 1975).

Skin sensitizing effects

Isoeugenol is a component of the so-called fragrance mix with which almost all patients with suspected contact eczema are routinely tested. Since the individual components of the mix are often tested in patients showing positive reactions to the mix, relatively extensive clinical experience with this substance is available.

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Currently a 1% isoeugenol preparation in petrolatum is commonly used for patch test purposes.

In a multi-centre study in 9 dermatologic clinics a positive reaction to 1% isoeugenol in petrolatum was observed in 20 of 1072 (1.9%) consecutively tested patients and 10 further reactions were rated irritative. In this study the quota of positive reactions to the fragrance mix amounted to 8.3% (89/1072 patients; in addition, 29 irritative reactions) (Frosch et al. 1995). In various other studies a positive reaction to isoeugenol was observed in approximately 20% of all patients with a suspected allergic reaction to the fragrance mix (Table 1), while especially patients

Table 1 Recent reports on allergic reactions to isoeugenol in patch tests in larger groups of patients with contact eczema or suspected contact allergy and a positive reaction to the fragrance mix (FM)

Persons tested	Concentration (vehicle)	Results	Comments	References
226 patients	1% (petrolatum)	reactions in 45/226 patients (19.9%)	test period: 7/1989–6/1999; in 283/2600 patients reactions to FM, 226 were tested with the constituents	Brites et al. 2000
1112 patients	1% (petrolatum)	reactions in 231/1112 patients (20.8%)	test period: 1984–1998; in 1811/23660 patients reactions to FM, 1112 were tested with the constituents	Buckley et al. 2000
677 consecutively tested patients	5% (petrolatum)	reactions in 15/677 patients (2.2%)	test period: 9/1991–12/1991; in 61/677 patients reactions to FM	De Groot et al. 1993
162 patients	1% (petrolatum)	reactions in 27/162 patients	test period: 1987; reactions in 15/64 men and 12/98 women; reactions to FM in 9.7% of 1845 patients	Enders et al. 1989
112 patients	1% (petrolatum)	reactions in 16/112 patients (14.3%)	test period: 1994–1998; in 112/757 patients reactions to FM; test preparation not clearly documented	Hendriks and van Ginkel 1999
367 patients	1% or 2% (petrolatum)	reactions in 68/367 patients (18.5%)	test period: 1979–1992; in addition, 17× questionable and 2× irritative reactions; in 449/8218 patients reactions to FM, 367 of them were also tested with the constituents; until 1983 tested with 2% isoeugenol in petrolatum	Johansen and Menné 1995

Table 1 (Continued)

Persons tested	Concentration (vehicle)	Results	Comments	References
167 patients	4% (petrolatum)	reactions in 13.8% of patients	test period: not specified; irritative reactions in 3.6%; in half of the patients a fragrance sensitization was suspected before testing based on patch test results or anamnestic findings	Larsen et al. 1996
63 patients	5% (petrolatum)	reactions in 14/63 patients	test period: 11/1983–6/1984; a total of 63/1200 patients had reactions to FM with 2% of each individual constituent	Santucci et al. 1987
54 patients	1% (petrolatum)	reactions in 12/54 patients	test period: 10/1984–6/1985; in a total of 54/1500 patients reactions to FM with 1% of each individual constituent	Santucci et al. 1987
991 patients	1% (petrolatum)	111× 1+, 62× 2+/3+ (a total of 17.5%)	test period: 1996–1999; reactions to FM in 11.4% of 35599 patients; 4900 patients had been tested with FM and the individual constituents, 991 reactions to the FM (566× 1+, 349× 2+, 76× 3+)	Schnuch et al. 2002
160 patients	1% (petrolatum)	positive reactions in 14.8% of patients	test period: 7/1998–12/1999; in 294/3604 patients reactions to FM, 160 of them had been tested with the individual constituents; in 2/104 patients reactions to isoeugenol without simultaneous reaction to FM	Temesvari et al. 2002

with a distinct reaction to the mix reacted to the individual component (Schnuch et al. 2002).

Reactions to isoeugenol are regularly far more frequently reported than reactions to eugenol (see also the documentation "Eugenol" 2013, a translation of the German documentation from 2003). Sixteen of 24 patients in whom a sensitization to isoeugenol could be reproduced in patch tests also reacted to 0.2% isoeugenol in ethanol in the Repeated Open Application Test (ROAT); 15 within 15 days. Ten of the 16 patients also reacted to 0.05% isoeugenol in ethanol no later than after 28 days. In a patch test most of those volunteers showed reactions to isoeugenol concentrations of 0.1% or less, and one person even to 0.0005% isoeugenol in ethanol (Andersen et al. 2001).

Table 2 Results of experimental studies with isoeugenol in guinea pigs

Method species sex	*i.d. induction* injection volume; concentration (vehicle)	*Epicutaneous induction* day, application; concentration (vehicle)	*Challenge* day, application; concentration (vehicle)	Number of animals with positive reactions	Comments	References
Open Epicutaneous Test, Himalayan white-spotted, ♀ + ♂	–	day 0-day 20, 0.1 ml each open; 10% on 8 cm2 (not specified)	day 21 and day 35: 0.025 ml open; 1% on 2 cm^2 (not specified)	positive (no further details)	6–8 animals and controls used; positive evaluation of the test if at least 2/8 animals had reacted	Klecak et al. 1977
Occlusive Epicutaneous Test (modified Buehler Test), Hartley, ♀	–	2 weeks, each 3× per week, 48 h occlusive, 10% (petrolatum)	day 28, 48 h occlusive, 0.1% and 1% (petrolatum)	0.1%: 8/20 1%: 16/20	reading after 72 h; in pretests irritative reactions in 4/10 and 1/10 animals to 10% and 5% isoeugenol and in 0/10 animals to both 2% and 1% isoeugenol	Ishihara et al. 1986; Itoh 1982
Modified Buehler Test, Hartley, not specified	–	1st and 2nd week, each 3× per week, 6 h occlusive, 4% (petrolatum); 3rd week 3× 6 h occlusive, 1% (petrolatum) [ear swelling test]	day 35, 6 h occlusive, 2% (petrolatum)	24 h reading: 5/10; 48 h reading: 1/10	after repeated challenge same incidences; in pretests no irritative reactions, however, after 2 weeks induction reduction of concentration from 4% to 1 % due to observed "irritations"; after first challenge reactions in 2/10 and 1/10 controls, no reaction after second challenge	Kaminsky and Szivos 1990

Table 2 (Continued)

Method species sex	*i.d. induction* injection volume; concentration (vehicle)	*Epicutaneous induction* day, application; concentration (vehicle)	*Challenge* day, application; concentration (vehicle)	Number of animals with positive reactions	Comments	References
Modified Buehler Test, Hartley, not specified	–	1st and 2nd week, each 3× per week, 6 h occlusive, 4% (petrolatum); 3rd week 3 × 6 h occlusive each on the abraded skin, 1% (petrolatum)	day 35, 6 h occlusive, 2% (petrolatum)	24 h: 2/10; 48 h: 1/10	after second challenge reactions in 7/10 and 2/10 animals	Kaminsky and Szivos 1990
Modified Buehler Test, Hartley, not specified	–	1st and 2nd week, each 3× per week, 6 h occlusive, 30% (petrolatum); 3rd week 3× 6 h occlusive, 20% (petrolatum)	day 35, 6 h occlusive, 2% (petrolatum)	24 h: 8/10; 48 h: 4/10	after second challenge reactions in 9/10 and 2/10 animals	Kaminsky and Szivos 1990
Modified Buehler Test, Hartley, not specified	–	3 weeks 1× per week each, 6 h occlusive, 3% (petrolatum) [ear swelling test]	day 35, 6 h occlusive, 1% (petrolatum)	24 h: 5/8; 48 h: 4/8	during induction few "irritations" observed; reactions in 1/10 and 0/10 controls; a second challenge has not been performed	Kaminsky and Szivos 1990

Table 2 (Continued)

Method species sex	*i.d. induction* injection volume; concentration (vehicle)	*Epicutaneous induction* day, application; concentration (vehicle)	*Challenge* day, application; concentration (vehicle)	Number of animals with positive reactions	Comments	References
Modified Buehler Test, Hartley, not specified	–	3 weeks 1× per week, 6 h occlusive on the abraded skin, 3% (petrolatum)	day 35, 6 h occlusive, 1% (petrolatum)	24 h: 9/10; 48 h: 5/10		Kaminsky and Szivos 1990
Modified Buehler Test, Hartley, not specified	–	3 weeks 1× per week, 6 h occlusive, 30% (petrolatum)	day 35, 6 h occlusive, 1% (petrolatum)	24 h: 7/10; 48 h: 6/10		Kaminsky and Szivos 1990
Modified Buehler Test, Hartley, not specified	–	3 weeks, each 1× per week, 6 h occlusive, 1% (petrolatum) [test with Hilltop Chamber]	day 35, 6 h occlusive, 1% (petrolatum)	24 h: 1/9; 48 h: 0/9 (the same after 2nd challenge)	reactions in 1/10 controls after 1st challenge; not after 2nd challenge	Kaminsky and Szivos 1990
Modified Buehler Test, Hartley, not specified	–	3 weeks, each 1× per week, 6 h occlusive on the abraded skin, 1 % (petrolatum)	day 35, 6 h occlusive, 1% (petrolatum)	24 h: 3/9; 48 h: 2/9	2nd challenge: reactions in 3/9 and 1/9 animals	Kaminsky and Szivos 1990

Table 2 (Continued)

Method species sex	*i.d. induction* injection volume; concentration (vehicle)	*Epicutaneous induction* day, application; concentration (vehicle)	*Challenge* day, application; concentration (vehicle)	Number of animals with positive reactions	Comments	References
Modified Buehler Test, Hartley, not specified	–	3 weeks, each 1× per week, 6 h occlusive, 30% (petrolatum)	day 35, 6 h occlusive, 1% (petrolatum)	24 h: 7/9; 48 h: 3/9	after 2nd challenge: reactions in 8/9 and 2/9 animals	Kaminsky and Szivos 1990
Draize Test, Himalayan white-spotted, ♀ + ♂	d 0: 0.05 ml, d 1–9: 0.1 ml; 0.1% (isotonic saline solution)	–	day 35 and day 49: i.d. 0.05 ml; 0.1% (isotonic saline solution)	positive (no further details)		Klecak et al. 1977
Optimization Test, Pirbright white; ♀ + ♂	day 0, day 2 and day 4 100 µl 0.1% (30% ethanol) each; day 7, day 9, day 11,day 14, day 16 and day 18 100 µl 0.1% in FCA each (30% ethanol in isotonic saline solution)	–	day 35, i.d. 100 µl 0.1% (30% ethanol in isotonic saline solution) day 49, 24 h occlusive, 0.5% (petrolatum)	17/20 20/20	reactions in 7/20 controls after the i.d. challenge with the vehicle; reading after 24 h (i.d. challenge) and 48 h (epicutaneous challenge)	Maurer 1985; Maurer et al. 1979
Cumulative Contact Enhancement Test (CCET), Pirbright and Hartley, not specified	day 7, 2× 100 µl FCA/water	on day 0, 2, 7 and 9, 24 h 0.2 ml occlusive; Pirbright: 10%, 30% (ethanol) or 100%; Hartley: 100%	day 21, 24 h occlusive; not specified (ethanol)	Pirbright 10%*: 6/6, 30%*: 6/6, 100%*: 2/6 Hartley 5/10	no reactions in 6 Pirbright controls; no details regarding controls in the Hartley guinea pigs; *: induction concentration	Tsuchiya et al. 1985

Table 2 (Continued)

Method species sex	*i.d. induction* injection volume; concentration (vehicle)	*Epicutaneous induction* day, application; concentration (vehicle)	*Challenge* day, application; concentration (vehicle)	Number of animals with positive reactions	Comments	References
Maximization test, Dunkin-Hartley, not specified	day 0, 2× 100 µl substance each, substance in FCA and FCA; 0.1% (isotonic saline solution with 0.01% dodecyloxy-benzolsulphonate	day 7, 48 h occlusive; 25% (acetone/poly-ethylene glycol 400, 7:3)	day 21: 24 h occlusive; 5% (acetone/polyethylene glycol 400, 7:3)	10/10	no reactions in 5 FCA-pretreated control animals	Hilton et al. 1996 b
Maximization test, Himalayan white-spotted, ♀ + ♂	day 0, 2× 100 µl substance each, substance in FCA and FCA; 5% (not specified)	day 8, 48 h occlusive; 25% (petrolatum)	day 21: 24 h occlusive; subirritative concentration (no further details)	positive (no further details)		Klecak et al. 1977
Modified maximization test, Pirbright-white, ♀ + ♂	day 0, 4× 100 µl FCA	day 0, 24 h occlusive, 3% (petrolatum); day 7, open; 10% sodium lauryl sulphate in petrolatum; day 8, 48 h occlusive; 3% (petrolatum)	day 21: 24 h occlusive; 0.5% (petrolatum)	10/10		Maurer and Hess 1989

Table 2 (Continued)

Method species sex	*i.d. induction* injection volume; concentration (vehicle)	*Epicutaneous induction* day, application; concentration (vehicle)	*Challenge* day, application; concentration (vehicle)	Number of animals with positive reactions	Comments	References
FCA test, Himalayan white-spotted, ♀ + ♂	day 0, 2, 4, 7 and 9: 0.1 ml; 50% (FCA)	–	day 21 and day 35: 24 h occlusive; subirritative concentration (petrolatum)	positive (no further details)	control animals pretreated 5× with 0.05 ml FCA	Klecak et al. 1977
"Adjuvant Patch Test (APT)", Hartley, ♀	day 0, 4× 100 µl FCA	day 0, day 1 and day 2, 24 h occlusive after abrasion of skin; 10% (ethanol) day 7, open 10% sodium dodecylsulphate in petrolatum d 8, 48 h occlusive, 0.2 ml 10% (ethanol)	d 21: open, 10 µl 0.1–10% (ethanol)	0.1%: 4/5 0.5%: 5/5 1%: 5/5 5%: 5/5 10%: 5/5	no details regarding reactions in controls; readings after 24, 48 and 72 h	Yanagi et al. 2001
"Adjuvant Patch Test (APT)", Hartley, ♀	day 0, 4× 100 µl FCA day 6, 2× 100 µl FCA	day 0, 72 h occlusive after abrasion of skin; 10% (ethanol) day 6, 48 h occlusive after abrasion of skin, 0.4 ml 10% (ethanol)	day 13: open, 10 µl 0.1–10% (ethanol)	5/5 each	no details regarding reactions in controls; readings after 24, 48 and 72 h	Yanagi et al. 2001
"Adjuvant Patch Test (APT)", Hartley, ♀	day 0, 4× 100 µl FCA	day 0, 72 h oclusive after abrasion of skin; 10% (ethanol) day 6, 48 h occlusive after abrasion of skin, 0.4 ml 10% (ethanol)	day 13: open, 10 µl 0.1–10% (ethanol)	5/5 each	no details regarding reactions in controls; readings after 24, 48 and 72 h	Yanagi et al. 2001

A maximization test with 8% isoeugenol in petrolatum did not lead to sensitization in 25 volunteers (Opdyke 1975). However, 28/78 volunteers had been sensitized in a maximization test with 8% isoeugenol, (no further data given) (Thompson et al. 1983). Ten occlusive tests, each between 48-72 hours with 0.2 g of a preparation of 8% isoeugenol in ethanol were given to volunteers. After a break of two weeks a 72 hour challenge test with the same preparation produced reactions in 9/73 persons (Marzulli and Maibach 1980).

In various studies isoeugenol and isoeugenol-containing fragrance preparations have been tested in humans, especially by means of the Human Repeated Insult Patch Test (HRIPT). However, due to the mixed exposure, studies with fragrance preparations are not used for this evaluation (Thompson et al. 1983). In the HRIPT induction treatment 56, 38 and 81 volunteers were treated 24 hours occlusively with 0.5%, 1% and 1.25% isoeugenol in ethanol over a period of 3 weeks, 3 days per week, on the upper arm. After a break of two weeks a challenge test using the same concentration was again applied on the upper arm, 0/56, 1/38 and 1/81 volunteers showed reactions (Thompson et al. 1983).

In the Lymphocyte Transformation Test (LTT), peripheral lymphocytes from 4 patients who had shown 1+– reactions to isoeugenol in the epicutaneous test produced positive results, yet, lymphocytes from one patient who had shown a 2+ reaction, produced negative results (Sieben et al. 2001).

Airway sensitizing effects

There are no data available for airway sensitizing effects of isoeugenol in man.

Animal experiments

In repeated 24 hours occlusive applications to rabbits and guinea pigs undiluted isoeugenol was found to be strongly irritative but was found non-irritant in the 48 hour occlusive application to the mini-pig (Motoyoshi et al. 1979). In 4/10 and 1/10 female Hartley guinea pigs an irritative reaction was induced after a 48 hours occlusive application with 20 mg of 10% and 5% isoeugenol in petrolatum, while none of the animals reacted to an isoeugenol concentration of 2% or less (Itoh 1982).

Skin sensitizing effects

Numerous experimental studies with guinea pigs both without the use of Freund's complete adjuvant (FCA) (Open Epicutaneous Test, Occlusive Epicutaneous Test, (modified) Buehler Test, Draize Test) and with the use of FCA (Maximization Test, FCA Test, Cumulative Contact Enhancement Test, Optimization Test) yielded throughout positive findings (see Table 2). In the Local Lymph Node Assay an

EC_3-value (necessary concentration to triplicate lymphocyte proliferation) of 1.3% was determined. The authors rated isoeugenol as a moderately effective allergen (Basketter et al. 2002). Various studies with CBA/Ca mice also yielded positive findings with concentrations of 2.5–25% isoeugenol in acetone/olive oil (4:1) (Basketter and Scholes 1992; Bertrand et al. 1997; Hilton et al. 1996 a, b; Kimber et al. 1991). Positive findings have also been reported for a maximization test (induction and challenge: 2% isoeugenol) that is however incompletely documented (Ishihara et al. 1986). Supporting evidence for the sensitizing effects was provided by the results of a study not carried out according to standardized methods (Yanagi et al. 2001; see Table 2). Further positive findings in the FCA test, the Open Epicutaneous Test and the Maximization Test (Tsuchiya et al. 1985) are, however, incompletely documented.

In a Draize Test only two "albino guinea pigs" (no further details) were administered an intracutaneous injections of 0.1 ml 0.1% isoeugenol in 1% ethanol on 10 consecutive days. Three weeks after the last injection both animals demonstrated a moderate reaction following the open challenge test with 1% isoeugenol in peanut oil (Griepentrog 1961).

The positive findings from a (modified) Mouse Ear Swelling Assay with 3 and 10% isoeugenol study are however only of limited relevance for evaluation due to missing standardization (Thorne et al. 1991).

Airway sensitizing effects

Six female BALB/c mice were treated on day 1 with 50 µl each 5%, 10% or 25% on the shaved flanks and on day 7 on the auricles with 25 µl each 2.5%, 5% or 12.5% isoeugenol in acetone/olive oil (4:1). On day 14 after the initial treatment the total serum IgE was examined. Only one of the animals initially treated with 10% isoeugenol demonstrated a slightly increased IgE value compared to the 10 control animals. On an average no group treated with isoeugenol showed a significant increase of the serum IgE compared to the control group (Hilton et al. 1996 b). Therefore, this not sufficiently validated study, does not provide any evidence of airway sensitizing effects of isoeugenol.

Manifesto

Based both on the extensive clinical experience and animal studies, isoeugenol shows a skin sensitizing potential. Therefore, isoeugenol is designated with an "Sh". There is no evidence of immunological effects of isoeugenol on the respiratory system. Therefore, isoeugenol is not designated with an "Sa".

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