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On the skin sensitisation potential of rosin and oxidised rosin

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ABSTRACT

In the EU rosin is classified as a skin sensitiser, apparently on the basis of its oxidation to sensitising agents. Rosin (gum, tall oil or wood) is not a skin sensitiser when examined in the guinea pig maximisation test (GPMT). Oxidised rosins are sensitisers 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 sensitiser 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 sensitiser in this test. Thus, oxidised rosin is not a potent inducing agent for skin sensitisation 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.

1. Introduction

Colophony (rosin) is frequently claimed to be one of the most common skin sensitisers. 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 sensitiser on grounds of the skin sensitisation 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)
Individual rosin acids (as % w/w of total rosin acids)			
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
Neoabietic 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 ~10 μm) 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, (Tygelsjő, 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 (~10 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 sensitiser (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 sensitiser (Karlberg et al.,1999).

Oxidised rosin is a sensitiser 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 sensitiser.

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 sensitiser in this test. Hausen found synthetically prepared abietic acid to be a weak sensitiser. 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 peroxo—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