Contact allergy to fragrances: current patch test results (2005–2008) from the Information Network of Departments of Dermatology*

WOLFGANG UTER1, JOHANNES GEIER2, PETER FROSCH3 AND AXEL SCHNUCH2

1Department of Medical Informatics, Biometry and Epidemiology, University of Erlangen/Nürnberg, Erlangen, Germany, 2Information Network of Departments of Dermatology (IVDK) at the University of Göttingen, Göttingen, Germany and 3Department of Dermatology, University of Witten/Herdecke and Klinikum Dortmund Gmbh, Dortmund, Germany

Background: Contact sensitization to fragrances is common both in clinical and in population samples. The spectrum of allergens is broad and diverse, and to some extent covered by a set of screening agents.

Objectives: To examine the current frequency of contact sensitization to fragrance allergens in patients routinely patch tested for suspected allergic contact dermatitis with the baseline series and special series.

Patients and methods: Between 2005 and 2008, 40 709 patients were patch tested in the departments of the Information Network of Departments of Dermatology (http://www.ivdk.org). Results with selected fragrances were analysed.

Results: Of all patients tested with the German baseline series, 15.1% reacted positively to fragrance mix (FM) I (6.6% positive), FM II (4.6% positive) or Myroxylon pereirae resin (balsam of Peru, 6.8% positive). Among the single constituents of FM I, Evernia prunastri [oak moss absolute (abs.)] was the leading allergen, and amyl cinnamal the least frequent allergen. Among fragrances not included in FM I or FM II, Evernia furfuracea (tree moss abs.) was the most common allergen.

Conclusions: For diagnostic purposes, it is necessary to combine several screening agents. The frequency of contact sensitization differs greatly between single fragrances.

Key words: clinical epidemiology; contact allergy; fragrances. © John Wiley & Sons A/S, 2010.

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*The following centres contributed data to this analysis (in alphabetical order): Aachen (C. Schröder, J. M. Baron), Augsburg (A. Ludwig), Basel (A. Bircher), Berlin Charité (M. Worm), Bern (D. Simon), Bielefeld (I. Effendy), Bochum (H. Dickel), Bochum BGFA (M. Fartasch), Dermatologikum (K. Reich, V. Martin), Dortmund (K. Kügler, P. J. Frosch, R. Herbst), Dresden (A. Bauer, P. Spornrnat-Ragall), Erlangen (V. Mahler), Essen (U. Hillen), Freudenberg (Ch. Szliska), Gera (J. Meyer), Graz (B. Krańke, W. Aberer), Greifswald (M. Jünger), Göttigen (J. Geier, Th. Fuchs), Halle (B. Kreft), Hamburg (E. Coors), Hannover (T. Schaefer, Th. Werfel), Heidelberg (M. Hartmann, U. Jappe), Heidelberg AGS (E. Weisshaar, T. L. Diepjen), Homburg/Saar (C. Pfohl), Jena (A. Bauer, S. Schliemann-Williams), Kiel (J. Brasch), Krefeld (M. Lilie, S. Wassilew), Mainz (D. Becker), Mannheim (Ch. Bayerl, D. Booken, H. Kurzen), Marburg (H. Löffler, M. Hertl), Minden (R. Stadler), München LMU (B. Przybilla, P. Thomas, R. Eben, T. Oppel, T. Schuh), München Schwabing (K. Ramrath, M. Agathos, M. Georgi), München TU (U. Darsow), Münster (B. Hellweg, R. Brehler), Nürnberg (A. Hohl, D. Debos), Osnabrück (Ch. Skudlik, S. M. John), Rostock (J. Trcka), Tübingen (T. Biedermann), Würzburg (A. Trautmann).

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From the background of a broad exposure to fragrances (1), this diverse group of chemicals is still a leading cause of contact sensitization among patients patch tested for suspected allergic contact dermatitis (2). Changes in the spectrum of potentially sensitizing fragrances do occur (3) and have led to the development of a second fragrance mix (FM), FM II (4). Moreover, exposure
may change in terms of reduced use concentrations of fragrance compounds, following regulation or amended production standards, as in the case of isoeugenol (codes and standards available at www.ifraorg.org). Hence, the pattern of contact sensitization observed in patch test patients needs to be continually analysed, to evaluate the effects of such interventions, and to identify new allergens that may emerge.

In the present article, the frequency of contact sensitization to fragrance allergens diagnosed in the departments of the Information Network of Departments of Dermatology (IVDK) (www.ivdk.org) in the past 4 years will be analysed (with the exception of FM II and its single constituents reported on elsewhere (5), and test results obtained with essential oils presented and discussed in a separate article). This period follows the study period of a previous analysis assessing the prevalence of contact sensitization to 26 fragrances (6) that have to be labelled according to EU regulations (7, 8).

Methods

The IVDK (www.ivdk.org), a contact allergy surveillance network in Germany, Switzerland and Austria, has been described elsewhere. Briefly, results for all patients patch tested in the participating departments are electronically recorded, along with important demographic and clinical data. The diagnostic procedure follows international guidelines (9) that have been further refined by the German Contact Dermatitis Research Group (10), of which all IVDK participants are members. All data are transmitted to the data centre in Göttingen in an anonymous format twice yearly, where they are checked and, if satisfying internal quality control criteria (11), analysed according to international guidelines (12) using SAS software (version 9.2; SAS Institute, Cary, NC, USA).

For the present analysis, data of all patients patch tested between January 2005 [July 2005 in the case of majantol, to avoid overlap with a previous publication on this allergen (13)] and December 2008 were included (n = 40 709 in the course of 41 656 consultations). In cases of multiple consultations of one patient, the strongest of the possible multiple test results was considered for analysis. Some fragrance compounds and sorbitan sesquioleate (SSO) had been temporarily patch tested in the ‘monitor series’ (14), that is in consecutive patients, whereas before or after they were tested in the context of special series, as the remaining fragrance compounds. As the prevalence of (positive) reactions in consecutive patients is not comparable to the prevalence when only selected patients are tested (expected to be higher in the latter case), and for better comparison with a previous study based on consecutive patients, results are presented separately. Allergens were provided by Almirall-Hermal/Trolab, Reinbek, Germany. As the composition of these series changed, and for other reasons, such as the temporary unavailability of allergens, the number of patients actually tested with the allergens varies. Weak (+) to strong (+++) positive patch test reactions on the third day after application of the test or, if this was not read, after the fourth day were aggregated as ‘positive’ outcomes and contrasted with non-positive (non-allergic) reactions, comprising negative, doubtful and irritant reactions. For information on INCI nomenclature and CAS numbers, the ‘CosIng’ database (http://ec.europa.eu/enterprise/cosmetics/cosing/, last accessed December 2009) and the list of fragrances that have to be labelled in the EU (7, 8) were utilized.

Results

The distribution of important clinical and demographic variables in subgroups of patients reacting positively to different screening agents is shown in Table 1. Evidently, there are differences with regard to a ‘typical’ patient profile; hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) is not as much associated with older age and leg dermatitis as is Myroxylon pereirae. The frequencies of irritant or doubtful, weak positive and stronger positive patch test reactions to the fragrance allergens included in the baseline series are shown in Table 2. The test preparations of FM I and the eight single constituents all contain SSO as an emulsifier. It is thus possible that, in cases of sensitization to SSO (which is tested at 20% petrolatum as part of the FM I breakdown test series), positive reactions to the FM and its constituents, respectively, will be caused by SSO, although its concentration in the fragrance allergens is considerably lower. Moreover, irritant (false-positive) reactions to SSO itself may occur and – less likely, owing to the lower SSO concentration already mentioned – to fragrance allergens containing SSO. Among the 8858 patients tested with FM I and SSO, 1.1% + to +++ reactions to SSO were noted. Interestingly, 37% of these 93 positive SSO reactions were observed in patients negative to FM I (containing 5% SSO). Unfortunately, 1707 patients with a positive FM I reaction (19.3%) were not further tested with the breakdown test including SSO. However, all patients positive to SSO were excluded from further analyses concerning FM I and its eight single constituents. The results in the subgroup of 8765 patients tested with at least SSO and FM I, and negative to the
former, regarding the single FM I constituents are presented in Table 3. For better comparison with other studies testing either consecutive patients, or using a special test series (e.g. breakdown after positive reaction to FM I in the baseline series), the results are stratified for this test context.

Detailed results regarding FM II and its single constituents, including HICC, will be reported elsewhere (5). Patch test results with other fragrance compounds, tested in an aimed fashion as part of different special series, with the few exceptions noted, are presented in Table 4. The number of patients tested with the single compounds differs because of changes in the composition of the DGK test series in this period.

Addressing concomitant reactivity between FM I and its single compounds in those 4167 patients tested with FM I and all single constituents, including SSO, and not reacting +, ++ or +++ to SSO, Table 5 illustrates a consistent relationship between intensity of positive reactions to the screening mix and the proportion of positive reactions to a single compound. Evidently, sensitization to multiple single compounds is not uncommon in patients with a +++ reaction to FM I. Moreover, only 6 of these 62 patients (9.7%) did not react to any of the eight single compounds, as compared with 21.7% with negative breakdown test results among patients with a ++ reaction to FM I, and 52.3% among patients with a + reaction to FM I. Overall, the proportion of patients with non-positive reactions to all single constituents but a positive reaction to FM I was 38.8% in this analysis, excluding patients with positive reactions to SSO.

Cross-reactivity between cinnamal and cinnamyl alcohol was marked [Cohen’s $\kappa = 0.58$, 95% confidence interval (CI) 0.49–0.67]. Regarding the eight constituents of FM I, in three other pairs of allergens, concomitant reactivity was at least weak (eugenol versus isoeugenol, $\kappa = 0.29$, 95% CI 0.20–0.37; hydroxycitronellal versus geraniol, $\kappa = 0.25$, 95% CI 0.15–0.35; isoeugenol versus Evernia prunastri, $\kappa = 0.21$, 95% CI 0.15–0.27), whereas in the other cases it was negligible ($\kappa < 0.2$). Moreover, in the mere 534 patients tested with both compounds, positive reactions to $\alpha$-amyl cinnamal (2 positive) and amyl cinnamyl alcohol (1 positive) were (i) very rare and (ii) completely discordant in the other cases.

Colophonium and oil of turpentine have been regarded as ‘fragrance-associated’ allergens, because they contain terpenes and terpene oxides similar to...
those found in some fragrance compounds. Hence, the association of positive reactions to FM I with positive reactions to these two allergens, and to FM II for comparison, was examined. Quantified as odds ratios (ORs), bivariate analyses identify a marked association—see Table 6, column ‘a’. Adjustment for age and sex in a logistic regression model for each of the three allergens does not alter the OR estimates much (Table 6, column ‘b’). In contrast, the inclusion of the first two allergens (Table 6, column ‘c’), and then finally all three allergens (Table 6, column ‘d’), into one model, along with age and sex, yields strong evidence of mutual confounding of the association of oil of turpentine and colophony, respectively, with FM I, and also confounding by FM II. In contrast, the much stronger association between FM I and FM II remains entirely unaffected whether or not colophonium or oil of turpentine is considered.

**Discussion**

The present analysis of patch test data from a clinical surveillance network provides a current perspective on the frequency of sensitization to different screening and single fragrance allergens, thus supplementing previous reports from this group (6, 16, 17). For the first time, the new screening allergen FM II has been included [detailed results with FM II and its single constituents will be reported elsewhere (5)]. As compared with FM I and *M. pereirae*, the demographic and clinical profile of sensitized patients seems to be different, with leg dermatitis under-represented and hand dermatitis over-represented in the HICC-positive patients. The reasons for this, such as different exposure or susceptibility factors, warrant further investigation. The frequencies of contact sensitization to fragrance allergy screening allergens (Table 2) are similar to current results from other areas, namely, with more positive reactions to *M. pereirae* than to FM I.

Regarding FM I, the percentage of positive reactions has been calculated on the basis of all patients tested in a first step (Table 2), for comparability with other published results. However, as SSO is added to FM I and its single constituents, ‘positive’ reactions may result from sensitization to SSO, which is why it has been recommended to always test SSO along with FM I in the baseline series (18). Unfortunately, this recommendation has not been taken up broadly [for instance, the current European baseline series does not include SSO (19)]. The German Contact Dermatitis Research Group has included SSO in the breakdown test series of single FM I ingredients. If this series was to be applied to patients with a previous positive reaction to FM I on a regular basis, a putative false-positive reaction resulting from SSO sensitization could be recognized. Unfortunately, often for organizational or logistic reasons, which presumably apply elsewhere too, breakdown testing is not applied regularly. Conversely, the breakdown test is sometimes applied without a prior positive patch test reaction to FM I on a regular basis, a putative false-positive reaction resulting from SSO sensitization could be recognized. Unfortunately, often for organizational or logistic reasons, which presumably apply elsewhere too, breakdown testing is not applied regularly. Conversely, the breakdown test is sometimes applied without a prior positive patch test reaction to FM I, merely on the basis of the patient’s history, previous tests or other reasons. All in all, the proportion of patients positive to FM I is higher in the subgroup of 8765 patients tested with SSO than in all 36 961 patients tested with FM I. In this article, we have taken a conservative approach by restricting further analyses

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**Table 3.** Results with single constituents of FM I (8765 patients tested with at least sorbitan sesquioleate (SSO) and FM I and not having a +, ++ or +++ reaction to SSO), stratified for test context:

<table>
<thead>
<tr>
<th>Allergen (CAS no.)</th>
<th>% Test context</th>
<th>Number tested</th>
<th>% (IR)</th>
<th>% +</th>
<th>% ++ / +++</th>
<th>% pos. std.</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM I</td>
<td>8</td>
<td>B</td>
<td>8765</td>
<td>3.03</td>
<td>6.92</td>
<td>4.23</td>
<td>10.12</td>
</tr>
<tr>
<td><em>Eugenia</em> <em>prunastri</em> (Oak moss absolute)</td>
<td>1</td>
<td>B</td>
<td>1213</td>
<td>1.90</td>
<td>1.24</td>
<td>0.99</td>
<td>1.81</td>
</tr>
<tr>
<td>Sp</td>
<td>4482</td>
<td>1.49</td>
<td>3.30</td>
<td>3.24</td>
<td>5.59</td>
<td>(4.90–6.27)</td>
<td></td>
</tr>
<tr>
<td><em>Isoeugenol</em> (97-54-1)</td>
<td>1</td>
<td>B</td>
<td>1214</td>
<td>1.24</td>
<td>1.48</td>
<td>0.33</td>
<td>1.62</td>
</tr>
<tr>
<td>Sp</td>
<td>5747</td>
<td>1.25</td>
<td>2.04</td>
<td>1.51</td>
<td>3.41</td>
<td>(2.90–3.92)</td>
<td></td>
</tr>
<tr>
<td><em>Hydroxycitronellal</em> (107-75-5)</td>
<td>1</td>
<td>B</td>
<td>1214</td>
<td>0.66</td>
<td>0.99</td>
<td>0.16</td>
<td>1.17</td>
</tr>
<tr>
<td>Sp</td>
<td>4359</td>
<td>0.89</td>
<td>2.82</td>
<td>0.48</td>
<td>2.95</td>
<td>(2.43–3.47)</td>
<td></td>
</tr>
<tr>
<td><em>Cinnamal</em> (104-55-2)</td>
<td>1</td>
<td>B</td>
<td>1214</td>
<td>1.98</td>
<td>1.40</td>
<td>0</td>
<td>1.43</td>
</tr>
<tr>
<td>Sp</td>
<td>4527</td>
<td>1.46</td>
<td>1.90</td>
<td>1.06</td>
<td>2.64</td>
<td>(2.16–3.13)</td>
<td></td>
</tr>
<tr>
<td><em>Cinnamyl alcohol</em> (104-54-1)</td>
<td>1</td>
<td>B</td>
<td>1214</td>
<td>1.73</td>
<td>0.66</td>
<td>0</td>
<td>0.73</td>
</tr>
<tr>
<td>Sp</td>
<td>4502</td>
<td>0.80</td>
<td>1.62</td>
<td>1.00</td>
<td>2.36</td>
<td>(1.89–2.83)</td>
<td></td>
</tr>
<tr>
<td><em>Eugenol</em> (97-53-0)</td>
<td>1</td>
<td>B</td>
<td>1214</td>
<td>1.65</td>
<td>0.41</td>
<td>0.08</td>
<td>0.44</td>
</tr>
<tr>
<td>Sp</td>
<td>4801</td>
<td>1.19</td>
<td>1.15</td>
<td>0.46</td>
<td>1.57</td>
<td>(1.19–1.95)</td>
<td></td>
</tr>
<tr>
<td><em>Geraniol</em> (106-24-1)</td>
<td>1</td>
<td>B</td>
<td>1214</td>
<td>1.89</td>
<td>0.49</td>
<td>0.08</td>
<td>0.39</td>
</tr>
<tr>
<td>Sp</td>
<td>5695</td>
<td>1.26</td>
<td>0.83</td>
<td>0.23</td>
<td>0.87</td>
<td>(0.63–1.10)</td>
<td></td>
</tr>
<tr>
<td><em>α-Amyl cinnamal</em> (122-40-7)</td>
<td>1</td>
<td>B</td>
<td>1214</td>
<td>1.15</td>
<td>0.25</td>
<td>0</td>
<td>0.26</td>
</tr>
<tr>
<td>Sp</td>
<td>4375</td>
<td>0.53</td>
<td>0.55</td>
<td>0.07</td>
<td>0.61</td>
<td>(0.36–0.86)</td>
<td></td>
</tr>
</tbody>
</table>

B, baseline attachment (monitor series); CI, confidence interval; FM, fragrance mix; Sp, special breakdown series of FM I; ?, doubtful reaction; IR, irritant reaction.

Age and sex standardization (pos. std.) according to (12).
regarding FM I (constituents) to those patients who were tested with SSO and by excluding those with positive reactions to SSO, which limited the number of evaluable patients (Tables 3 and 5).

A comparison of test results in patients who were tested routinely, along with the baseline series, and in those patients who were tested in a more aimed fashion confirms the notion that the yield of positive reactions is always (much) higher in the latter setting (Table 3). In this study, however, the general effect is enhanced by the fact that a – fairly limited – proportion of patients was tested with the single constituents, prompted by a positive screening test with FM I, and not only guided by, for example, a history suggestive of perfume intolerance. The results in consecutively tested patients (context ‘B’) are largely similar to those of a previous analysis (6) and to those of a recent report from Groningen, The Netherlands (20) – the somewhat higher sensitization prevalences noted in the latter study presumably result from a slightly more targeted application of the allergens.

In a recent experimental study, protein–cinnalamid adducts were detected in skin homogenates treated with cinnamal and cinnamyl alcohol but not in those treated with α-amyl cinnamal. This suggests that there is a common hapten involved in cinnamal and cinnamyl alcohol sensitization, in line with our observation of a marked concordance upon patch testing and previous results of Buckley et al. (21) (κ = 0.44, 95% CI 0.37–0.51, recalculated), and that metabolic activation (to cinnamal) is involved in the latter. Conversely, there does not appear to be a common hapten for cinnamal and α-amyl cinnamal (22), again in line with the observations in the present clinical study.
Regarding the other ‘annex’ fragrance allergens (8) (except for the ingredients of FM II, see above), a comparison with previous data is difficult, as they have previously been applied in consecutive patients (6). The few allergens yielding a sensitization prevalence >0.5% in the present study, namely *Eternia furfuracea* (tree moss absolute), butylphenyl methylpropional and amyl cinnamyl alcohol, were at least slightly more common allergens than previously found in consecutive patients. In the remainder, some non-systematic variation of the altogether low sensitization prevalence is noted. Again, benzyl benzoate did not cause any positive reaction, like α-isomethyl ionone in the present study (previously 1 case in 2004 consecutive patients) (6). Majantol, which is not one of the ‘annex’ allergens, was found to elicit positive patch test reactions in 0.5% of consecutive patients (13), which is close to the 0.36% presently observed with majantol tested in the ‘monitor series’. Among those allergens not listed in (8), salicylaldehyde stands out, in that it caused 12 + and two ++ reactions, whereas in previous studies on a larger number of patients, only single cases were observed (23, 24). In contrast, menthol, vanillin and benzaldehyde appear to be quite rare allergens.

In the 1999 Scientific Committee on Cosmetic Products and Non-Food Products opinion, benzyl alcohol is classified as an allergen frequently causing allergic reactions, having been found to cause allergic reactions in 1.2–15% of patients with eczema resulting from cosmetic products (8). The current International Fragrance Association (IFRA) standard recommends a limit ranging from 0.2% in deodorants, to 1.4% in hand creams, and 5% in rinse-off hair conditioners (http://www.ifra.org, last accessed December 2009). The German ‘Rote Liste’ (http://www.rote-liste.de, last accessed November 2009) lists 205 drug specialties (products) containing benzyl alcohol as an excipient, pointing to topical medications as a possible source of exposure, in addition to (perfumed) cosmetics.

The relative importance of the eight single constituents of FM I (Table 5) is very similar to the ranking, and also the proportions, found in a previous similar analysis (17), except for eugenol and cinnamyl alcohol swapping their rank. This ‘internal’ stability is particularly remarkable considering the overall drop in sensitization prevalence of FM I (17).

The IFRA recommended in 1992 that the use of unmodified colophonium in perfumes should be abandoned; however, it is unknown whether modified forms of colophonium are used in perfumes. Irrespective of this, colophonium and oil of turpentine are regarded as markers of contact allergy to those fragrances that contain similar terpenes and terpene oxides, such as essential oils from *Pinaceae*, even though their maximum level of peroxides has been restricted to 10 mmol/l (25). Still, the role of colophonium in the detection of fragrance contact allergy is considered to be minor in comparison with *M. pereirae*, FM I and FM II (24, 26). In a number of previous studies, significant relationships were found between colophonium and FM I (27) and between FM I and oil of turpentine (17, 27), respectively. Our present results regarding the association with FM I as a main marker of fragrance contact allergy seem to indicate the following:

(1) Considerable overlap between colophonium and oil of turpentine exists (note the change of association estimate from column ‘b’ to ‘c’ in terms of mutual confounding) with regard to representing perfume-associated terpene (oxide) contact allergy.

### Table 6. Results of a bivariate analysis [crude odds ratio (OR)] and adjusted logistic regression analyses (adjusted ORs) addressing the association between contact sensitivity to FM I (+ to +++ versus non-positive) as outcome and oil of turpentine and colophonium (and FM II, in the final model), respectively, as ‘explanatory factors’ in the 8452 patients tested with all four allergens and sorbitan sesquioleate and not reacting positively to the latter.

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>(a) Crude</th>
<th>(b) Adjusted for age and sex</th>
<th>(c) Adjusted for age, sex and the respective other allergen</th>
<th>(d) Adjusted for age, sex and the respective other two allergens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colophonium</td>
<td>2.7 (2.0–3.4)</td>
<td>2.6 (2.0–3.3)</td>
<td>1.9 (1.5–2.5)</td>
<td>1.5 (1.1–2.1)</td>
</tr>
<tr>
<td>Oil of turpentine</td>
<td>5.8 (4.2–8.0)</td>
<td>5.5 (4.0–7.6)</td>
<td>4.2 (3.0–5.9)</td>
<td>3.0 (2.0–4.5)</td>
</tr>
<tr>
<td>FM II</td>
<td>11.8 (9.6–14.4)</td>
<td>11.7 (9.5–14.3)</td>
<td>–</td>
<td>11.7 (8.7–13.1)</td>
</tr>
</tbody>
</table>

CI, confidence interval; FM, fragrance mix.

(a) Bivariate analysis, that is, not adjusted for any other factor.

(b) Little change (<10%) from crude OR; that is, there is no confounding by age or sex of the relationship between FM I and the three other allergens.

(c) Mutual adjustment of colophony and oil of turpentine reduces the strength of association with FM I in both cases; that is, mutual confounding exists. FM II not considered.

(d) Further adjustment for reactivity to FM II slightly reduces the association between colophony and FM I, but markedly reduces the association between oil of turpentine and FM I; that is, there is considerable confounding (>50% change in estimate) of the latter association by FM II.
allergens, even though the added value for the patient is limited, in view of the as yet very partial declaration of fragrance ingredients in consumer products.

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Address:
Wolfgang Uter
Department of Medical Informatics Biometry and Epidemiology
University of Erlangen/Nürnberg
Waldstr. 6 D-91054 Erlangen
Germany
Tel: +49 9131 8522750
Fax: +49 9131 8522721
e-mail: wolfgang.uter@imbe.med.uni-erlangen.de