



On the skin sensitisation potential of rosin and oxidised rosin

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ABSTRACT

In the EU rosin is classified as a skin sensitizer, apparently on the basis of its oxidation to sensitising agents. Rosin (gum, tall oil or wood) is not a skin sensitizer when examined in the guinea pig maximisation test (GPMT). Oxidised rosins are sensitizers in the GPMT. Oxidised gum rosin was further tested in the mouse local lymph node assay (LLNA) and the Buehler test, but is not a sensitizer in either of these tests. Further, the outcome of the LLNA can be used to assess the potency of oxidised rosin as an inducing agent in humans, and oxidised rosin is, at most, a weak sensitizer in this test. Thus, oxidised rosin is not a potent inducing agent for skin sensitization unless the dermal barrier is bypassed and/or there is deliberate use of Freund's Complete Adjuvant to induce greater susceptibility.

The material used for human patch testing ('colophony') is in oxidised form. A re-examination of epidemiological studies suggests that patients in dermatological clinics show higher response rates than do the general population or those occupationally exposed to presumably oxidised rosin. Thus, the differences seen in susceptibility in the regulatory tests may be reflected in the human population.

These results are discussed in terms of possible testing and classification strategies for dealing with existing chemicals, with particular reference to the new European Union legislation.

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1. Introduction

Colophony (rosin) is frequently claimed to be one of the most common skin sensitizers. This information is based on data obtained using human patch testing with standard series of test substances, one of which is described as colophony (rosin).

Rosin is a natural product derived from pine trees. It is a resin. Depending on the source, the rosin may be gum rosin (from living trees), tall oil rosin (a by-product of pulping) or wood rosin (from pine tree stumps). Geographically, rosin comes from China, USA, Indonesia, Russia, Scandinavia and Portugal. A clear problem with rosin is that it oxidises on contact with air. This oxidation requires access to oxygen, thus it is much more rapid when powdered rosin is exposed to air, as compared to rosin in massive form. Indeed, sealed barrels of rosin can be stored for considerable periods of time with little, if any air oxidation occurring.

Rosin is transported mainly in massive form, either at high temperature as liquid or at room temperature as a supercooled liquid (a glass like material), both of which are circumstances in which oxidation is limited. Some 'rosin' is marketed for specific uses in small blocks (e.g., violinists' rosin). However, much of the 'rosin' placed on the market is chemically modified in order to improve

its technical performance. Simultaneously, this alters the potential for oxidation. Most of the public exposure to 'rosin' is exposure to chemically modified 'rosins'.

Non-oxidised rosin has been classified by the EU as a skin sensitizer on grounds of the skin sensitization potential of oxidised rosin (Karlberg et al., 1999). The classification was last updated in the 21st Adaptation to Technical Progress in 1994 (Directive 94/69/EC). Much of the data on which this classification is based makes use of non-standard tests using test material that was poorly defined in terms of its oxidation status. In this paper we publish the results of properly conducted regulatory tests on rosin and oxidised rosin. The tests were undertaken in order to gain further insights into the validity of the assay systems and of the human data. The results of the tests on non-oxidised rosin are negative and those on oxidised rosin are test-dependent. Thus two questions must be raised. The first is 'which regulatory test is the more relevant to the human system?' The second is whether a substance should be classified on the basis of information on its own hazard or on the basis of the hazard of a clearly differentiable substance that may form during storage.

2. Materials and methods

Gum, wood and tall oil rosin were obtained from Dérivés Résiniques et Terpéniques, Dax, France, Hercules, USA and Arizona

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Table 1
Composition of the gum, tall oil and wood rosins

Substance	Gum rosin (% w/w)	Tall oil rosin (% w/w)	Wood rosin (% w/w)
<i>Individual rosin acids (as % w/w of total rosin acids)</i>			
Abietic acid	45.2	44.2	49.7
Dehydroabietic acid	3.3	18.1	8.5
Dihydroabietic acid	0.6	2.7	0.9
Isopimaric acid	3.6	6.6	13.5
8,5-Isopimaric acid	0.3	1.4	4.2
Levopimaric acid	0.4	2.5	0.2
Neobietic acid	14.0	3.9	4.7
Palustric acid	19.2	7.8	8.6
Pimaric acid	7.4	3.7	5.9
Sandopimaric acid	1.5	2.5	1.9
Dimers	1.0	<0.1	4.2
Others	3.5	6.6	1.0
Total rosin acids in the sample (as % w/w of total material in sample)	93.7	93.4	91.5
Peroxide number (ppm)	13	30	88

Each substance was assayed by glc in three different laboratories and the average value of these results is shown.

Chemicals, Sweden. The gum rosin was a deliberate mixture of 85% Chinese gum rosin and 15% Brazilian gum rosin stored for use as a representative rosin for testing purposes. The wood rosin was 100% US material containing and tall oil rosin was 45% Scandinavian, 10% French, 45% US material. The compositions are in Table 1. The rosins were stored at -20°C under nitrogen to minimise oxidation. Naturally oxidised gum rosin (Dérivés Résiniques et Terpéniques), and wood rosin were produced by exposing ground rosin (particle size $\sim 10\text{ }\mu\text{m}$) to air at room temperature for 3 months. Two samples of clinical patch test material (20% colophony [gum rosin] in white petrolatum) were obtained, one (BD) from Bio Diagnostics (Upton on Severn, UK) and the other (CD) from Chemotechnique Diagnostics, (Tygelsjö, Sweden). Hexyl cinnamic aldehyde (in corn oil) was used as positive control. Olive oil was used as vehicle for test material and served as control. Test material was prepared at room temperature, a measured volume of vehicle being added to a weighed amount of test material. All containers were flooded with nitrogen to prevent further oxidation.

The guinea pig maximisation test (GPMT), mouse local lymph node assay (LLNA) and Buehler assay were performed in accordance with the relevant regulatory guidelines (Tests B6 and B42, respectively, of Annex V of Directive 67/548/EEC) in independent Contract Research Organisations and were subjected to Good Laboratory Practice audit. Preliminary irritation tests were conducted. In the guinea pig maximisation test, each laboratory used its normal procedure. Intradermal administration was conducted on day 1, topical induction on day 8 and 24 h topical challenge was applied on day 21, with observation at 24 and 48 h post-removal of the patch. Sodium lauryl sulphate was applied 24 h prior to the topical induction application of test substance in the CTL series, it was not used in the Scantox series. In the Buehler assay, topical application was conducted on days 0, 7 and 14. Substance was applied for 6 h under occlusive dressing. Challenge (also a 6 h exposure) was conducted 2 weeks later. Sodium lauryl sulphate pretreatment is not a requirement for this test and was not undertaken. In the mouse LLNA, 25 μL of test substance was applied to the dorsal surface of the ear for each of 3 days. On day 5 the mice received tritiated thymidine 5 h before sacrifice. The draining auricular lymph nodes were excised and pooled for each experimental group and tritium incorporation determined using liquid scintillation counting.

In a time course study, pelleted or powdered gum rosin was subjected to natural oxidation. The powdered material was passed through a 0.55 mm sieve. Pellets were obtained by breaking up a thin layer (2–4 mm depth) rosin, prepared by cooling molten rosin

under nitrogen. Samples were weighed at various times after preparation. Peroxide number was determined before and during oxidation. At least duplicate samples were taken at each time point and peroxide content measured by titration. Samples ($\sim 10\text{ g}$) were dissolved in 50 ml acetic acid:chloroform (2:1 v/v), 5 ml of 1 M potassium iodide solution added and the solution allowed to react for 5–30 min in the dark. After dilution with 100 ml water, 2.5 ml 0.5% starch was added and the solution titrated against 0.01 M sodium thiosulphate. A solvent blank and a hydrogen peroxide standard were similarly titrated. Samples were also examined by Fourier transform infra red spectroscopy (FTIR) (Perkin Elmer system 2000) of KBr pellets containing 2% rosin sample.

3. Results and discussion

3.1. Rosin and oxidised rosin

As rosin is a natural product, its composition varies with source. When tested in the two guinea pig tests for skin sensitisation it is clear that non-oxidised rosin is not a skin sensitizer (Tables 2 and 3). Rosin from three widely different sources, namely gum rosin, wood rosin and tall oil rosin, were examined in the guinea pig maximisation test (GPMT), thus any variation in composition is unlikely to have affected these results. This position was eventually accepted by the regulatory authorities in the EU who, nevertheless indicate that the substance should be labelled because it oxidises readily and oxidised rosin is a known skin sensitizer (Karlberg et al., 1999).

Oxidised rosin is a sensitizer when tested in the GPMT (Table 2). The results confirm those obtained by Karlberg (1991) using a more severe test. She identified that minimally air exposed Swedish tall oil rosin was not sensitising in her 'Freund's Complete Adjuvant Test' (FCAT), an adjuvant based guinea pig test employing three intradermal administrations of test substance and Freund's Complete Adjuvant, but for which there is no longer an EU or OECD agreed test guideline. Air exposed (oxidised) tall oil rosin was a clear sensitizer.

If 'gum rosin' (which gave a positive sensitisation reaction, but for which the oxidation status was not recorded) was used as challenge there was no significant response in minimally oxidised tall oil rosin induced guinea pigs (Karlberg, 1991). A clear cross-reaction response with air exposed 'tall oil rosin' induced guinea pigs was observed.

Abietic acid is a major component of all three types of rosin (Zinkel and Russel, 1989). Abietic acid and colophony have been examined in the Freund's Complete Adjuvant Test (FCAT) and GPMT assays (Karlberg et al., 1985; Hausen et al., 1989). Karlberg compared two samples of abietic acid and gum rosin (oxidation status not stated). Both the commercially produced abietic acids gave a positive response in the GPMT, but, when purified, the pure abietic acid was not a skin sensitizer in this test. Hausen found synthetically prepared abietic acid to be a weak sensitizer. This illustrates the confusing nature of the data in much of the early literature and the need to clearly characterise the oxidation status of the material being tested.

There is substantial evidence that a variety of relatively unstable species, notably epoxy and peroxy-compounds and hydroperoxides, are responsible for induction when tested in the FCAT. The materials present in oxidised 'rosin', include several oxidation products of notable sensitisation potential (Hausen et al., 1990, 1993; Gäfvert et al., 1992, 1994; summarised in Lepoittevin and Karlberg, 1994). Generally, these materials were synthesised chemically, but the materials identified in Hausen et al. (1993) were generated on storing purified, crystallised rosin in daylight at room temperature for 1–6 months. Gäfvert et al. (1992, 1994) also identified that some oxidation products cross-reacted with one another; the peroxide of

Table 2
Harrpa GPMT studies with rosins

Test material	Test reference	Induction Id	Induction topical	Challenge	Result (responders)	EU classification
Gum rosin	CTL/F/147	3% Corn oil/DMF	30% Corn oil/DMF	3%, 10%, 30%	Test: 0/19, 0/19, 0/19; control: 0/10, 0/10, 0/10	Negative
		10% Corn oil/DMF	30% Corn oil/DMF	0%, 3%, 10%, 30%	Test: 1/20, 0/20, 0/20, 0/20; control: 0/10, 0/10, 0/10	Negative
Gum rosin	CTL/P/4921	10% Olive oil	75% Paraffin wax	0%, 10%, 30%, 75%	Test: 0/20, 0/20, 1/20, 2/20; control: 0/10, 0/10, 0/10, 0/10	Negative
Gum rosin	Scantox 25212/A	0.3% Coconut oil	50% Coconut oil	50%	Test: 1/20; control: 0/10	Negative
Tall oil rosin	CTL/E/0186	10% Olive oil	40% Olive oil	3%, 10%, 30%, 40%	Test: 0/20, 0/20, 1/20, 1/20; control: 0/10, 0/10, 0/10, 0/10	Negative
Wood rosin	CTL/P/5234	10% Olive oil	30% Olive oil	10%, 30%	Test: 0/20, 1/20; control: 0/10, 0/10	Negative
Wood rosin	CTL/P/5224	10% Olive oil	40% Olive oil	25%, 40%	Test: 1/20, 1/20; control: 0/10, 0/10	Negative
Gum rosin, naturally oxidised	CTL/P/5415	3% Olive oil	75% Olive oil	3%, 10%, 25%, 50%	Test: 2/20, 6/20, 14/20, 19/20; control: 0/10, 0/10, 2/10, 4/10	Positive
Gum rosin, naturally oxidised	Scantox 25212/B	0.3% Coconut oil	50% Coconut oil	50%	Test: 20/20; control: 3/10	Positive
Wood rosin, naturally oxidised	CTL/E/0183	10% Olive oil	40% Olive oil	3%, 10%, 30%, 40%	Test: 0/20, 0/20, 3/20, 8/20; control: 0/9, 0/9, 0/9, 0/9	Positive
Disproportionated gum rosin	CTL/E/0185	10% Olive oil	40% Olive oil	3%, 10%, 30%, 40%	Test: 0/20, 0/20, 0/20, 0/20; control: 0/10, 0/10, 0/10, 0/10	Negative
Hydrogenated gum rosin	CTL/E/0184	10% Olive oil	40% Olive oil	3%, 10%, 30%, 40%	Test: 0/20, 0/20, 0/20, 0/20; control: 0/10, 0/10, 0/10, 0/10	Negative
Superoxidised' gum rosin	CTL/P/4920	10% Olive oil	75% Olive oil	1%, 3%, 10%, 30%	Test: 0/19, 0/19, 0/19, 0/19; control: 0/10, 0/10, 0/10, 0/10	Negative

Positive controls (hexyl cinnamic aldehyde) 9/19 (CTL) 7/10 (Scantox) both satisfactory.

A positive response is a grade 1 or higher response at the appropriate site. Readings were taken at 24 and 48 h.

Test reference CTL indicates that the study was conducted at Central Toxicology Laboratories, Macclesfield, UK, Scantox indicates that the study was conducted at Scantox Laboratory, Ejby, Denmark. Pretests to determine irritancy were performed differences between laboratories represent inter-laboratory differences.

Table 3
HARRPA Buehler assays with rosins

Test material	Test reference	Induction	Challenge	Result	EU classification
Gum rosin	CTL/F/148	30%	30, 10, 3%	Test: 0/20; control 0/20	Negative
		10% (3% DMF in corn oil) ^a	30, 10, 3%	Test: 0/19; control 0/10	
Oxidised gum rosin	PSL 20436	75% (Moistened with olive oil)	75% (Olive oil)	Test: 0/20; control 0/10	Negative

Positive historical controls (hexylcinnamaldehyde) gave the expected positive result. Test reference CTL indicates that the study was conducted at Central Toxicology Laboratories, Macclesfield, UK, PSL indicates that the study was conducted at Eurofins Product Safety Laboratories, Dayton, NJ, USA.

^a 30% was the claimed maximum solubility.

15-dehydroabiatic acid cross-reacted with 15-hydroperoxyabiatic acid and the α - and β -forms of 13,14-epoxyabiatic acid cross-reacted. Gäfvert et al. (1994) further identified that multiple antigens can be generated. However, these materials are chemically and thermally unstable. In all cases the testing to determine if these oxidation products could induce sensitisation in animals involved dosing procedures bypassing the skin barrier and employed adjuvant to maximise the likelihood of induction occurring. Hausen (1998) mentions that for his tests he has modified the FCAT and the GPMT “with the purpose of determining most favourably the sensitising capacity of moderate and weak allergens.” Thus the likelihood of these materials being capable of induction when applied to the surface of the skin remains uninvestigated.

When tested in a relatively new test, the mouse LLNA, oxidised gum rosin was not a sensitiser (Table 4). The substance was tested at concentrations up to 25%, the highest concentration in the stan-

Table 4
Mouse LLNA study with oxidised gum rosin

Dose (% w/v)	Stimulation index	Conclusion
0	1.0	Does not meet regulatory Criterion Not a sensitiser
1	1.01	
2.5	0.97	
5	1.58	
10	1.26	
25	2.87	

Material dosed in acetone:olive oil 3:1 (v/v). The highest dose used was determined by solubility, precipitate was present. The regulatory criterion for a positive is a Stimulation Index of 3. Study conducted at Central Toxicology Laboratories, Macclesfield, UK.

dard series that could be tested, thus, although the effective concentration for a 3-fold stimulation index (EC3 for a 3-fold SI), by extrapolation is just greater than 25%, no SI exceeded the regulatory criterion of 3. There could be a species difference or the use of adjuvant and the lack of intradermal dosing that might be the cause for this difference between the LLNA and the GPMT and FCAT. However, the species difference argument fails, as a recently performed Buehler assay also gave negative results (Table 3). There are two possible explanations for the results seen. The Buehler test and the LLNA assay may both be less sensitive than the GPMT and, in particular, the FCAT, possibly because of increased susceptibility to the sensitisation process caused by the administration of Freund's Complete Adjuvant. Alternatively, it is necessary to bypass the epidermis if the active species formed in the oxidation process are to induce a sensitisation reaction.

Only the mouse LLNA has actually undergone formal independent assessment to determine the accuracy of its predictions. The EC3 values have been shown to correlate closely with data on human potency (Basketter et al., 2007). An EC3 value of greater than 25% indicates that oxidised rosin is not a potent sensitiser. Thus two questions must be asked:

- what do the results from these tests imply?
- what is the relevance to human sensitisation?

3.2. The material used in human testing

From the above data, rosin is not a sensitising agent in either the GPMT or the Buehler test. Oxidised ‘rosin’ clearly acts as a

Table 5

Cross-reactivity of testing substance and oxidised gum rosin in the GPMT

Test material	Induction intradermal	Induction topical	Challenge	Responders	Classification
Gum rosin, naturally oxidised	3% Olive oil	75% Olive oil	20%	Test: 10/10; control 3/5	Positive
Colophony (Chemotechnique Diagnostics)	3% Olive oil ^a	75% Olive oil	20%	Test: 10/10; control 2/5	Positive
Colophony (Bio Diagnostics)	3% Olive oil ^a	75% Olive oil	20%	Test: 5/10; control 1/5	Positive
Olive oil	3% Olive oil ^a	75% Olive oil	Olive oil	Test: 1/10; control 0/5	Negative

Hexylcinnamic aldehyde was used as positive control and gave the expected result. A positive result is based on the regulatory classification (Health and Safety Commission, 2002) of >30% of the animals (compared to controls) responding. Study conducted at Central Toxicology Laboratories, Macclesfield, UK.

^a Induction was with oxidised gum rosin; control was with the omission of test substance in induction phase.

complete sensitising agent in the GPMT, but is not a sensitiser in the Buehler assay or the mouse LLNA. The active species in the colophony (rosin) used for patch testing in humans therefore is unlikely to be gum rosin, but may be oxidised gum rosin.

We examined whether the material used in human testing is capable of eliciting a response in the GPMT when oxidised gum rosin is used as inducing agent. Two samples from different sources were examined; both were clearly positive using the criteria set out in the regulatory guidelines (Table 5).

Chemical examination of the samples confirmed that they contained oxidation products of rosin, as previously suggested by Sadhra et al. (1996). Analysis of naturally oxidised gum rosin and of one sample of material intended for use in human testing by Fourier transform infrared spectroscopy indicated that the spectra were identical and that a strong OH absorption at 3411 cm^{-1} , characteristic of naturally oxidised rosin, was present. When the oxidation process was followed in a sample of rosin being oxidised naturally, this band developed over a period of approximately 50 days. Measurement of hydroperoxide by a standard technique indicated that the amounts present in one samples of the material intended for human testing was >85% (w/w comparison) of that present in maximally naturally oxidised powdered rosin. A similar value was obtained for the second sample from a different source. By comparison, there was little natural oxidation of pelleted rosin within the same time frame (Fig. 1).

3.3. Human data

3.3.1. Studies in humans

Commercial preparations of colophony (rosin) used for patch testing have been investigated by Sadhra et al. (1996, 1998), using human patch testing. In their first paper these authors examined the 'unmodified rosin' (equal mixture of American, Chinese and Portuguese), as placed into commercial patch test preparations, and the corresponding patch test preparation. Of 35 subjects previ-

ously positive to the commercial preparation, three showed no reaction to the material from which the preparations were made. These 35 patients were also tested for cross-reactivity with a series of resin acids: abietic acid (two preparations, 60% and 54%, respectively, cross-reacted); levopimaric acid (17% cross-reacted), palustric acid (14% cross-reacted), neoabietic acid (9% cross-reacted) and pimaric acid (6% cross-reacted). The materials were apparently used as supplied, since no attempt appears to have been made to store the materials under conditions preventing oxidation. However, unlike with the animal study, the nature of the inducing agent cannot be further identified.

In the second paper, the authors demonstrated that the resin acids in 'fresh' unmodified rosin, kept under inert conditions, underwent progressive and substantial oxidation over 4 weeks, and that the dermatological activity increases significantly with time. The only substance in non-oxidised rosin claimed as having significant allergenicity was abietic acid, which they declared was weak, but important allergen. They also found that nominally raw Portuguese rosin cross-reacted with fewer (11/20 versus 17/20) patch test positives (nominally rosin sensitive individuals) than did a sample of the same rosin allowed to oxidise for 4 weeks. Although claimed as 'fresh', and stored under nominally inert conditions, neither the physical state nor the oxidation state of the 'fresh' material was stated.

In all of these human studies the inducing agent is unknown. Although probably not the preferred explanation, the studies are consistent with the position that rosin (non-oxidised or oxidised) is not an inducing agent. They are also consistent with the position that, unless oxidised, rosin is not an eliciting agent.

3.3.2. Occupational exposure

Anecdotal information suggests that the prevalence of skin sensitisation in factories handling rosin is no higher than that in the general population.

A study has been carried out on a human population in a tall oil rosin factory (Farm et al., 1994; Farm, 1998). No exposure measurements were conducted. However, photographic evidence from elsewhere suggests that exposure is likely to have been significant. In a factory of 180 present workers and 30 former employees, 163 (90.5%) and 17 (48.6%) participated in the study, which consisted of questionnaire, clinical examination and patch testing with a standard series of test materials and additional rosins. There were 10 cases of hand eczema, and seven subjects were patch test positive to gum rosin (oxidation state unstated) in the standard series. One person reacted to tall oil rosin and none reacted to the additional rosins (glycerol ester of tall oil rosin, pentaerythritol ester of tall oil rosin, glycerol ester of maleic adduct of tall oil rosin and polymerised tall oil rosin, maleopimaric acid and glycerol triabietate) tested. The authors admitted that the frequency of reaction to the gum rosin in the standard series was about the same as that for patch test positive patients at their dermatology clinic. The conclusion has to be that, despite the limited size of the study, the study gave no evidence that the material, to which workplace exposure to tall oil rosin was likely, is a skin sensitising agent.

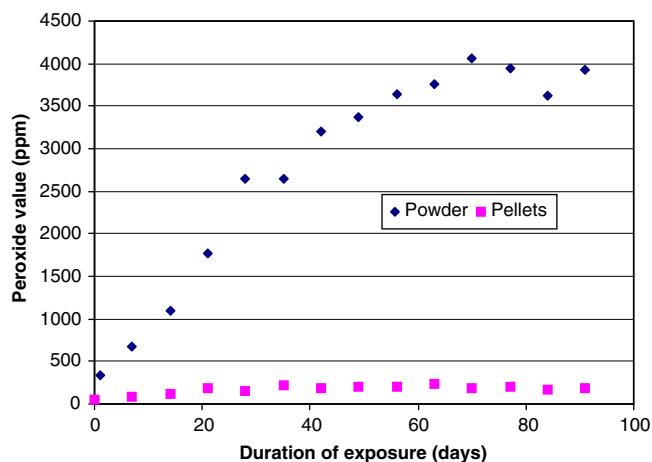


Fig. 1. Natural oxidation of powdered and pelleted rosin.

A Swedish study has been conducted in opera house artistes (Farm et al. 1995; Farm, 1998). Colophony ('rosin') is used by dancers and violinists. Dancers rosin is (presumably powdered) Portuguese colophony, and therefore is likely to be oxidised. The argument is that both dancers and opera singers may also be exposed to colophony through make-up. However, no exposure measurements were conducted. Totals of 116 singers and dancers and 16 make-up artistes, 132 of the 186 (71%) currently on payroll on 1 February 1993 and regularly working through the Spring, were included in the study. They were interviewed, examined and patch tested. More than half had a history of intolerance to cosmetics. Colophony gave positive patch tests in three subjects (2.3%), two singers and one dancer. Two of these subjects were also sensitive to dancer's 'rosin' on patch test, the other had a history of sensitivity to dancer's 'rosin'. This is less than the frequency of sensitisation seen in dermatological clinics (see below). A criticism of this paper is that only current members were examined; whether this represented a 'survivor' population due to many of those affected moving away from the stage was not investigated. Nevertheless, the prevalence of 'rosin' induced skin sensitisation was below that for European patients referred to skin clinics. Thus the circumstances in which presumably high exposure to 'rosin' (probably oxidised rosin) occurred failed to elicit a higher incidence of skin sensitisation, supporting the proposition that oxidised rosin does not induce skin sensitisation.

3.3.3. General public

A prevalence study investigating contact allergy in a general population (Nielsen and Menne, 1992). Based on the results for 773 adult Danes, prevalence rates of 0.4% in men and 1.0% in women (average 0.7%) were obtained. The relationship between exposure and effect was not investigated further.

A second study has been conducted on a population from northern Norway (Dotterud and Smith-Sivertsen, 2007). They examined a total of 1236 people, 546 men and 690 women. Prevalence rates for colophony were 0.9% in men and 1.4% in women (average 1.2%).

3.3.4. Possibly enhanced susceptibility general public

Human prevalence testing has been conducted with 'rosin' (or colophony) on those presenting at dermatological clinics. The substance has been included in both North American and European standard series of test allergens. Based on the evidence above, the actual substance responded to is oxidised 'rosin'. Based on the information available concerning amounts placed on the market, exposure to inducing levels of 'rosin' (in practice, oxidised 'rosin') is probably considerably less than exposure to chemically modified rosins and other chemically related species.

Gäfvert et al. (1996) refer to a total 912 patients from five European clinics who were being patch tested for sensitisation to maleopimaric acid. Maleopimaric acid (maleinised rosin) is a known (and potent) sensitiser in animals (Gäfvert, 1994; Nillson et al., 2002; Clarke et al., 2007). Of these 912, 39 (4.2%) were positive for 'rosin', but there was considerable inter-clinic variation with results varying from 1.3% to 6.9%. As the degree of oxidation is related to sensitising potential, and was uncontrolled, at least part of this variation may be due to the test material. Additional studies gave values of 1.3% for 665 patients attending a Portuguese clinic and 5.5% of 1072 patients attending a Swedish clinic. Interestingly, the authors conceded that most (general public) exposure is to modified rosin and that additional cases are revealed following challenge with chemically modified rosins. However, in choosing maleopimaric acid as the main modified rosin for examination, Gäfvert et al. introduced a new structure that specifically alerts for sensitisation, an acid anhydride (see e.g., Barratt and Basketter, 1996) into the rosin, so it would be predicted that there is only limited cross-reactivity.

A study in England involved 1553 new patients (704 males, 849 females) attending a skin hospital between 1983 and 1988 (Sadhra et al., 1996). These patients were patch tested with the European Standard Series. Of these 61 (3.9%; 31 male, 30 female) were recorded as 'rosin' sensitive.

In a German study an epicutaneous test has been performed on some 12 911 patients tested between 1 October 1996 and 30 September 1999 in a study by the German Contact Allergy Group and the Information Network of Departments of Dermatology (Geier and Hausen, 2000). A total of 515 patients (4.0%) reacted to colophony (oxidation status not stated). Severity of response could be examined in this study. A weak response was seen in 271 (2.10%) and a clear response in 244 (1.89%).

The studies published by the North American Contact Dermatitis Group divide the total population into three time periods (Marks et al., 1998). The testing was conducted using a 'standard' tray. A total of 50 allergens were used. Over the period 1994–1996, some 2.6% of the 3113 patients attending patch test clinics were sensitive to the colophony test preparation. Between 1992 and 1994 some 1.9% of 3540 patients were sensitive and between 1985 and 1989 2.2% of 3948 patients responded. Colophony was not one of the 12 most frequent contact allergens.

The prevalence rates obtained in the studies quoted are summarised in Table 6. Additional studies on patch test clinic populations, reviewed in (Downs and Sansom, 1999) yielded equally variable results (1.2–7.0%). It would appear that the results from the dermatological clinics, although highly variable, gave higher prevalence rates than those from occupationally exposed populations or the general population. Whether the populations from the dermatological clinics were representative of the general public and whether they were exposed to rosin were not investigated further, thus the relationship between exposure and frequency of response could not be examined in these studies.

It can be suggested that in all of the studies on patients at dermatological clinics patients were selected on the basis that there was a reason for testing, i.e., that they were susceptible populations. Some evidence supporting this comes from Smith et al (2000). Based on data which indicated that 78% of those with a positive patch test for colophony also responded to <2.5% sodium lauryl sulphate whereas only 44% of a matched control group responded, they suggest that those presenting at dermatological clinics may be more susceptible to sensitisation. The population

Table 6

Summary of the prevalence rates of colophony ('rosin') positive tests in different populations

Type of population	Prevalence rate %	Reference
General population	0.7%	Nielsen and Menne (1992)
General population	1.2% (Men 0.9%; women 1.4%)	Dotterud and Smith-Sivertsen (2007)
Presumed exposure to colophony/tall oil		
Tall oil factory	0.6 % to tall oil rosin	Farm et al. (1994), Farm (1998)
Dancers and violinists	2.3/4.3%	Farm et al. (1995), Farm (1998)
Population where investigation required (skin clinics)	UK—3.9% Europe—1.3–6.9% [Portugal—1.3% Germany—1.3% France—3.1% UK—6.0% Denmark—5.7;6.9% (2 clinics)] Germany—4.0% USA—~2.5%	Sadhra et al. (1996), Gäfvert et al. (1996). Geier and Hausen (2000) Marks et al. (1998).

being examined was a group selected on the basis that they should be investigated further for allergic contact dermatitis.

3.3.5. Summary

Most studies in humans say nothing about the inducing agent. In studies on those occupationally exposed to 'rosin' exposure to rosin or oxidised 'rosin' is assumed, although the levels of exposure encountered have not been determined. The reasonable assumption is that uncontrolled exposure to higher levels of oxidised rosin is more likely in working populations than in the general population. These studies confirm that the results of the LLNA and Buehler assays, namely that oxidised rosin is not a potent sensitiser, are consistent with the conclusions generally concerning the relationship between potency in the LLNA and in humans. In other studies (i.e., in studies on those attending dermatological clinics), the inducing agent has not been determined. The inference is made that the eliciting agent is also the inducing agent. It can be suggested that studies based on populations from dermatological clinics were on individuals already in some way more susceptible to sensitisation, i.e., equivalent to adjuvant treated animals. Given that most rosin placed on the market is chemically modified, it is possible that the inducing agent is a related substance that cross-reacts with components in oxidised rosin, especially if the inducing dose(s) required is/are markedly higher than the eliciting dose.

4. Conclusions

Overall, this paper suggests that the situation in humans and animals is analogous. In animal studies, non-oxidised rosin is not a sensitiser. Studies in humans generally have used rosin containing oxidation products. The results for oxidised rosin depend on the evidence cited. When based on the epidemiology studies on workers and prevalence studies in the general population, prevalence is low, suggesting low potency. Animal tests using dermal administration give a negative response when oxidised rosin is tested. One test, the mouse LLNA, can be used for potency, and indicates that oxidised rosin is not a potent sensitiser. Thus, these two sources of information are congruent. However, when based on prevalence studies on patients tested when presenting at clinics (i.e., probably a particularly susceptible population), what was probably oxidised rosin elicited a response relatively frequently. Further, results of animal tests based on adjuvant treatment and intradermal dosing (i.e., models using animals rendered more susceptible to induction and/or exposed more to reactive intermediates) oxidised rosin is sensitising and should be classified. This paradox needs further investigation. As important, these results clearly indicate that different regulatory tests can give different responses for the same substance.

One question is 'which test result should be given precedence' when there is conflicting data and/or inadequate information. This circumstance can only happen when a substance has been marketed for some time. However, it is a possible scenario with the registration and testing requirements under the European Union REACH regulation (Regulation 1907/2006). When classification is based on human populations from dermatology clinics and a test that is likely to over-classify, it should be permissible to undertake further, more appropriate testing if the worker experience or the prevalence in normal populations suggests a miss-match. The results of the further test (which should be the mouse LLNA as it is currently the most validated test in terms of identifying potency Basketter et al., 2000, 2007) would then be used for classification. This would constitute a 'weight of evidence' approach, biased towards giving the more relevant test greater credibility. It would also permit direct comparisons with substances about to be mar-

keted within the European Union, for which the mouse LLNA is now the preferred study.

These studies may also indicate that there is a danger when classifying a substance on the basis of the intrinsic properties of another substance. It might be more effective to apply the procedures in Directive 1999/45/EEC (the Preparations Directive) to sensitising impurities. If the amount of the oxidised, sensitising material exceeds the threshold in the Directive, the appropriate classification/labelling provisions should be applied. In the present case this implies that, if the oxidation product is liable to be present at >1% in the preparation following storage under reasonable conditions for a sensible period, alerting to this fact should be undertaken in the form of application of the classification. If the oxidation product is liable to be present at >0.1% in the preparation, then the requirement to label 'Contains (name of sensitising substance). May produce an allergenic reaction' could be adapted to 'May contain (name of sensitiser) as an oxidation product. May produce an allergic reaction.' That this requirement can be applied to impurities is included in the Directive concerned with criteria for classification and labelling (Directive 2001/59/EEC). This approach would be accurate and consistent with the procedures adopted for other human health hazards.

Finally, great care should be taken when solely using human population data as the basis for classification. Unlike experimental data in humans, much of the data from these sources does not distinguish between low potency sensitisers with widespread exposure and high potency sensitisers with minimal exposure. Further, extrapolation from predisposed populations to general populations may add to the difficulties. Because of the continuing nature of sensitisation, once induced, it is probably unethical to conduct experimental studies deliberately examining for skin sensitisation in humans. With the coming of the LLNA we should use its ability to distinguish potency in order to distinguish between strong and weak sensitisers, and adjust classification criteria accordingly.

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