SKIN SENSITISATION OF ROSIN AND ITS DERIVATIVES IN RELATION TO THEIR CHEMISTRY ©

A MONOGRAPH

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BIOGRAPHIES OF THE AUTHORS

H Paul A Illing was formerly an independent consultant specialising in toxicology and risk assessment for occupational health, environmental pollution and consumer protection (1999-2015). For much of the time he was consultant to the Hydrocarbon and Rosin Resin and Pine Chemicals Producers Association (HARRPA) supervising the skin sensitisation programme and to H4R Consortium for the REACH Registrations of rosin and rosin related chemicals.

Dr. Illing founded his consultancy following 18 years in Government chemicals risk assessment. This included periods as Head of Toxicology and Head of Biocides and Biological Agents in the UK Health and Safety Executive and first Secretary of the Government/Research Councils Initiative on Risk Assessment and Toxicology (since renamed the Interdepartmental Group on Health Risks from Chemicals). He joined the Health and Safety Executive as a principal toxicologist at the start of the first Europe wide regulatory scheme (for New Substances) in 1982 and he implemented several subsequent regulatory schemes. Prior to this he was in pharmaceuticals research, undertaking studies on the disposition and metabolism of candidate drug substances.

Dr. Illing trained at Universities of St Andrews (BSc), Dundee (PhD), Mainz (Postdoctoral fellow) and Central Lancashire (MSc Env. Tox.).He holds fellowships from the Royal Society of Biology, the Royal Society of Chemistry and the Royal Institute of Public Health and is now a retired Fellow of the Institution of Occupational Safety and Health. He has published extensively on toxic risk assessment and management, and is author of 'Toxicity and Risk' (Taylor and Francis, 2001), and chapters in 'Handbook of Regulatory Toxicology' (Springer, 2014), 'General and Applied Toxicology' (Macmillan. first and second editions, 1993 and 2000), 'Fundamental Toxicology for Chemists'/'Fundamental Toxicology' (Royal Society of Chemistry, 1996 and 2006), 'Practical Guide to Chemical Safety Testing' (RAPRA, 2003) and 'Alternatives to Animal Testing' (Issues in Environmental Science and Technology vol. 23, Royal Society of Chemistry, 2006). He is also an Honorary Lecturer in the Centre for Occupational and Environmental Health, University of Manchester.

Dr. Illing was a Titular Member of Committee VII (Chemistry and Health) of IUPAC (2011-2014; Associate Member 2009-2010)) and a member of the RSC Environment, Health and Safety Committee and Occupational and Environmental Toxicology Group Committee. He was the RSC nominated member of the UK Home Office Poisons Committee (2012-2014) and the DEFRA Chemicals Stakeholders Forum (2003-2009). Dr. Illing is now retired.

Leon Rodenburg received his PhD from the University of Leiden in 1986. He performed environmental research for five years at The Netherlands Organisation for Applied Scientific Research, and in 1991 he joined Hercules to become a regulatory expert for the Adhesives business. Eastman Chemical acquired this in 2001 and Leon worked there until his retirement in September 2016.

Dr. Rodenburg's main fields of interest were food contact regulations, classification and labeling of hazardous substances and the REACH regulations.

He was the project leader in the Hydrocarbon and Rosin Resin and Pine Chemicals Producers Association (HARRPA) for the investigation of the skin sensitisation properties of rosin and its derivatives. He presented the results of these investigations at several conferences.

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SUMMARY

The issue of skin sensitisation from rosin and rosin derivatives has been the subject of much discussion and debate over many years. That discussion has been complicated by differences in testing methods, improper sample preparations that rendered some data equivocal and by the complex nature of the substances themselves. The purpose of this document is to present more recent test data conducted in a standardised fashion on this family of substances, and to present those data in a manner related to the function chemistry of those substances. It will be shown that rosin and rosin derivatives can be divided into groups based on their function chemistry and that the propensity toward skin sensitisation is related to this functional chemistry.

This document is divided into two major sections. The chemistry section provides a clearer picture of the complexity of this group of substances and how they are grouped based on their functional chemistry. The second section then takes each chemical group and presents relevant skin sensitisation data for that group including in some cases, older data that may have been equivocal or invalid for some reason with explanation as to why these data may be less than reliable.

Rosin is demonstrably not a skin sensitiser in any of the tests conducted. Rosin in the physical forms used in practice (e.g. pelletised or drummed) does not appreciably oxidise. Naturally oxidised rosin, prepared in the laboratory by prolonged exposure of powdered rosin to air at ambient temperature (since no commercial product exists), is sensitising only in the GPMT (Guinea Pig Maximisation Test), but not the mouse LLNA (Local Lymph Node Assay) nor the Buehler assay. Superoxidised rosin, prepared in the laboratory by exposure to oxygen under high pressure, is not a sensitiser. Hydrogenated rosin cannot be easily oxidised so it is not a sensitiser by any test. Although, in an inadequately described test the potassium salt of hydrogenated rosin gave a marginally positive result, on a weight of evidence basis, rosin and hydrogenated rosin salts, and rosin and hydrogenated rosin esters are not sensitising. Disproportionated rosin and formaldehyde-treated rosin are also not sensitising.

Rosin adducts, formed by reaction with maleic anhydride or fumaric acid, are clear sensitisers. Esterification of the acid anhydride results in a considerable reduction in the skin sensitising potential, but some residual activity remains.

Predictably, large molecules generally do not penetrate the skin and therefore the phenolic resins are not skin sensitisers.

INTRODUCTION

Like all chemicals, rosin and its derivatives are regulated by law. They are included in the definition of substances of "Unknown or Variable composition, Complex reaction product or Biological material" (UVCB) used by both the United States Environmental Protection Agency (US EPA) and the European Union European Chemicals Agency (EU ECHA).

One of the less desirable properties of rosin is the potential for oxidation. Oxidation discolours the substance and may result in modification of the toxicological properties. The conjugated diene structure also provides desirable reactive sites for chemical modifications. Hence the conjugated double bond system is key to understanding the chemistry of rosin.

Hydrogenation and adduct formation are reactions taking place across the conjugated double bond system, and hence prevent or reduce oxidation. Other chemical modifications may alter the reactivity of the double bond system. Rosin, oxidised rosin and chemically modified rosins have been grouped on the basis of their chemistry into three main categories, each consisting of a number of groups, namely:

Starting materials and groupings where the conjugated diene structure is retained -

- Resin acids and rosin acids
- Rosin salts
- Formaldehyde treated rosin
- Rosin esters

Chemically modified rosins wherein the conjugated diene structure has been modified or eliminated -

- Hydrogenated rosin
- Dehydrogenated/disproportionated rosin
- Hydrogenated rosin salts
- Hydrogenated rosin esters
- Rosin adducts and adduct salts
- Formaldehyde modified rosin adducts
- Rosin adduct esters

Other chemical modifications (a miscellaneous group of substances) -

- Phenolic modified rosin adducts
- Decarboxylated rosin

Early studies (pre 1991) on the skin sensitisation potential of rosin and chemically modified rosins used a variety of testing techniques and of test materials. In many cases, so-called ‘rosin’ was at least partially oxidised through storage under inappropriate conditions that allowed the rosin to form hydroperoxides and therefore the results of these tests are unreliable.

More recently, rosin, oxidised rosin and chemically modified rosins have been grouped on the basis of their chemistry. Their skin sensitisation potential has been assessed using Organisation for Economic Co-operation and Development (OECD) regulatory test procedures, principally the Buehler test, the Guinea Pig Maximisation test (GPMT) and the mouse LLNA (local lymph node assay). In general, the testing is consistent with the known chemistry of rosin, oxidised rosin and chemically modified rosins. The results are

remarkably consistent across a wide range of substances tested, including nominally the same substance from different sources, and across testing laboratory and test method.

ROSIN CHEMISTRY

What is rosin?

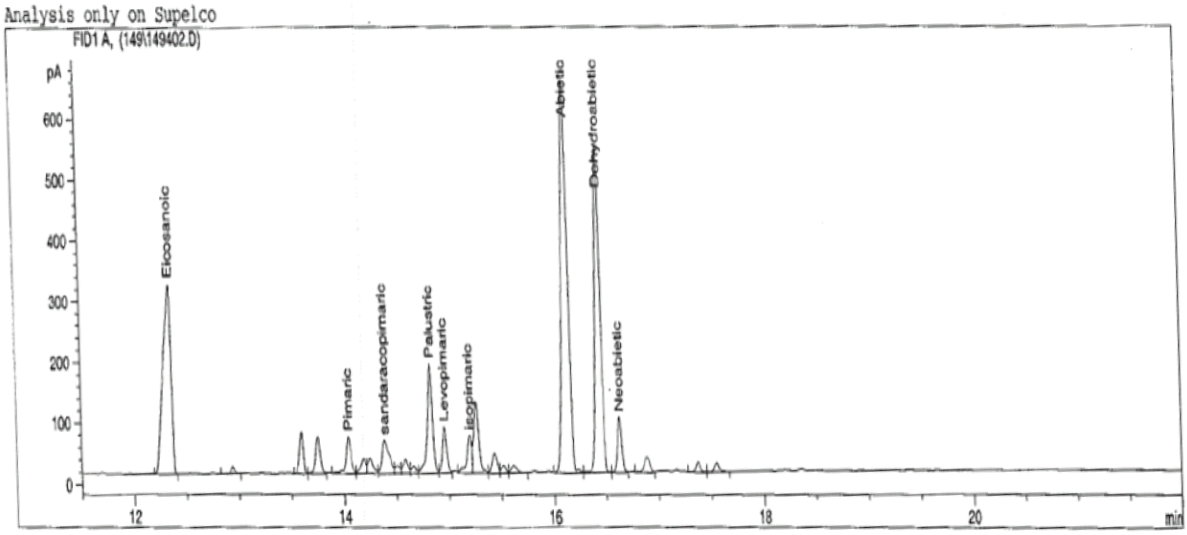
Rosin is a complex naturally occurring substance obtained from trees, typically pine trees. It is a light amber glassy solid at room temperature. Rosin has been used commercially for thousands of years, for example, in caulking the seams in wooden ships. The substance consists mainly of diterpenic carboxylic acids containing one or two double bonds and, in some resin acids, an aromatic ring. The chemical properties make possible many different rosin derivatives and give rise to the useful physical properties of rosin. Rosin and rosin derivatives are used in a wide variety of applications such as adhesives, varnishes, printing inks and coatings, paper sizing, lubricant additives, plasticising agents, air entrainment aids and even food (chewing gum) and food additives (citrus drink clouding agents and fruit waxes).

Rosin has a broad melting range, is very poorly soluble in water and has very low vapour pressure. The sample used for registration in the EU had a melting range of 66-93^{o}C, a solubility of 0.9 mg/kg water and a vapour pressure calculated as 0.06 mbar at 20 ^{o}C (see ECHA website).

Composition

There are more than 20 different isomeric structures of resin acids, most of which have the general formula of $C_{19}H_{29}COOH$. Below is a typical chromatogram of rosin illustrating the complex nature of rosin (Figure 1).

Figure 1: Gas chromatogram of rosin



In chemical nomenclature the terms "rosin" and “rosin acids and resin acids” are essentially synonymous. Abietic acid is generally the predominant resin acid in rosin and is often used to illustrate the typical structure of resin acids. The structure of abietic acid and some other common resin acids are shown under the next section. The ratio of the various resin acids in rosin varies depending upon the region from which it is obtained, the process used to isolate it, the species of tree from which it came and even in some cases, the climate in which the tree grows. However, the chemistry is similar across the family.

The total acid content of rosin is typically 90-95% depending upon the source of the rosin and the manufacturing process. The remaining components are commonly called “neutrals” or “unsaponifiables” because these components do not have the carboxylic acid functionality and are generally less reactive. The “neutral fraction” is generally composed of diterpene hydrocarbons, alcohols, esters, or aldehydes. The neutral fraction is typically <10% and relatively unimportant relative to the resin acid components. As rosin is defined as a UVCB, the neutral fraction is a part of the substance[1].

[1] Generally the consortium dealing with EU REACH registrations (H4R) has aimed at consistency, and treated rosin as a UVCB substance and hence chemically where the source is biological and the process is a synthesis). The Guidance states that:

> ‘A description of the chemical process shall be a generic description of the type of process (esterification, alkaline hydrolysis, alkylation, chlorination, substitution etc.), together with relevant process circumstance’.

However, it is possible that some independent registrants under the EU REACH programme may have treated rosin as a single chemical substance and hence chemically modified substances derived from rosin as ‘multi-constituent substances’ rather than UVCBs. According to the guidance the definition of a multi constituent substance is:

> ‘A multi-constituent substance is a substance consisting of several main constituents present at concentrations generally ≥ 10% and < 80% (w/w). A multi-constituent substance is named as a reaction mass of two or more main constituents. A multi-constituent substance is the result of a manufacturing process’.

For commercial reasons the source of rosin has been indicated by using trivial names, referring more to the manufacturing process than to chemical differences. “Gum rosin” is the term used for rosin that is derived from tapping live trees. “Tall oil rosin” is the term used for the rosin that is derived from tall oil, a product that is set free during the pulping of tree trunks for the paper industry. “Wood rosin” is rosin that is obtained from the extraction of tree stumps and the root system that are left behind after the harvesting of pine trees for timber and paper making.

A synonym for rosin is “colophony”, after the ancient Greek city (now in Turkey) called Colophon.

Further confusion was added when European Inventory of Existing Commercial Chemical Substances (EINECS) was set up in the late 1970’s and early 1980’s. Companies submitted entries for rosin based on the available Chemical Abstracts Registry Numbers (CASRN’s), but without coordination between producers and importers[2]. The result was that four CASRN’s were used for rosin: 8050-09-7 rosin; 8052-10-6 rosin, tall oil; 73138-82-6 resin acids and rosin acids; and 94114-23-5 resin acids and rosin acids, tall-oil”.

A detailed look at the composition of rosin and the source it is obtained from, i.e. live tree tapping (gum rosin), wood pulping (tall oil rosin) or tree stump extraction (wood rosin), indicates that there are slight differences in resin acid distribution. Table 1, taken from the chapter by Soltes and Zinkel in the book Naval Stores [Zinkel and Russell, 1989], shows the compositions.

[2] As these registrations generally lack CAS numbers, unless they are known to the H4R Consortium or the Pine Chemicals Association, data from them cannot be evaluated. No such data was available at the time of publication of this monograph.

Table 1: Typical composition of common resin acids in some US rosins

		Rosin	
Resin acid[1]	Tall oil[2]	Wood	Gum
Pimaric	4.4	7.1	4.5
Sandaracopimaric	3.9	2.0	1.3
Communic	1.0	---	3.1
Levopimaric	---	---	1.8
Palustric	8.2	8.2	21.2
Isopimaric	11.4	15.5	17.4
Abietic	37.8	50.8	23.7
Dehydroabietic	18.2	7.9	5.3
Neoabietic	3.3	4.7	19.1

[1] Percent of acid fraction

[2] Also contains fatty acids and other minor resin acids such as the secodehydroabietic acids

One is tempted to regard the differences as significant but the resin acid distribution is much more dependent on species of the pine tree, geographical area, climate and season. Table 2 below, also taken from Soltes and Zinkel (1989) demonstrates this. Analysis of the resin acids in the oleoresin shows significant variation in resin acid distribution, depending on the species of the tree: abietic acid ranges from 8.6 % in Pinus taeda to 37 % in Pinus halepensis, levopimaric and palustric acid ranges from 12 % in Pinus peuce to 64 % in Pinus taeda.

Table 2 also shows the acid distribution in gum rosin from different geographical areas. Taking the same resin acids, abietic acid ranges from 22 % in American and Honduran rosin to 53.3 % in Mexican rosin. Levopimaric and palustric acid range from 9.8 % in Mexican rosin to 30 % in Portuguese rosin. These numbers illustrate clearly that rosin is a true UVCB.

Also when rosin is heated during processing, for example, during distillation, the abietic-type resin acids tend to equilibrate. The levopimaric acid disappears and abietic acid becomes the predominant resin acid and the aromatic dehydroabietic acid also increases. (Chen 1992, p 142-143)

Table 2: Principal resin acids in typical pine oleoresins and some commercial gum rosins[1]

Samples	Pimaric	Sandara-copimaric	Communic	Levo-pimaric/ Palustric	Iso-pimaric	Abietic	Dehydro-abietic	Neo-abietic
Oleoresin	Per Cent of Acid in Acid Fraction							
P. elliottii var. elliottii	5.1	1.8	3.1	37	21	9.7	3.7	16
P. elliottii var. densa	3.8	1.9	3.1	38	21	12	3.7	16
P. palustris	5.4	1.1	0	52	10	9.4	8.3	13
P. taeda	8.7	2.2	0	64	T	8.6	6.3	9.5
P. ponderosa	7.6	2.9	0	40	15	11	8.2	11
P. halepensis	0	1.2	0	39	10	37	1.5	9.7
P. brutia	0	1.2	0	44	10	32	2.5	10
P. pinaster	8.0	2.0	0	39	12	14	4.2	18
P. caribaea	4.2	2.2	0	49	8	10	8.6	16
P. peuce	1.8	1.0	0	12	32	35	0.8	14
Rosins								
American	5.1	1.8	2.8	25	17	22	5.7	20
American[2,3]	5.4	1.8	1.8	20.3(1.4)	14.2	27.9	7.1	16.3
Brazilian[2]	4.7	1.7	3.2	11.4(0.3)	18.2	36.3	5.4	
Burmese	7.9	3.0	0	44	8.3	30	6.0	2.2
Chinese	9.2	2.7	0	22	1.5	44	4.3	15
French	10	2.2	0.3	22	7.0	36	4.9	17
Greek	0	1.9	0	14	11	50	4.5	13
Honduran	9.6	2.2	0	21	17	22	12	15
Indian	9.2	1.5	0	11	20	38	2.0	18
Mexican[2,4]	6.8	1.2	0	9.8(0.3)	12.9	53.3	7.8	6.1
Portuguese	8.8	1.9	0.7	30	5.3	32	5.1	16
Portuguese[2]	8.3	1.4	0	20.4(11.7)	4.5	27.7	5.8	17.2
Russian	7.8	2.4	0	27	5.6	35	5.3	17
Spanish	8.7	1.5	0	27	0	36	1.9	24
Turkish	0	1.3	0	24	13	41	5.1	15

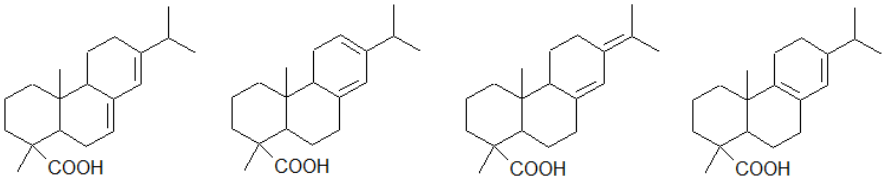
[1] Data from Soltes and Zinkel, 1989.
[2] Palustric values given first, levopimaric values are in ().
[3] Also contains small amounts of imbricataloic acid, as well as imbricataloic and isocupressic acids and their acetates
[4] A distilled resin

As the variability of the composition of rosin is a natural phenomenon typical of UVCB substances, it is reasonable to say that there are no significant differences between gum, wood and tall oil rosin and that the three types of rosin are essentially the same in their major uses. There should thus be no distinction between them or their derivatives from these three sources for regulatory purposes. In fact, the United States Environmental Protection Agency (USEPA) has consolidated the three rosins into one TSCA Inventory

entry (CASRN 8050-09-7) (Lau 1992). This also underlies the coordinated naming of chemically modified rosins achieved as a consequence of the institution of REACH, and, in particular the Substance Information Exchange Forum (SIEF) and lead registrant concepts in the European Union (EU). For REACH purposes all rosins are considered to be the same.

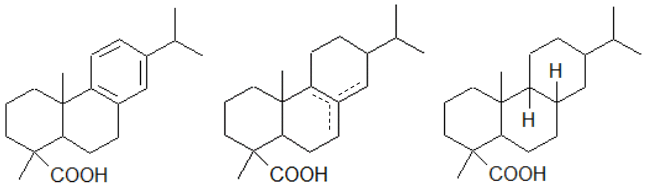
Resin Acids in Rosin

The four predominant and most important resin acids in rosin, commonly called the "abietic-type" resin acids, are shown below.

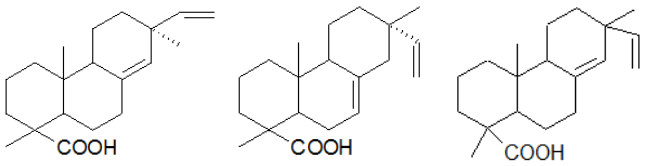


Abietic acid | Levopimaric acid | Neoabietic acid | Palustric acid

These resin acids are of importance because in addition to the carboxylic acid functionality, they also have conjugated double bonds. The key importance of this will be discussed later under the section on "Reaction Chemistry". Depending upon source and method of manufacture, rosin typically contains 50-70% of these abietic-type resin acids. Additional structures for some of the other important resin acids are shown below.



Dehydroabietic acid | Dihydroabietic acid | Tetrahydroabietic acid



pimaric acid | isopimaric acid | Sandaraco pimaric acid

The carboxylic acid group and the unsaturation make several chemical reactions possible, each leading to properties that lead to a wide variety of applications such as adhesives, printing inks and coatings, paper sizing, lubricant additives, plasticising agents, air entrainment aids and foods (chewing gums) and food additives (emulsifiers – E445[3]).

[3] Approved food additive in EU Regulation (EC) No 1333/2008 on food additives

Addition reactions across the unsaturated bonds

Oxidation

The two conjugated double bonds of resin acids provide desirable reactive sites for chemical modifications. However, they also render the molecule susceptible to oxidation. This causes discolouration of the product and other undesirable changes in properties, so procedures for producing, shipping, storing and using rosin are purposely designed to eliminate the presence of air.

Minor (1965) demonstrated that of the pure resin acids, only abietic acid reacts with oxygen. The weight increase after 90 days of exposure to air is reported to be 9.3 %, while none of the other pure acids showed any weight increase. Thus minimising the abietic acid content of rosin or its derivatives stabilises the product.

This can best be observed in the oxygen absorption by rosin and rosin derivatives shown in Table 3:

Table 3: Typical oxygen absorption of various types of rosin and rosin derivatives (source Kennedy et al., 1989)

Rosin substance	Weight-% O_2 Absorption
Unmodified rosin	9
Partially dimerised rosin	5
Polymerised rosin	1
Hydrogenated rosin	< 1
Pentaerythritol ester of rosin	< 1

As explained later, dimerisation or polymerisation of rosin involves the reaction of the double bonds between two rosin molecules. No conjugated diene system remains after the formation of the dimer, so it is difficult for it to oxidise. The same is true in hydrogenated rosin, where the double bonds are eliminated by hydrogenation. In the case of rosin esters, disproportionation takes place during the esterification. The disproportionation reaction, as will be explained below, involves the hydrogenation and dehydrogenation of rosin and thus a reduction in conjugated double bonds.

It is known from unpublished studies in the 1960's conducted by Hercules, that rosin oxidises to form either peroxide (-C-O-O-C-) or hydroperoxide (-C-O-O-H), depending on the reaction conditions. The method of oxidation plays a dominant role in whether oxidised rosin is or is not a skin sensitiser.

"Naturally oxidised rosin", produced by exposure of powdered rosin to air at room temperature for many days, contains hydroperoxide functionality (see Figure 3). The presence of hydroperoxide in naturally oxidised rosin can be unambiguously demonstrated by IR spectroscopy (see Figure 3). The reference substances cumylhydroperoxide and dicumylperoxide, shown in Figure 2, were used to identify the hydroxy stretch vibration in a hydroperoxide at 3411 cm^{-1}, which is absent in the spectrum of dicumylperoxide.

Figure 2: Infrared spectra of cumylhydroperoxide and dicumylperoxide, reference spectra

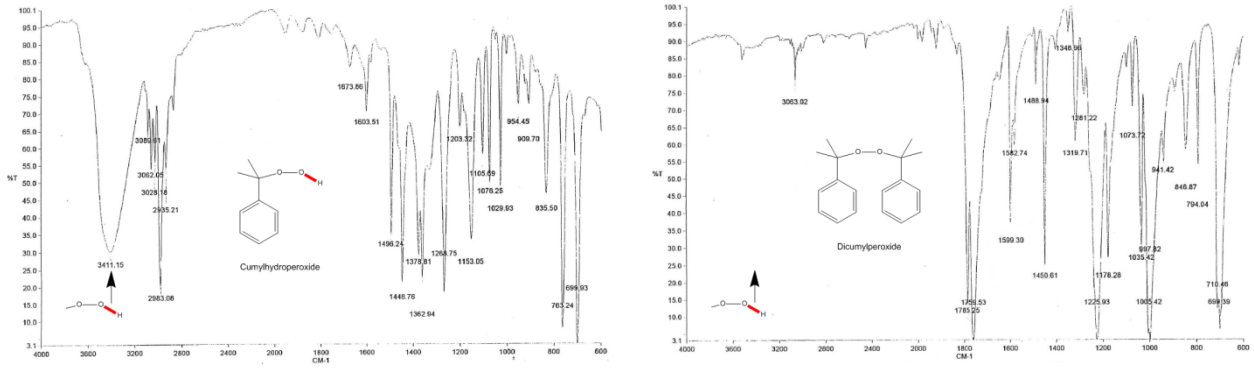
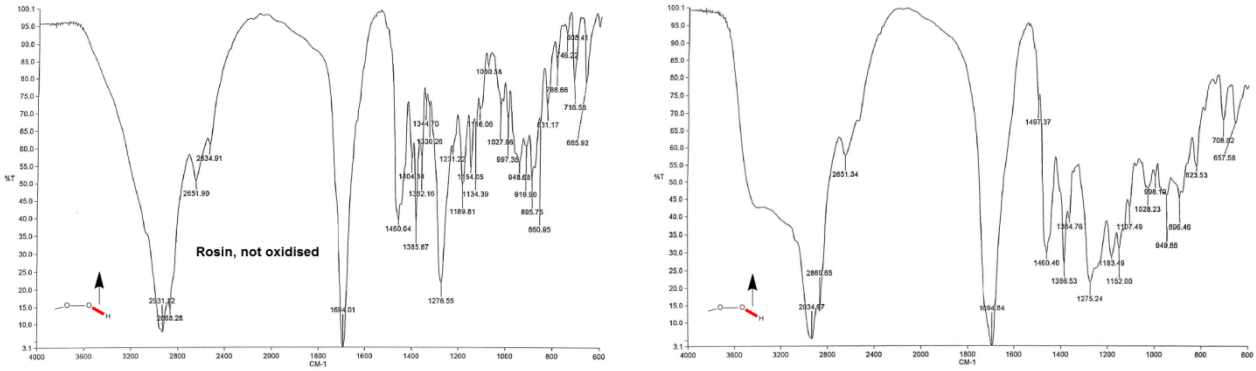
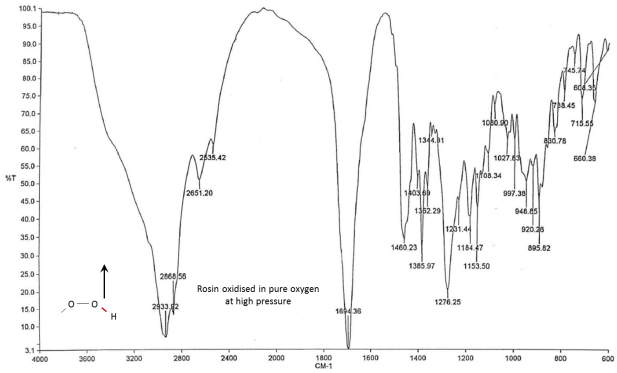


Figure 3: Infrared spectra of rosin and powdered rosin after exposure to ambient air for 105 days



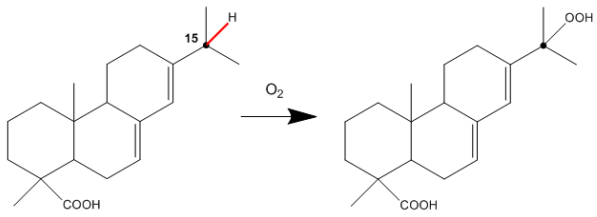
However, **"superoxidised rosin",** produced by oxidation in an atmosphere of pure oxygen at high pressure (15 bar or 220 psi), contains only peroxide functionality, as demonstrated by infrared spectroscopy (see Figure 4) and is not a sensitiser.

Figure 4: Infrared spectra of powdered rosin exposed to pure oxygen at high pressure



Reaction with oxygen causes the disappearance of the conjugated double bond chromophore (Chen 1992, p 150) and the generation of the 15-hydroperoxide among other oxidation products. The hydrogen atom

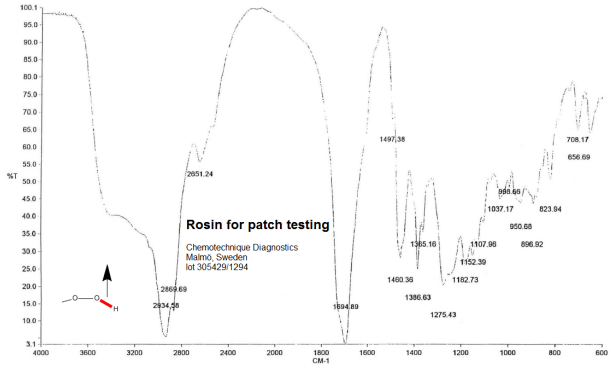
on position 15 of abietic acid (see figure 5) is both tertiary and allylic to the conjugated diene moiety so that free radicals are easily formed (Karlberg, 1988).



abietic acid 15-hydroperoxyabietic acid

So-called "rosin" is sold as a solution for patch testing in dermatological clinics. IR spectra show that these samples are heavily oxidised and not representative of true rosin.

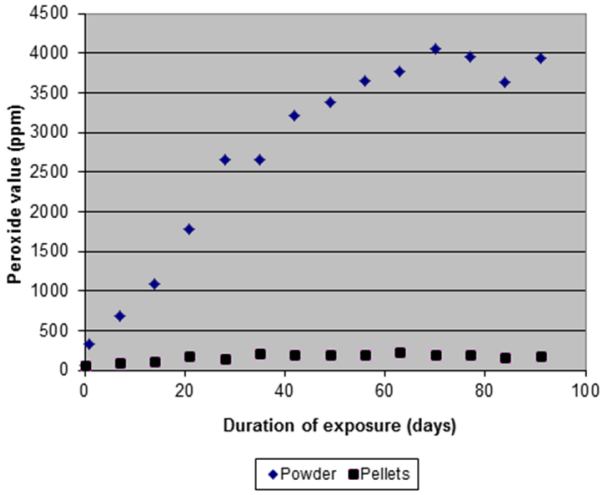
Figure 5: Infrared spectrum of rosin solution as offered for sale to dermatological clinics



The physical form of rosin also has a dominant effect on whether or not the rosin oxidises (see Figure 6). In a time course study, pelleted or powdered gum rosin were both subjected to natural oxidation (i.e. exposed to air at room temperature) (Botham et al, 2008). 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 and the peroxide number was determined before and during oxidation. At least duplicate samples were taken at each time point and peroxide content measured by titration.

This study demonstrates that rates of oxidation of rosin depend upon its physical form. If the rosin has a high surface area it is oxidised relatively readily in air. If the substance is in massive block form or in the form of pellets oxidation may occur at the surface, but does not occur throughout the rosin.

Figure 6: Peroxide Value of Pelletised and Powdered Rosin vs. Oxidation Time



(from Botham et al., 2008)

From the graph it can be concluded that, if the surface/volume value increases, there is much more oxidation. In the graph it can be seen that for powdered material a plateau is reached after 60 – 70 days. This phenomenon can be explained by the fact that hydroperoxides are labile substances. Apparently, after 60 – 70 days, formation and breakdown have come to equilibrium. The hydroperoxides formed decompose to acid, alcohol and aldehyde groups. There is substantial evidence that a variety of relatively unstable species, notably epoxy and peroxo-compounds and hydroperoxides, are produced as a consequence of this oxidation process (Gafert et al., 1994).

Much rosin is also transported as a hot liquid under nitrogen, which also minimises oxidation.

Hydrogenation

Hydrogenation is one way to reduce the active unsaturated sites thereby lessening the probability of oxidation (Soltes and Zinkel, 1989). Partial hydrogenation to saturate one of the conjugated double bonds is relatively easy to achieve but full hydrogenation to saturate the second double bond is more difficult due to lower reactivity and steric hindrance. Commercially available hydrogenated rosin has varying degrees of hydrogenation but is generally not fully hydrogenated. However, simply reducing the concentration of conjugated diene structure, especially that of abietic acid, is sufficient to greatly reduce the potential for oxidation and improve the stability of the products. Hydrogenation does not otherwise alter the structure and nor does it not affect the carboxylic acid functional group, and is often a useful “first step” for applications requiring lighter colour or higher oxidative stability of the finished product. In particular, the 15-hydroperoxide of abietic acid cannot be formed.

Hydrogenated rosin and its ester derivatives are used in adhesives and sealers, cosmetics, electronics, paints and coatings, and inks and paper. They have FDA approval for a number of food-related and medical uses. They are also used in personal care products such as depilatory waxes. In the EU these resins are approved by the European Food Safety Authority as additive to food contact plastics (Regulation (EU) No 10/2011).

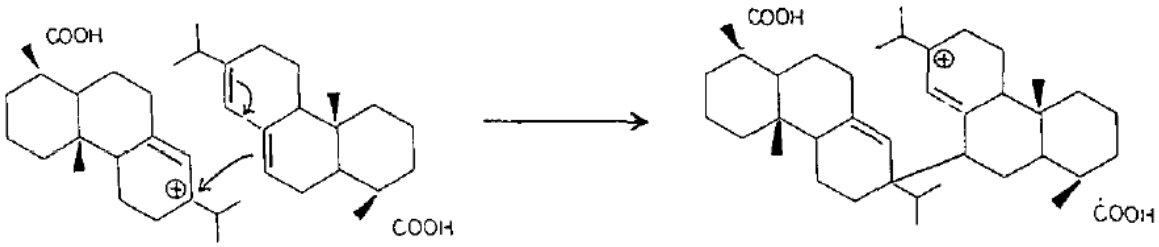
Dehydrogenation/Disproportionation

This process is sometimes used reduce the conjugated double bonds in some resin acids, thereby making the resulting disproportionated rosin less susceptible to oxidation (Soltes and Zinkel, 1989; Chen 1992, p

150). This process usually involves a catalyst that is capable of transferring hydrogen. The reaction converts two dienes to a hydrogenated resin acid and a dehydrogenated resin acid, thus altering the ratios from that of the original rosin. Disproportionated rosin may contain more than 50 weight-% of dehydroabietic acid. Because of the lack of abietic-type acids to react with free radicals, disproportionated rosin finds much use as an emulsifier in synthetic rubber manufacture

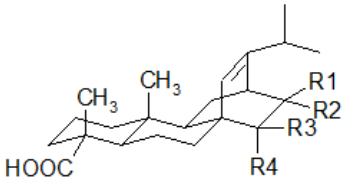
Dimerisation

Treatment of rosin with acid catalysts such as sulphuric acid and boron trifluoride generates a dimer of rosin called "dimerised rosin" or (incorrectly) "polymerised rosin." (Chen 1992, pp. 151-154). It appears that the dimers are formed mainly by carbon-carbon bond formation between the diene resin acids. At least 12 structures of dimer are present. Dehydroabietic acid is a byproduct. The net result is a non-crystalline, higher melting product that is less susceptible to attack by oxygen that can be further reacted to form adhesive, lacquer, varnish or ink resins.



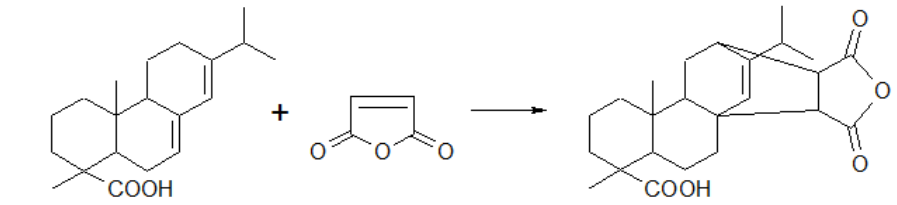
Diels-Alder Addition Reactions leading to Maleopimaric Acid

Diels-Alder addition can be used to form what are commonly called "rosin adducts" or "fortified rosin" (Mayr et al., 1984; Soltes and Zinkel, 1989; Wiyono et al, 2007, Wiyono and Tachibana, 2008; Chen, 1993 pp. 155-158). This type of reaction is used to place additional functionality on the rosin molecule. Adduction reactions are typically carried out using heat and acid catalyst but are also self-catalyzed by the carboxylic acid group. (see Soltes and Zinkel, 1989). Because Diels-Alder adduction requires rosin molecules with a homoannular conjugated diene structure, only the levopimaric acid can be adducted. The different diene resin acids are in equilibrium in the presence of an acid catalyst. A small concentration of levopimaric acid is constantly generated, and reacts with the dienophile to form the same adduct structures. Examples of some Diels-Alder dienophiles and their resulting structures are given below.

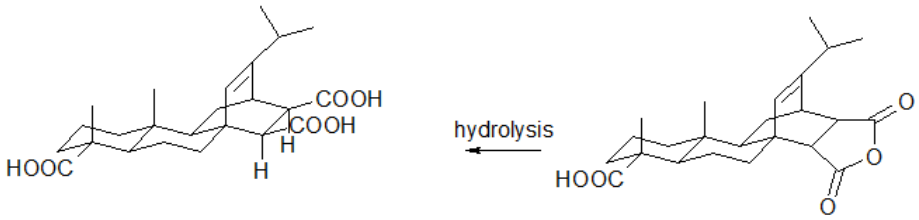


Reactant	Structure
Maleic anhydride	R1, R3 = COOH (endo, endo); R2, R4 = H
Fumaric acid	R2, R3 = COOH (exo, endo); R1, R4 = H
Acrylonitrile	R3 or R4 = CN (mixed endo and exo); R1, R2 = H
Acrylic acid	R3 or R4 = COOH (mixed endo and exo); R1, R2 = H

From maleic anhydride maleopimaric acid is formed, which contains an anhydride ring in the endo position. The reaction with fumaric acid is slower than that with maleic anhydride but the products are lighter in colour and higher melting. Fumaropimaric acid can easily isomerise and cyclise to endo-maleopimaric acid, and, in fact, maleopimaric acid is an impurity in fumaropimaric acid preparations. Irrespective of starting dienophile the eventual product on continued heating is maleopimaric acid (Wiyono et al, 2007).



abietic acid maleic anhydride maleopimaric acid anhydride



maleopimaric acid anhydride

Rosin adducts find use in various ink and coating resins where the polyfunctionality allows higher molecular weight resins to be formed. Obviously hydrogenated rosin cannot form these adducts as the relevant diene structure has been removed by the hydrogenation.

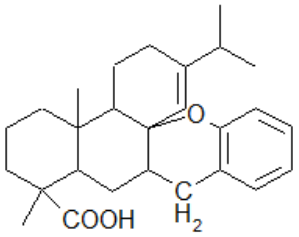
Formaldehyde-Treated Rosin

Rosin is often reacted with 0.5 to 4% by weight of formaldehyde in the presence of an acid catalyst to lower its tendency to crystallise. The softening point of rosin is about 80 °C, while crystallised rosin melts at 110-130 °C often causing problems in the manufacturing process by blocking pipelines.

The chemistry of rosin reaction with fomaldehyde is complex and depends upon the stoichiometry and reaction conditions. Many different structures have been proposed (Strazsins 1989; Bicu and Mustata 1993,1994; Chen 1992, pp159-160). However, the most definitive study of commercial formaldehyde-treated rosin was published by McGuire and Suchanec (1994). They found that in addition to unmodified rosin, this product contains two stereoisomers of 7-methyldehydroabietic acid, 14-methyldehydroabietic acid, dimethyl substituted dehydroabietic acid, 7-hydroxymethylabietic acid and abietic acid substituted by both hydroxymethyl and methyl groups. The authors postulated that these results can be reasonably explained by a Prins reaction at the C-7 and/or C-14 positions to create hydroxymethyl derivatives followed by dehydration and aromatization.

Phenol/Formaldehyde Addition

The phenol/formaldehyde addition is another important modification that can occur with rosin (Soltes and Zinkel, 1989; Belgacem and Candini 2008). This modification has wide application in the printing ink, coating and adhesives industries. Condensates of alkylphenols such as tert-butylphenol and para-nonylphenol with formaldehyde are reacted with rosin esters and rosin adduct esters yielding polymers of very complex structures (Challinor 1993; Kang et al 2000). One representative structure is shown below.



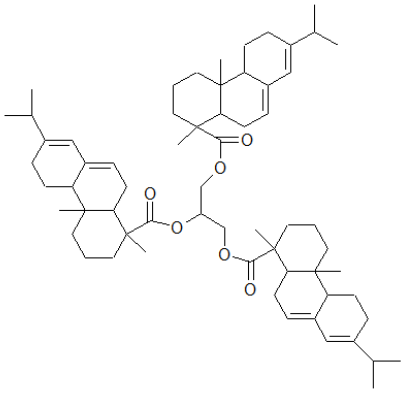
Abietic acid reacted with phenol and formaldehyde

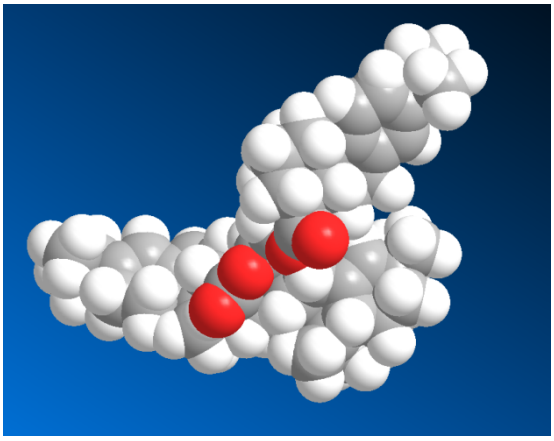
Reactions at the Carboxyl Group

The carboxylic acid functionality allows various chemical reactions such as esterification and salt formation. The most important of these various reactions and derivatives are discussed below.

Esterification

Rosin and hydrogenated rosin can undergo esterification with alcohols or polyols such as methanol, glycerol, pentaerythritol and triethylene glycol (Soltes and Zinkel, 1989). As hydrogenation does not significantly affect the carboxylic acid functionality the resulting esters are essentially similar irrespective of whether rosin or hydrogenated rosin was the starting material. The diagrams below show the structure of the typical product of the esterification reaction between rosin and glycerol, the glycerol triester of rosin.





Glyceryl triabietate, the space filling structure on the right shows how bulky this ester is. The steric hindrance in the molecule shields the ester function from external reagents. Also, the bulk of the molecule surrounding the ester is a cyclic aliphatic hydrocarbon moiety, explaining the very low water solubility of this type of substance.

Esterification is usually conducted at elevated temperatures under an inert atmosphere that prevents oxidation taking place (Soltes and Zinkel, 1989). In most industrial esterification processes a catalyst is added to partially disproportionate the rosin and to lighten the colour; this significantly reduces the concentration of abietic acid present leading to a product with improved oxidation resistance (see for example Johnson (1987)). Steric hindrance may limit the extent of esterification. The major use for rosin esters is as adhesive tackifiers - principally in hot-melt adhesive systems used in case sealing, baby diapers etc.

Esterification of Adducts

Esters can also be made with adducted rosin (see under "Diels-Alder Addition"). Because the rosin adduct is multifunctional, its esterification with polyols such as glycerol, pentaerythritol and triethylene glycol can, but not necessarily result in polyester formation. Depending upon the ratio of carboxylic (from the rosin adduct) to hydroxyl (from the polyols) groups, complex polymeric and cross-linked structures are possible.

The inverse reaction, that is, esterification of the rosin first with a polyol followed by reaction with maleic anhydride or fumaric acid in the second step leads to approximately the same substance. The principle is also applicable to the glycerol esters of fumarated and maleated rosin.

Salt Formation

Salts can be made from rosin, disproportionated rosin, hydrogenated rosin or adducted rosin. They can be split into 2 groups. The salts of monovalent cations (e.g. sodium, potassium [Na^+, K^+]) are usually called "soaps" and the salts of divalent cations (e.g. calcium, magnesium, zinc [Ca^{2+}, Mg^{2+}, Zn^{2+}]) are usually called "resinates". Na^+ and K^+ salts of maleic or fumaric adducted rosin are commonly used as sizing agents in the manufacture of paper. Ca^{2+}, Mg^{2+} and Zn^{2+} resinates often find application in the ink and coating industry.

Whereas the salts of monovalent cations are partially soluble in water and stable at high pH (typically > 9), the salts of divalent cations are highly insoluble in water (Ca-salt: 43 mg/l, Mg-salt: 65 mg/l; Ca/Zn-salt: 18 mg/l) but relatively soluble in non-polar solvents and oils. Due to their difference in water solubility, the two types of salts will not have the same behaviour environmentally. The very low solubility of the salts of divalent cations is similar to the solubility of the resins they are synthesised from. For that reason, the behaviour of the starting resins and their divalent salts is expected to be the same. For the monovalent cation salts, the pH determines whether you have the free acid (R-COOH), the salt ($R\text{-}COO^-\ Na^+$) or a mix of the two. Hence, the monovalent cation salts will be evaluated separately.

The neutral fraction of rosin is never soluble in water, regardless of the pH. Therefore, monovalent resinates will never be clear solutions.

Summary

- Rosin is a substance of complex and variable composition derived from a biological source (UVCB).
- Many of the names given to rosin and chemically modified rosins were the result of uncoordinated submissions of entries to chemical inventories and refer to the manufacturing process, not to chemical/toxicological differences.
- The conjugated double bond system is key to understanding the chemistry of rosin and its chemically modified derivatives. The conjugated diene structure enhances the oxidation that discolours the substance and that can modify the toxicological properties.

- Elimination of the conjugated double bond system by hydrogenation, disproportionation, dimerisation or adduct formation prevents or reduces oxidation.

Justification for Grouping Rosin and Rosin Derivatives into Families

Generally, when there are large numbers of closely related substances an attempt can be made to group substances together in order to minimise testing in animals. In the case of chemically modified rosins this can be conducted on the basis of the chemistry behind the modification. This approach has been used for both the United States High Production Volume (US HPV) chemicals program and for the EU Registration, Evaluation and Authorisation of CHemicals (REACH) registrations. Although both schemes are tonnage based, the EU scheme includes substances produced at lower tonnages than those in the US HPV program and excludes polymers.

The US HPV challenge program included test plans for:

- Rosins and rosin salts;
- Rosin esters;
- Rosin adducts and adduct salts.

These groups were modified for the EU REACH programme to reflect the wider range of chemicals being examined. The key addition was a grouping entitled:

- Rosin adduct esters.

Outlined below are the chemicals included in the groupings. It should be noted that, as skin sensitisation testing has been carried out on representative substances for each group, there will be substances listed for which experimental testing has not been conducted. Also, there may have been testing on low production volume chemically modified rosins that have not yet been registered but are clearly members of certain groups. Small volume chemicals may be essential to a test program as they may represent 'worst case' scenarios.

Rosin, Hydrogenated Rosin and Their Salts

Rosin is described in EINECS under two types: rosin (EC No 232-475-7/CASRN 8050-09-7) and tall oil rosin (EC No 232-484-6/CASRN 8052-10-6). Rosin and tall oil rosin are also listed in EINECS under EC No 277-299-1/CASRN 73138-82-6 as "Resin acids and Rosin acids" and EC No 302-657-1/CASRN 94114-23-5 as "Resin acids and Rosin acids, tall-oil". As explained above, these different names refer to manufacturing processes, not to chemical/toxicological differences. For regulatory purposes rosin (EC No 232-475-7 or CASRN 8050-09-7) is the term that is used.

Rosin has two reactive sites: the carboxylic acid group and double bonds. In the hydrogenation process the double bonds, and hence the reactivity related to the double bonds is removed. Hydrogenation is applied by industry to stabilise rosin against oxidation. Therefore, industry is convinced, and has convinced the European Chemicals Agency (ECHA) that the toxicology of rosin can be regarded as the 'worst case' scenario for hydrogenated rosin. This is also the reasoning applied to the comparison of rosin derivatives and hydrogenated rosin derivatives, where the derivatisation takes place with the same chemical. Thus the toxicity of the glycerol ester of rosin would be the worst-case scenario for the toxicity of the glycerol ester of hydrogenated rosin.

The CASRN of rosin includes catalytically disproportionated rosin. A disproportionation reaction involves two dienes, where one of the dienes dehydrogenates, delivering the hydrogen to the other diene, which hydrogenates. Disproportionated rosin may contain more than 50 weight-% of dehydroabietic acid, an aromatic ring containing molecule. As disproportionation reduces the amounts of material with conjugated diene structures it deactivates the potential for addition reactions involving conjugated diene structures and hence the likelihood of toxicological interactions, i.e. rosin is the 'worst case' scenario.

As discussed earlier, rosin may react with itself at the double bonds to form a molecule carrying the trivial names "rosin dimer" and "polymerised rosin". In fact no polymerisation reaction is involved and thus this trivial name is incorrect, rosin dimer is a C_{40}-terpene containing two double bonds and two carboxylic acid groups. It is believed that rosin dimer and its salts also belong to this family. The dissociation constant of the carboxylic acid group is not believed to be influenced by the extension of the molecule. Due to the size of the molecule, it is believed that rosin dimer is less biologically available than rosin. Therefore, it is reasonable to expect that rosin dimer will be less biologically active than rosin.

Both the salts of monovalent cations (e.g. sodium, potassium) and the salts of divalent cations (e.g. calcium, magnesium, zinc) are present in this family. Members of this family included in the EU REACH programme are shown in Table 4:

Table 4: Rosin, hydrogenated rosin and rosin salts

CASRN	Name
001740-19-8	[1R-(1α,4aβ,10aα)]-1,2,3,4,4a,9,10,10a-octahydro-7-isopropyl-1,4a-dimethylphenanthren-1-carboxylic acid {Abietic acid}
008050-09-7*	Rosin. A complex combination derived from wood, especially pine wood. Composed primarily of resin acids and modified resin acids such as dimers and decarboxylated resin acids. Includes rosin stabilized by catalytic disproportionation.
008052-10-6	Tall oil rosin. A complex combination derived from tall oil. Composed primarily of tricyclic monocarboxylic acids, mainly abietic and dehydroabietic acids. Includes tall-oil rosin stabilized by catalytic disproportionation (now included in CAS 8050-09-7).
009007-13-0	Resin acids and Rosin acids, calcium salts
009010-69-9	Resin acids and Rosin acids, zinc salts
061790-50-9*	Resin acids and Rosin acids, potassium salts
061790-51-0*	Resin acids and Rosin acids, sodium salts
065997-05-9	Rosin, oligomers
065997-06-0*	Rosin, hydrogenated
068334-35-0	Resin acids and Rosin acids, calcium zinc salts
068648-50-0	Rosin, dimers, calcium salts
068990-01-2	Resin acids and Rosin acids, hydrogenated, potassium salts
068990-02-3	Resin acids and Rosin acids, hydrogenated, sodium salts
070879-76-4	Resin acids and rosin acids, calcium magnesium zinc salts
084776-85-2	Resin acids and Rosin acids, tall-oil, potassium salts (now included in CAS 61790-50-9)
085409-26-3	Resin acids and Rosin acids, tall-oil, sodium salts (now included in CAS 61790-51-0)
91081-53-7	Rosin, reaction products with formaldehyde

*These substances were also included in this family in the US HPV program test plan.

The US HPV Challenge test plan also included in this family:

- CASRN 68425-06-1 Rosin, distillation overheads
- CASRN 68783-82-4 Rosin, low boiling fraction

Rosin esters

In rosin esters, the carboxylic acid group has been esterified with alcohols of various types. For the rosin industry the following alcohols are the most important: methanol, di- and triethylene glycol, glycerol and pentaerythritol.

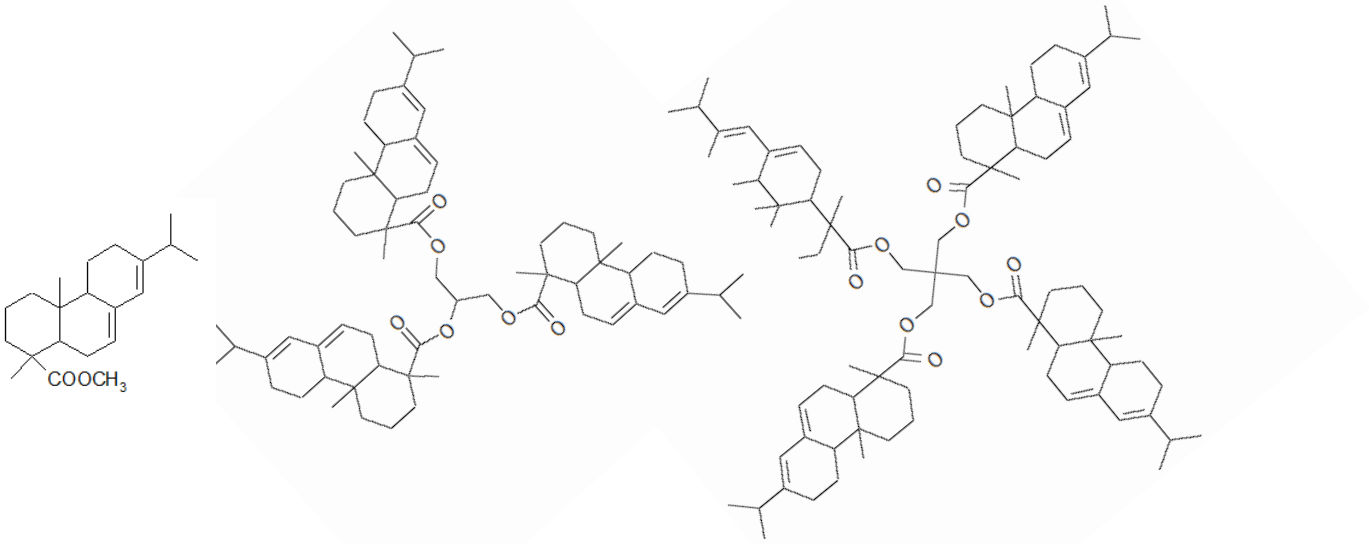
Considering the severity of the conditions needed to form the ester (long reaction time [hours], very high temperature [typically 200 °C]), it is clear that these esters are difficult to synthesise. Therefore, it is expected that the esters of rosin are very stable. The polyol esters, e.g. the glycerol and pentaerythritol esters, suffer from severe steric hindrance at the ester site. For the polyol esters, steric hindrance will strongly interfere with enzymatic hydrolysis.

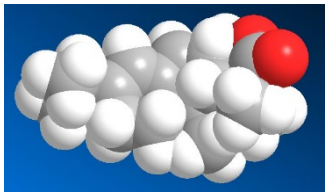
Any minimal hydrolysis that does occur would result in the rosin and the starting alcohol. Thus, under worse case scenarios, the toxicological and ecological effects of the minimal hydrolysis products could assessed by looking at the effects of the starting materials: rosin and the alcohol. The simplest of the rosin esters is the methyl ester, the smallest ester possible. If there is any reaction possible, it would be hydrolysis into the free rosin acids and methanol.

The same arguments can be applied to esters of hydrogenated rosin. In addition, hydrogenation takes away the reactive center at the double bond system. Hydrogenation leads to stabilization of the reactivity and will not otherwise affect the esterification of rosin.

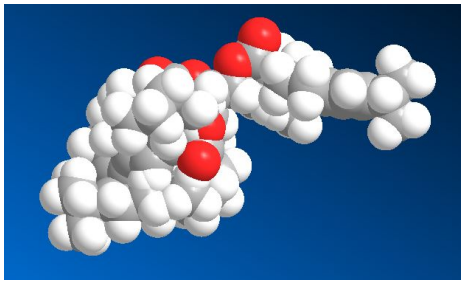
Rosin dimer is grouped into the family of rosin, thus its esters belong in this group. These are even less soluble and less prone to hydrolysis than the straight esters of rosin due to even more severe steric hindrance. The structures below give an impression about the steric hindrance in various esters.

Based on the aforementioned arguments, the worst-case scenario should be built on the methyl ester of rosin.

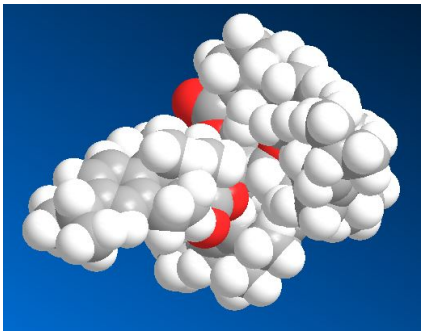




Methyl abietate



Glycerol triabietate



Pentaerythritol tetraabietate

Members of this family included in the EU REACH programme are listed in Table 5:

Table 5: Rosin esters (including hydrogenated rosin esters)

CASRN	Name
008050-15-5*	Resin acids and Rosin acids, hydrogenated, Me esters
008050-25-7	Resin acids and Rosin acids, esters with triethylene glycol
008050-26-8*	Resin acids and Rosin acids, esters with pentaerythritol
008050-31-5*	Resin acids and Rosin acids, esters with glycerol
036388-36-0	Bis[[1,4a-dimethyl-7-(1-methylethyl)tetradecahydrophenanthryl]methyl] phthalate (phthalate ester of hydroabietyl alcohol)
064365-17-9*	Resin acids and Rosin acids, hydrogenated, esters with pentaerythritol
065997-12-8	Resin acids and Rosin acids, polymerised, esters with pentaerythritol
065997-13-9*	Resin acids and Rosin acids, hydrogenated, esters with glycerol
068153-38-8*	Resin acids and Rosin acids, esters with diethylene glycol
068186-14-1*	Resin acids and Rosin acids, Me esters

068475-37-6	Resin acids and Rosin acids, polymerised, esters with glycerol
068512-65-2	Resin acids and Rosin acids, esters with ethylene glycol
068515-02-6	Rosin, oligomeric reaction products with isophthalic acid and pentaerythritol
068648-53-3	Resin acids and Rosin acids, hydrogenated, esters with triethylene glycol
084776-83-0	Resin acids and Rosin acids, esters with trimethylolpropane
084776-84-1	Resin acids and Rosin acids, tall-oil, esters with triethylene glycol (now included in CAS 8050-25-7)
085566-49-0	Resin acids and Rosin acids, tall-oil, esters with pentaerythritol (now included in CAS 8050-26-8)
085711-66-6	Resin acids and Rosin acids, esters with glycerol and diethylene glycol

*These substances were those included in this family in the US HPV program test plan.

Rosin Adducts and Rosin Adduct Salts

As stated earlier, the double bond system in resins acids can react by a Diels-Alder reaction with maleic anhydride or fumaric acid. The reaction of abietic acid with maleic anhydride leads to the formation of maleopimaric acid. This is an acid anhydride, which is an alerting structure indicative of a potential for skin sensitisation (Barratt and Basketter, 1996).

Due to the fact that rosin adducts can have three carboxylic acid groups, one may expect higher solubility into water and thus higher bioavailability relative to rosin. Both the salts of monovalent cations (e.g. Na^+, K^+) and the salts of divalent cations (e.g. Ca^{2+}, Mg^{2+}, Zn^{2+}) are present in this family. The same arguments as for rosin salts can be applied here.

The Diels-Alder adduction occurs only on those resin acids that contain conjugated double bonds. The conjugated resin acid content of rosin typically varies from 50-70%, thus even under the best of reaction conditions, 30-50% of the resin acid molecules remain unreacted simply because they cannot undergo this reaction. However, these resin acids are not abietic acid and do not tend to react with oxygen. Members of this family included in the EU REACH programme are listed in Table 6:

Table 6: Rosin adducts (and adduct salts)

CASRN	Name
008050-28-0*	Maleated rosin
065997-04-8*	Rosin, fumarated
068152-93-2	Tall oil, maleated (now included in CAS 8050-28-0)
068201-59-2*	Resin acids and Rosin acids, fumarated, sodium salt
068201-60-5	Resin acids and Rosin acids, maleated, sodium salts
068649-83-2*	Resin acids and Rosin acids, fumarated, potassium salts
085409-27-4*	Resin acids and Rosin acids, maleated, potassium salts
085409-30-9	Tall oil rosin, maleated (now included under CAS 8050-28-0)
085631-69-2	Tall-oil rosin, fumarated (now included under CAS 65997-04-8)
091722-01-9	Resin acids and Rosin acids, maleated, calcium salts
160901-14-4	Fatty acids, tall oil, oligomeric reaction products with maleic anhydride and rosin, calcium magnesium zinc salts
95009-65-7	Rosin, fumarated, reaction products with formaldehyde

*These substances were also included in this family in the US HPV program test plan.

The US HPV Challenge test plan also included:

- CAS 68554-16-5 Rosin, maleated/fumarated.

Products of Other Addition Reactions

This group includes the product formed by reaction of rosin with treated with formaldehyde at elevated temperatures. As it is likely to behave in a similar manner to rosin it is considered with the rosin group. Formaldehyde treated rosin is used in paper size and printing ink applications. It can also be reacted with fumaric acid and therefore is a molecule included in the adduct grouping above.

As noted previously formaldehyde-treated rosin leads mainly to the addition of a methyl group to the rosin molecule. This addition was deemed irrelevant for eco-toxicological studies so CASRN 91081-53-7, Rosin, reaction products with formaldehyde is grouped with the substances in Table 3 and CASRN 95009-65-7, Rosin, fumarated, reaction products with formaldehyde is grouped with rosin adducts in Table 5.

Rosin Adduct Ester

In principle, rosin adducts may form polyesters when reacted to polyols. Depending upon how much polyol in added, either low or high acid number adduct esters are obtained. “Acid number” is a measure of the unesterified carboxylic acid content of rosin or rosin adducts. The acid number is the amount of potassium hydroxide (in mg) to neutralise one gramme of resin. Thus, a rosin or rosin adduct which is highly esterified will have a low acid number and vice-versa. Maleic- or fumaric-modified rosin has a typical acid number in the range of 250-330.

There are three classes of adduct esters. One class is the "Alcohol solubles", which generally has acid values between 180-250 mg KOH/g, although there are grades that have acid values between 105-120 mg KOH/g. These contain very little polyol. All of these products, with an acid value greater than 120 mg KOH/g, can be regarded as "acid type", i.e. as having a sufficient number of acid groups present to behave as rosin adducts.

The second class of adduct esters tends to have acid values in the range of 30-70 mg KOH/g. The third class is called "alcohol insoluble" products, which have acid values below 25 mg KOH/g. The latter two classes would be expected to have similar properties and to behave similarly to rosin esters.

There seems to be a "cross-over" point in properties related to an acid value somewhere between 70-120 mg KOH/g. An acid number of 100 mg KOH/g is approximately in the middle of the separation between "ester-type" and "acid-type". Members of this family are:

Table 7: Rosin adduct esters

CASRN	Name
065997-11-7	Rosin, fumarated, oligomeric reaction products with pentaerythritol
068038-41-4	Rosin, maleated, polymer with glycerol
071243-68-0	Resin acids and Rosin acids, fumarated, decyl esters
091081-25-3	Resin acids and Rosin acids, maleated, mixed esters with diethylene glycol, glycerol and phthalic anhydride
092202-14-7	Rosin, fumarated, reaction products with glycerol and pentaerythritol
094581-15-4	Resin acids and Rosin acids, fumarated, esters with pentaerythritol
094581-16-5	Resin acids and Rosin acids, maleated, esters with glycerol
094581-17-6	Resin acids and Rosin acids, maleated, esters with pentaerythritol

094581-69-7	Rosin, maleated, polymer with pentaerythritol
097489-11-7	Resin acids and Rosin acids, fumarated, esters with glycerol
193293-72-0	Resin acids and Rosin acids, maleated, esters with diethylene glycol and triethylene glycol

Phenolic Modified Rosin Adducts

The resins in this family are formed by the reaction of rosin, formaldehyde, phenol and alkyl- and/or arylphenols. These products may be esterified with polyols. The same is true for rosin adduct modified with formaldehyde, phenol and alkyl- and/or arylphenols. These products meet the OECD/REACH polymer definition.

Members of this family include:

Table 8: Phenol modified rosin adducts

CASRN	Name
067700-45-2	Rosin, polymer with formaldehyde and phenol
068140-03-4	Rosin, maleated, polymer with p-tert-butylphenol, formaldehyde, glycerol and pentaerythritol
068152-62-5	Rosin, maleated, polymer with formaldehyde, nonylphenol and pentaerythritol
068152-70-5	Rosin, polymer with bisphenol A, formaldehyde and glycerol
068309-61-5	Rosin, maleated, polymer with formaldehyde, pentaerythritol and phenol
068425-03-6	Rosin, maleated, polymer with formaldehyde, pentaerythritol and phenol
071243-71-5	Rosin, maleated, polymer with p-butylphenol, formaldehyde, glycerol and nonylphenol
091081-50-4	Rosin, maleated, reaction products with bisphenol A, formaldehyde and pentaerythritol
092202-16-9	Rosin, maleated, reaction products with p-tert-butylphenol, formaldehyde and pentaerythritol
092202-17-0	Rosin, maleated, reaction products with formaldehyde, pentaerythritol and 4-(1,1,3,3-tetramethylbutyl)phenol
129595-12-6	Rosin, maleated, polymer with bisphenol A, p-tert-butylphenol, formaldehyde, nonylphenol and pentaerythritol
132778-09-7	Rosin, polymer with p-tert-butylphenol, formaldehyde, maleic anhydride, pentaerythritol and soybean oil

Decarboxylated Rosin

The removal of the carboxylic acid group can be complete or incomplete. The complete removal of the acid group leads to a cyclo-olefin, i.e. alkylated decahydrophenanthrene. This is a type of hydrocarbon that is outside the scope of substances covered by the consortium on rosin resins. However, it should be noted that rosin contains a “neutral” fraction, which includes similar naturally occurring decarboxylated diterpene structures.

Incomplete removal of the carboxylic acid group leads to a complex mix of resin acids and alkylated decahydrophenanthrenes. The resin acid part is comparable to unmodified rosin and should be cross-read with rosin.

Members of this family include (see Table 9):

Table 9: Decarboxylated rosin

CASRN	Name
8050-18-8	Rosin, decarboxylated

Summary and Conclusions

Rosin, oxidised rosin and chemically modified rosins have been grouped on the basis of their chemistry into four categories:

Starting materials and groupings where the conjugated diene structure is retained -

- Resin acids and rosin acids (Category 1)
- Rosin salts (Category 1)
- Formaldehyde treated rosin (Category 1)
- Rosin esters (Category 2)

Chemically related manufactured substances whereby the conjugated diene structure has been modified -

- Hydrogenated rosin (Category 1)
- Dehydrogenated/disproportionated rosin (Category 1)
- Hydrogenated rosin salts (Category 1)
- Hydrogenated rosin esters (Category 2)

Chemically modified substances whereby the conjugated diene structure has been modified, resulting in maleopimaric acid formation -

- Rosin adducts and adduct salts (Category 3)
- Formaldehyde modified rosin adducts (Category 3)
- Rosin adduct esters (Category 4)

Methods for Identification of Skin Sensitisation

There are two key questions when evaluating skin sensitisation studies. They are:

- Was the substance tested the substance stated?
- What type of test was used?

Substances Tested

With rosin, often the substance tested was neither protected against oxidation on storage nor fully characterised. This is a greater problem when, in particular, the substance was stored in powdered form. Thus, older animal tests on 'rosin' (notably those conducted before the problem was recognised, i.e. before 1993) are unlikely to be valid. Studies dating from before 1993 have only been used if these problems are unlikely to have affected the results.

It is notable that research articles of this period, notably those by Karlberg et al. (e.g. Karlberg et al., 1991; 1996) often use "fractionated" rosin without giving sufficient detail about the method of fractionation nor what the exact composition of these fractions was. When rosin as a whole was used, Karlberg's group described it as rosin of "pharmaceutical quality", without further specification. In one of her publications she writes that "the content of oxidation products should be kept at a constant and rather high level." (Karlberg and Gafvert, 1996). In the same publication she writes that "the major resin acids, abietic acid and dehydroabietic acid, have very low allergenic activity. It has been shown that oxidation products of these acids are important allergens." This was published after industry brought to her attention that (powdered) rosin was particularly sensitive to oxidation. Industry does not produce oxidised rosin or sell it into the market. Such studies are therefore not relevant to rosin itself.

It should also be noted that some of the animal studies conducted on behalf of industry included use of heat and or other unacceptable methods (e.g. dissolution in n-hexane prior to addition of the intended solvent) to obtain solutions for testing. These studies are included in the tables but discounted from the discussion.

In practice, the tests conducted by industry and the results obtained were commercially confidential information restricted to a particular chemical company, so there was much duplication of testing. With the requirement to pool data under the EU REACH regulatory scheme and the making public of summaries of the test data, it is now possible to collate the data from different sources. In some cases this has revealed that a large number of tests have been conducted on a specific material, usually by several contract research organisations on behalf of different clients. This implies that rosins from different sources (and hence a range of compositions) have been tested. Fortunately, there is an impressive consistency in the findings.

Animal Study Methods

The guinea pig has been the species of choice for predicting skin sensitisation for several decades. Two types of tests were developed: adjuvant tests in which sensitisation are potentiated by the injection of Freund's complete adjuvant, and non-adjuvant tests. Originally, the OECD guidelines (of 1981) outlined four adjuvant based tests and three non-adjuvant tests, but later (1992) this was reduced to two tests, the Guinea Pig Maximisation Test (GPMT) of Magnusson and Kligman (1969) as the adjuvant based test and the Buehler assay (Buehler, 1965) as the non-adjuvant test. The former required 30% positive responders for the substance to be considered a sensitising chemical. The latter required 15% of the animals to respond for classification. The former was preferred in Europe, whilst the latter was preferred in the USA. Maurer also indicates that there was an impression that the Buehler test was less sensitive than the adjuvant tests (Maurer, 1996).

Earlier studies on rosin often used the 'Freund's Complete Adjuvant Test' (FCAT), another adjuvant based test. The FCAT is an adjuvant based guinea pig test employing three intradermal administrations of test substance and Freund's Complete Adjuvant, whereas the GPMT uses one intradermal administration of Freund's Complete Adjuvant and of test compound and a single subsequent administration of test compound by the dermal route (Maurer, 1996). Hausen (Hausen et al., 1990, 1993, 1998) used this non-OECD test method, and he described it as "a further development of the test methods named [GPMT] with the purpose of determining most favourably (i.e. obtaining a positive result whenever possible) the sensitising capacity of moderate and weak allergens." The relationship between the scale used by Hausen for scoring the results and the criteria laid down in the EU Regulation[4] on Classification, Labelling and Packaging of hazardous substances and mixtures (CLP) is unknown. Nevertheless, it is clear that this test is intended to be a more severe test than the GPMT.

Unlike the GPMT and FCAT tests, there is no attempt to bypass the skin barrier in the Buehler test. This would seem to be more consistent with how individuals would be exposed.

More recently a third test, the mouse Local Lymph Node Assay (LLNA), for which a guideline was originally adopted by the OECD in 2002 and a revised guideline issued in 2010 (OECD 2010), has become the preferred test for skin sensitisation potential in the EU. As well as reducing the number of animals required for a test, the LLNA can be used to examine potency. Like the Buehler assay, this test does not bypass the skin barrier.

In 2015 in vitro/in chemico preliminary assessment test guidelines (OECD Guidelines 442) were approved for use as preliminary tests for skin sensitisation, although the in vivo test remained definitive.

[4] Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures

Human Studies

Prevalence studies identifying rosin as a skin sensitiser suffer from two problems. The first is that, while the challenge agent should be known, the inducing agent is unknown.

The second, specific to rosin, is that, the material used in dermatological clinics as challenge agent is obtained as a solution and when examined, it turns out that the test material contains oxidised rosin. Thus the available prevalence studies are of little value in examining whether rosin is a human skin sensitiser. Animal tests (GPMT) with this challenge material confirmed cross reactivity with oxidised rosin (see Table A1) and chemical examination of samples of the challenge agent used in human prevalence studies confirmed that they contained oxidation products of rosin (see above), as previously suggested by Sahdra et al (1996). Most, if not all, human prevalence studies suffer from this problem and therefore are of little or no use when evaluating the skin sensitisation potential of rosin.

The only satisfactory testing in humans is when the identity of both inducing and challenge material is clear, and, in the case of rosin, precautions are taken to ensure that the material tested is not significantly oxidised. This implies that a properly conducted human repeated insult patch test (HRIPT) is required. Although normally the UN Globally Harmonised System and the European Union indicate that human patch testing (in which only the challenge substance is known) can be used for classification of a substance, such tests must use the appropriate challenge substance, i.e. rosin, not partly (or fully) oxidised rosin. Ethical considerations means that repeated insult patch test has not been performed using rosin although there are some data for certain chemically modified rosins.

Sources and Quality of Studies

The studies examined are identified from the European Chemicals Agency (ECHA) website, Botham et al. (2008) and Illing et al (2009) and using information supplied by the Hydrocarbon Resins, Rosin Resins And Pine Chemicals Producers Association (HARRPA) and the Pine Chemicals Association (PCA). When a study reference number is quoted the study report has been made available, otherwise the extensive summary given on the ECHA website is the source. Unless otherwise stated, tests conducted on behalf of industry after the introduction of standardised protocols and Good Laboratory Practice (in the early 1980s) have been conducted in accordance with the appropriate protocol and subjected to good laboratory practice audit. This includes the studies published in Botham et al (2008) and Illing et al (2009). Unless otherwise indicated, purely academic studies have not been subject to these requirements.

The quality of the studies has been assessed using the Klimisch grading system (Klimisch et al., 1997; Appendix 1). The appendix tables list studies that could be/have been graded Klimisch grade 1 (acceptable without reservation) or 2 (acceptable with reservation). In addition there are a small number of studies that are included in the Appendix tables that should be graded Klimisch grade 3 (not reliable) as the method used to prepare the substance for administration was inappropriate. These inappropriate methods have been noted, but the studies are included in this document for completeness sake. Also, a small number of studies on humans have been assigned Klimisch grade 4 (not assignable) because of a lack of detail concerning the procedures used.

Test Results

Groupings

The groupings outlined above have been aggregated for the purposes of examining the influence of structure on skin sensitisation. The groupings are:

- Rosin, oxidised rosin and rosin adduct with formaldehyde
- Hydrogenated rosin and disproportionated rosin
- Rosin salts
- Hydrogenated rosin salts
- Rosin esters
- Hydrogenated rosin esters

(This is to show the influence of oxidation and of hydrogenation on skin sensitisation potential).

- Rosin adducts
- Rosin adduct esters
- Phenolic modified rosin
- Decarboxylated rosin

Rosin, Oxidised Rosin and Rosin Adduct with Formaldehyde

Table 10: Summary of test results – Rosin, oxidised rosin, disproportionated rosin, hydrogenated rosin and rosin adduct with formaldehyde.

Name	Adjuvant based (*GPMT)	LLNA	Buehler
Rosin	*Negative (9)	Negative	Negative (2)
Naturally oxidised rosin	*Positive (4)	Negative	Negative
Disproportionated rosin	*Negative (3)+	ND	ND
'Superoxidised' rosin	*Negative (1)	ND	ND
Hydrogenated gum rosin	*Negative (1) Negative (2)	ND	ND
Resin acids and rosin acids, potassium salt	ND	Negative	ND
Resin acids and rosin acids, calcium salt	ND	Negative	ND
Rosin, reaction product with formaldehyde	*Negative	ND	Negative

[Parentheses include the number of independent studies. ND - No data. Individual data in Appendix Tables A2-A4. + two studies were negative, but of limited value due to responses in controls.]

There are a large number of skin sensitisation tests that have been conducted on rosin (Table 10). Most of the early tests are unreliable as they make use of non-standard tests and/or test material that was poorly defined in terms of its oxidation status or partially oxidised. Examples of probably partially oxidised material include the 'unmodified rosin' of Sadhra et al. (1996; 1998) and the 'minimally air exposed tall oil rosin' of Karlberg (1991). In addition, there was considerable confusion concerning the naming of rosin. Hence a multiplicity of tests has been conducted on samples of adequately characterised commercial rosin using the main acceptable regulatory tests for skin sensitisation. The tests have been conducted in a number of laboratories and have employed rosin from several sources.

Irrespective of the source (gum, wood or tall oil) rosin is clearly non-sensitising in any of the tests: the GPMT, the mouse LLNA or the Buehler assay.

Naturally oxidised rosin (finely powered rosin exposed to air at room temperature for many days) is sensitising only in the GPMT, but not the LLNA or Buehler assays. Superoxidised rosin (powdered rosin exposed to oxygen under pressure) is NOT a sensitiser under the GPMT.

It was eventually accepted by the regulatory authorities in the EU that rosin, if not oxidised, was not a skin sensitiser (Karlberg et al., 1999, ECB, 2000). However, the EU continued to classify rosin as a sensitiser.

It bears emphasising again that rosin did not show sensitising properties in any of the three tests and naturally oxidised rosin showed it in only one of the three tests - the GPMT. When examining oxidised rosin, initially it was thought that either there was a species difference between guinea pig and mouse or it was 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 the FCAT). However, the species difference argument fails, as the recently performed Buehler assay gave negative results (Table 10). There are two possible explanations for the results seen. The Buehler test and the LLNA assay may both 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 may be necessary to bypass the epidermis if the active species formed in the oxidation process are to induce a sensitisation reaction.

When tested in the FCAT (a particularly severe test for skin sensitisation) the materials present in oxidised ‘rosin’, included several oxidation products (including hydroperoxides) of notable sensitisation potential (Hausen et al., 1990; 1993; Gäfvert et al., 1992; 1994; summarised in Lepoittevin and Karlberg, 1994). However, these products 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", i.e. a modification using unrealistically severe exposure conditions not representative of those encountered in practice. Thus the likelihood of these materials being capable of induction when applied to the surface of the human skin remained uninvestigated.

Formaldehyde-treated rosin was negative in the GPMT and LLNA. This is unsurprising given that the commercial substance is similar to the original rosin, but with addition of small amounts of methyl, dimethyl and hydroxymethyl substituted resin acids (see earlier discussion).

Hydrogenated Rosin and Disproportionated Rosin

The data summarised in Table 10 for hydrogenated rosin (one negative GPMT test and two 'other' adjuvant based tests) indicate clearly that hydrogenated rosin does not have the potential for skin sensitisation in these tests. Although old, a study by Karlberg et al (1988) also indicated that hydrogenated rosin was not a sensitiser in the FCAT. It only caused sensitisation at the highest challenge concentration in a GPMT study. Also, it was less effective as a challenge agent when compared to 'rosin' in 'rosin sensitive' patients. A human repeated insult patch test conducted at the Industrial Biotest Laboratories in 1977 was also negative, but, as the laboratory is known to have falsified earlier results, must be graded Klimisch grade 3 (not reliable). Also, three GPMT studies indicate that disproportionated rosin is not a skin sensitiser. The weight of evidence is clear and anticipatable from structural considerations – the hydrogenation substantially reduces the quantity of conjugated diene, rendering oxidation to chemical species with a skin sensitisation potential unlikely.

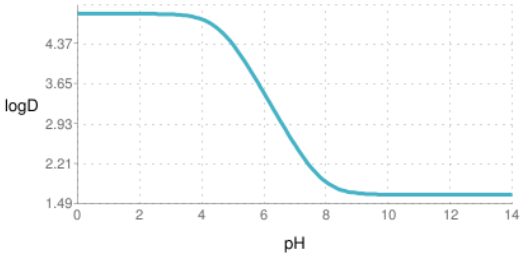
Rosin Salts

Only LLNA studies have been conducted on rosin salts (Table 10). The studies indicate that they are non-sensitisers. This can be anticipated on the basis of the results for rosin and a consideration of the solubilities of rosin and the rosin salts and the dissociation constants associated with the resin acids in rosin/rosin salts.

As for all carboxylic acids, resin acids are weak in nature; it is the pH of the medium and its buffering capacity that determine whether a resins acid is present as the free acid or the conjugated salt. Soltes (1989) reports a K_a value of $5.4 * 10^{-6}$, or a pK_a of 5.27. The ECB Summary Fact Sheet (Draft; ECB, 2008) identified values of pK_a of <5.7-6.4, between 5.7 and 7.25 (abietic acid 6.4-7.15), 6.4 and 5.7 (abietic acid and dehydroabietic acid) and 7.15 and 7.25 (abietic acid and dehydroabietic acid) for this dissociation constant This fits very well with what one would expect for carboxylic acids. As for all carboxylic acids, the monovalent salts are soluble in water, whereas the divalent salts are insoluble. Many values have been reported for the solubility of both the sodium and potassium salts of rosin (i.e. the monovalent salts). They are mutually miscible with water (Dinwoodie, 2003; ECHA). However, the consequent solution has a relatively high pH (9.6). The potassium salt of hydrogenated rosin is also very soluble (69 g/L) (Woolley and Mullee, 2004, ECHA). The addition of acid leads to precipitation, alkali to solubilisation. This is due to conversion from unionised to the anion with the unionised material being the acid and hence insoluble, and the ionised material being the anion in alkaline solution (with its associated monovalent metal cation).

The log octanol/water can be calculated for any pH value. Compounds having ionisable groups exist in solution as a mixture of different ionic forms. The ionization of those groups, thus the ratio of the ionic forms depends on the pH. Since logP describes the hydrophobicity of one form only, the apparent logP value can be different. The octanol-water distribution coefficient, logD represents the compounds at any pH value.

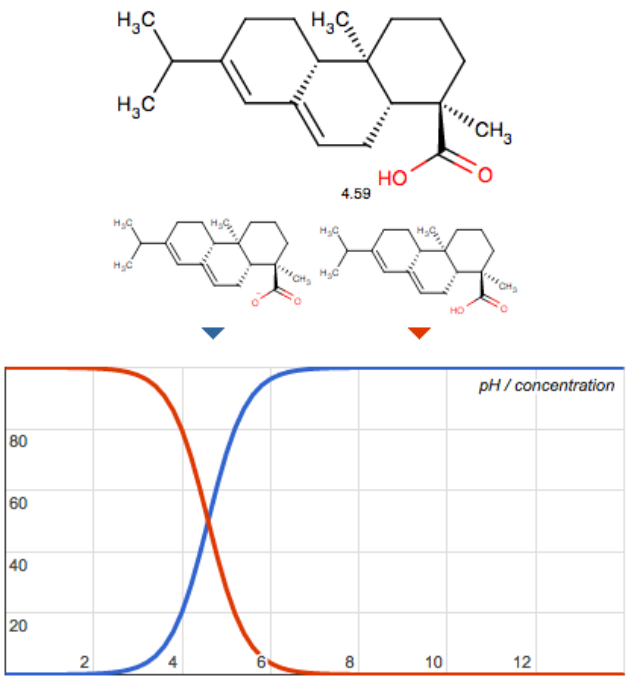
$$\boldsymbol{log\ Doct/water = log\ (\frac{[solute]octanol}{[solute]ionised\ water + [solute]neutral\ water})}$$



(source: http://www.chemicalize.org/structure/#!mol=structureId%3A1793002328338&source=fp

To the satisfaction of ECHA, it was demonstrated with a titration curve (see figure 7) that the rosin and its salts are basically the same and that the pH of the medium determines whether the medium contains free acid, the salt or both. It was claimed that the inflection point identified was due to precipitation as the ionised material was converted to unionised material and precipitated. This study confirms that it is very difficult to segregate dissociation constant and precipitation.

Figure 7: Calculated titration curve of abietic acid



(source: http://www.chemicalize.org/structure/#!mol=structureId%3A1793002328338&source=fp)

The resin acids are insoluble. The salts are freely soluble in water, but the solution yielded is alkaline. Hence, at values below the pK_a one would expect the solubility to be that of the acid, i.e. insoluble (≤ 1 mg/L). At values above the pK_a the solubility would be that of the anion (very soluble/miscible). It should be noted that approximately 10 % of rosin consists of so-called "neutrals", constituents that do not contain

a carboxylic acid group. These constituents are insoluble in water at any pH-value. Solutions of rosin monovalent salts are always turbid, because of the insoluble neutral fraction. Separating the effects of solubility from those of dissociation cannot be performed in a completely satisfactory manner. It also means that essentially any test for skin sensitisation potential using the salt as starting material is, in effect, a test on the free acid. Not surprisingly, the results of the tests on the salts are like those on the free acids and indicate a lack of sensitising potential.

Hydrogenated Rosin Salts

One LLNA test was conducted with the potassium salt of hydrogenated rosin (Appendix Table A3). The result was a marginal positive, seen only at the highest dose tested. The test has to be graded Klimisch grade 3 (not satisfactory) as it was conducted on a laboratory batch not made by the producer and used a non-standard solvent, ethanol:water (4:1 v/v). Ethanol as a solvent is known to yield high measured activity for weak sensitisers in this assay (Laiko et al., 2004), and the salt has detergent properties that are likely to enhance penetration. It can be anticipated from a comparison of the results from tests with rosin and hydrogenated rosin (Table 10), tests with the esters of rosin and hydrogenated rosin (Table 11) and the reasoning given for rosin salts (above) that, on a weight of evidence approach, the result was a false positive and that hydrogenated rosin salts are non-sensitising.

Rosin Esters

Table 11: Summary of test results – Rosin and hydrogenated rosin esters

Name	Adjuvant based	LLNA	HRIPT
Resin acids and rosin acids, methyl ester	ND	ND	Negative (2)#
Resin acids and rosin acids, esters with triethylene glycol	*Negative		
Resin acids and rosin acids, esters with pentaerythritol	*Negative (5) *Positive (1)	Negative	ND
Resin acids and rosin acids, esters with glycerol	*Negative (5)	Negative	Negative
Resin and rosin acids, hydrogenated, methyl ester	*Negative (3)	ND	Negative (2)~
Resin acids and rosin acids, hydrogenated, esters with pentaerythritol	ND	ND	Negative
Resin and rosin acids, hydrogenated, esters with glycerol	*Negative Negative (2)	ND	Negative

ND = no data. Individual data in Appendix Tables A5-A7

* indicates that the tests were GPMT, non starred were other adjuvant based tests that were acceptable at the time the study was conducted . Parentheses includes the number of tests

Studies Klimisch 4 – reliability not assignable

~ One study Klimisch grade 4 – reliability not assignable

There are many tests on rosin esters (Table 11). GPMT studies have been conducted on the ester with triethylene glycol, the ester with pentaerythritol (six studies, four of which used satisfactory dosing procedures and two, including the one positive study, used inappropriate methods for preparing the dosing solutions and are therefore Klimisch grade 3) and the ester with glycerol (five studies, one of which was over-heated during solution preparation, i.e. used an inappropriate preparation procedure and is Klimisch grade 3 – the result was borderline). LLNA studies have been conducted on the pentaerythritol ester and the glycerol ester of rosin. In addition three human studies have been conducted using the HRIPT

assay, only one of which has been included in the EU registrations. The two studies not included (both on the methyl ester) were negative, but Klimisch grade 4 – reliability not assignable. All satisfactory tests gave negative results, thus the weight of evidence is clear: rosin esters are not sensitising. This is consistent with a study using the cumulative contact enhancement test, an adjuvant based guinea pig test, on chemically synthesised glycerol triabietate (Shao et al., 1993). It is also in line with predictions based on the chemistry associated with esterification and the results with rosin.

Hydrogenated Rosin Esters

Three GPMT studies have been conducted on the methyl ester of hydrogenated rosin and one on the glycerol ester (Table 11). Two tests with another adjuvant-based assay have been conducted on the glycerol ester of hydrogenated rosin. Also, three apparently adequate human studies (one per ester) have been conducted using the HRIPT (Human Repeated Insult Patch Test) assay and the methyl, pentaerythritol and glycerol esters. A second study on the methyl ester was Klimisch grade 4 – reliability not assignable. All tests gave negative results. These results are consistent with the results for the rosin esters. Given that the reason for hydrogenation is to remove/reduce the reactivity of the conjugated diene, and thus reduce the susceptibility to oxidation to a great extent, these results can be predicted on structural grounds.

Rosin adducts

The rosin adducts included in this group are those substances whereby adduct formation leads to the formation of maleopimaric acid. They therefore include the product of treatment of fumarated rosin with formaldehyde (Table 12). All are sensitisers.

Table 12: Summary of test results – Rosin adducts

Name	Adjuvant based (GPMT)	LLNA	Buehler
Rosin, fumarated	ND	Positive	ND
Rosin, maleated	Positive (2)	Positive	Positive
Rosin, fumarated, reaction product with formaldehyde	ND	Positive	ND

ND = no data. Individual data in Appendix Tables A8-A10. Parentheses includes the number of tests

There is a consistency across the tests conducted on maleated rosin adducts. It is sensitising in all three tests - the GPMT, the Buehler assay and the mouse local lymph node assay. Fumarated rosin behaves similarly in the mouse LLNA test, but has not been tested in the guinea pig tests. Formaldehyde-treated rosin, when also fumarated, gave a positive response in the LLNA. It should be noted that, as hydrogenation reduces or eliminates the same conjugated diene moiety, no hydrogenated rosin adducts can be made.

As stated earlier both maleic anhydride and fumaric acid react with rosin to yield, either directly or indirectly, maleopimaric acid anhydride. Structurally, maleopimaric acid is an acid-anhydride. The isomeric form of the maleopimaric acid is unclear, but appears to depend on the starting material. According to Soltes and Zinkel (1989), maleic anhydride gives the endo form and fumaric acid the exo, endo form. Nilsson et al (2002) state that maleic anhydride yields the exo form of maleopimaric acid and fumaric acid yields the endo form. The LLNA tests on these substances were both positive, and, when graded for potency, both were clearly strong sensitisers. Maleopimaric acid has been found to be a sensitiser using

the cumulative contact enhancement tests (this test induces using four dermal exposures to the test substance, intradermal administration of Freund's Complete Adjuvant on the same day as the third administration of test substance) and the Freund's Complete Adjuvant (Gafvert et al., 1995; Nilsson et al., 2002). A structural alert indicative of a potential for sensitisation is present, as, under appropriate conditions, maleopimaric acid can contain the acid anhydride structure, capable of acting as an acylating agent (Barratt and Basketter, 1996).

As shown earlier formaldehyde reacts with rosin to yield a complex mixture. In addition to unmodified rosin, this product contains two stereoisomers of 7-methyldehydroabietic acid, 14-methyldeydroabietic acid, dimethyl substituted dehydroabietic acid, 7-hydroxymethylabietic acid and abietic acid substituted by both hydroxymethyl and methyl groups. This product is not sensitising (see the section 'Rosin, Oxidised Rosin and Rosin Adduct with Formaldehyde' above). Hence the formaldehyde treated rosin is considered as a part of the rosin and rosin salts category for the purposes of understanding the influence of chemical modifications on skin sensitisation potential.

Although oxidised rosin is also a skin sensitiser in adjuvant based tests, the immunological response for maleopimaric acid is clearly different to that from oxidised rosin (Table 13). Oxidised rosin did not induce an immunological response in the Buehler assay and, when an immunological response was induced by maleopimaric acid, challenge with oxidised rosin did not elicit a sensitisation response. The positive control (maleated rosin as inducing and challenge agent) was, as expected, positive. The sensitisation properties of oxidised rosin are due to the hydroperoxide of abietic acid whereas those from the maleated/fumarated derivatives derive from the Diels-Alder adduct or more likely, the anhydride functionality introduced.

Table 13: Buehler assays with oxidised rosin and maleated rosin.

Inducer	Challenger	Result	Source
Oxidised rosin	Oxidised rosin	Negative	Table A4
Maleated rosin	Oxidised rosin	Negative	Table A10
Maleated rosin	Maleated rosin	Positive	Table 10.

These tests were conducted at Eurofins/PSL in 2006-7.

Rosin Adduct Esters

Rosin adduct esters have been examined in two tests, the GPMT and the LLNA (Table 14).

Table 14. Summary of test results – Rosin adduct esters

Name	Adjuvant based	LLNA
Resin acids and rosin acids, fumarated, esters with pentaeythritol	*Negative	Positive (2)
Resin and rosin acids, maleated, esters with glycerol	*Positive (1) *Negative (1)	ND
Resin acids and rosin acids, maleated, esters with pentaerythritol	*Positive (2)	Positive (3)
Resin acids and rosin acids, fumarated, esters with glycerol and pentaerythritol	ND	Positive

ND = no data. Individual data in Appendix Tables A11 and A12;* * indicates that the tests were GPMT, non starred were other adjuvant based tests that were acceptable at the time the study was conducted . Parentheses includes the number of tests

The results for esters of rosin adducts were borderline sensitising/non-sensitising. Thus some results were positive (sensitisers) and a few were negative. The glycerol ester of maleated rosin gave results in the GPMT test that depended on the amount of maleic anhydride reacted with the rosin, 16% was sensitising and 8% non-sensitising. The pentaerythritol ester of the fumaric acid adduct gave negative results in the GPMT, but weakly positive results in two LLNA test. The pentaerythritol esters of the fumarated and maleated adducts and the glycerol and pentaerythritol ester of the fumarated adduct gave weakly positive results in the LLNA. The acid value was taken into consideration in the LLNA assays with maleated rosin and pentaerythritol – in all cases it was sufficiently low for the substance to be considered as largely present as the ester.

Decarboxylated rosin and phenolic rosin resins

Two phenolic resins have been examined in the LLNA. Both were found 'not sensitising'. This is consistent with the polymeric nature of the products. Decarboxylated rosin was negative in the mouse LLNA assay (see Table 15):

Table 15: Decarboxylated Rosin and Phenolic Rosin Resins

Name	LLNA
Decarboxylated rosin	Negative
Phenolic reaction product of rosin (polymer)	Negative
Gum rosin, fumarated or maleinated, reacted with formaldehyde and nonylphenol, and esterified.	Negative

Individual data are from Appendix Table A13. Phenolic Modified Rosin

SUMMARY

Early studies (pre 1993) on rosin and chemically modified rosins used a variety of testing techniques and of test materials. Much of the testing was conducted with impure or unsatisfactorily characterised material and therefore the results of these tests are unreliable. In particular, frequently "rosin" was at least partially oxidised through storage under inappropriate conditions. Also, commercially available rosin samples designated for dermatological testing were demonstrated to be heavily oxidised and containing high concentrations of hydroperoxide.

More recently, rosin, oxidised rosin and chemically modified rosins have been grouped on the basis of their chemistry and their skin sensitisation potential. They have been assessed using regulatory test procedures, principally the Guinea Pig Maximisation test (GPMT) and the mouse LLNA (local lymph node assay). In general, the testing is consistent with the known chemistry of rosin, oxidised rosin and chemically modified rosins. Given the wide range of substances tested and the number of sources and test laboratories used the results are impressively consistent.

Rosin, rosin salts and rosin esters are not sensitisers. However, when oxidised under mild conditions by exposure to air at room temperature, the resulting oxidised rosin is sensitising in the GPMT, but not in the Buehler test or the LLNA. Although the potassium salt of hydrogenated rosin gave a marginally positive result in the one inadequate test conducted on it, hydrogenated rosin and hydrogenated rosin esters are not sensitizing. Thus, on a weight of evidence basis hydrogenated rosin, hydrogenated rosin salts and hydrogenated rosin esters also are not sensitisers. In hydrogenated rosin the diene structure is, at least partially, removed and hence the potential for oxidation reduced or lost. Even though it retains the conjugated diene structure, formaldehyde treated rosin is not easily oxidised and is not sensitising.

Rosin adducts formed by reaction with maleic anhydride or fumaric acid result in the elimination of the conjugated diene structure but produce moieties containing a new and clear alert for sensitisation. Esterification of the acid anhydride results in a considerable reduction in the skin sensitising potential, but some residual activity remains.

Predictably, large molecules generally do not penetrate the skin and therefore the phenolic resins are not skin sensitisers.

DISCUSSION

Classification Criteria

The classification criteria for skin sensitisation are currently those of the UN Globally Harmonised System (Fifth edition, UN ECE 2013) and are identical to those in the EU under Regulation 1272/2008 (as amended). The definition of a skin sensitiser is:

A substance is a skin sensitiser:

(a) If there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons
(b) If there are positive results from an appropriate animal test.

This category is sub-divided into two sub categories, A and B.

The guidance also states:

'Evidence should include

(a) Positive data from patch testing, normally obtained in more than one dermatological clinic
(b) Epidemiological studies showing allergic contact dermatitis caused by the substance: situations in which a high portion of those exposed exhibit characteristic symptoms are to be looked at with special concern, even if the number of cases is small.
(c) Positive data from animal studies
(d) Positive data from experimental studies in man
(e) Well documented episodes of allergic contact dermatitis, normally obtained in more than one dermatological clinic
(f) Severity of reaction may also be considered.

Evidence from animal studies is usually much more reliable than evidence from human studies. However, in cases where evidence is available from both sources and there is conflict between the results, the quality and reliability of the evidence from both sources must be assessed in order to resolve the question of classification on a case-by-case basis.'

Classification

In general, rosin and chemically modified rosins are not skin sensitisers in the standard regulatory tests when the results are set against the appropriate criteria. This applies to rosin, rosin salts and rosin esters, as well as hydrogenated rosin, its salts and esters. The exceptions are discussed below and are associated with addition reactions associated with the conjugated double bond system in the molecule. A rational structure-activity relationship can be drawn up to explain these conclusions.

Rosin and Oxidised Rosin

The animal testing conducted with rosin indicates clearly that it should not be classified as a skin sensitiser. It was classified originally on the basis of human experience, as revealed by patch testing. Unfortunately the 'rosin' used for skin sensitisation testing in human prevalence studies was, and is, in powdered form or as a solution and therefore contains notable quantities of oxidised material that gives positive results when tested in the GPMT. As examined in greater detail in Botham et al (2008), because this human testing

employed inadequately controlled challenge material it cannot support the classification of rosin as a skin sensitiser.

The EU in the form of the Commission Working Group on the Classification and Labelling of Dangerous Substances (Karlberg et al, 1999; ECB, 2000) has accepted this as scientifically correct, but believes that rosin should continue to be labelled as a sensitiser because, as the EU Working Group stated and wrote:

> "declassification, although scientifically justified, would decrease the level of protection within the present regulatory system and the available means of control",

This meant that the labelling should continue because they claimed that rosin oxidises readily and oxidised rosin is a known skin sensitiser. As discussed earlier, rosin does not oxidise readily when transported hot under nitrogen, or when pelletised or when in 'massive' form, i.e. in 200 kg drums. Rosin only oxidises when powdered rosin is exposed to air for many days under mild conditions and this is not how rosin is normally produced or used.

Further, if the rules concerning classification are adhered to and rosin-containing oxidised rosin is considered as mixture, then, if it contains 0.1% or more of oxidised rosin (or hydroperoxide) it should be labelled 'Contains X. May produce an allergic reaction' (EU Regulation 1272/2008 Annex II section 2.8 and predecessors).

At the time of the classification of rosin, oxidised rosin was listed as an individual substance on the EINECS (CASRN 100085-68-5, EC-Number 309-211-5). The EU authorities did not classify this substance, but instead classified rosin - incorrectly, in our opinion. Oxidised rosin is listed by ECHA but is not a REACH-registered substance - not surprisingly since no one produces it intentionally. It seems to be counterintuitive that the hazard of a substance are determined not by the substance itself, but by the hazard of a different and known chemical derivative.

Thus it is the industry opinion that rosin has been incorrectly classified as a skin sensitiser in Europe.

Rosin Adducts with Maleic Anhydride or Fumaric Acid and Their Esters

Potency can now be taken into consideration when applying the United Nations Globally Harmonised System for hazard communication (UN GHS) and the EU classification of skin sensitisers to adducts and adduct esters formed from fumarated or maleated rosin. The LLNA is the test best capable of assessing potency and the classification uses the Effective Concentration producing stimulation Index of 3 (EC3). The adducts, with their EC3 of values of 1.9% and 0.74% respectively, should be classified in the strong sensitiser category (1A - EC3 < 2%). The formaldehyde treated maleated product (EC3 5%) is in the upper reaches of the sensitising category (1B – EC3 > 2%), presumably because of a slight deactivation due to the introduction of a methyl group. It seems probable that the same underlying sensitisation mechanism is operating for all of these substances (presence of the anhydride or the Diels-Alder structure[5]), and that it is independent of and different from that for oxidised rosin (presence of the hydroperoxide of abietic acid). Rosin adducts are substances that have the clear potential to be human skin sensitisers.

When the esters are examined in the LLNA, for pentaerythritol esterified fumarated rosin the EC3 is (average) 23%. For pentaerythritol esterified maleated rosin the EC3 is (average) 25% and for the mixed

[5] If it were shown that pure fumaropimaric acid were not a sensitiser, it would be clear that it is the anhydride functionality that is the cause of sensitisation. Sadly this data is lacking. The fact that the esters are not sensitisers supports this contention to some degree since they contain no anhydride function.

pentaerythritol/glycerol ester with fumarated rosin the EC3 is not calculable but ~10%. The borderline for sensitising/non sensitising is an EC3 of 50% for a solid. This because it is recommended that the substance be administered in solution and the highest recommended concentration for solutions is 50%. Although consistently positive in the LLNA, mixed results (usually with the higher percentage of adduction agent being more likely to be positive) have been recorded when the GPMT has been employed. These substances are therefore classifiable as sensitiser (1B), but close to the borderline classifiable/not classifiable.

As a general principle, the adducts should be classified in the strong sensitiser category (1A - EC3 ≤ 2%) and the adduct esters in the sensitiser category (1B – EC3 >2%).

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APPENDIX 1: Definitions for Klimisch Gradings 1-3

Grade	Description	Definition
1	Reliable without restriction	Studies or data from the literature or reports which were carried out or generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline (preferably performed according to GLP) or in which all parameters described are closely related/comparable to a guideline method.
2	Reliable with restriction	This includes studies or data from the literature, reports (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.
3	Not reliable	This includes studies or data from the literature/reports in which there are interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g., unphysiologic pathways of application) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for an assessment and which is not convincing for an expert judgment.
4	Not assignable	This includes studies or data from the literature, which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.).

APPENDIX 2: Tabulated data for individual studies on skin sensitisation potential conducted on rosin and chemically modified rosins

All the data tabulated has been obtained from regulatory studies conducted on commercial grade materials or on material synthesised in laboratory scale syntheses using the commercial processes. All the studies included in the tables are classifiable as Klimisch Grade 1 or 2 standards unless otherwise stated, and were conducted using the appropriate test guidelines (or, when the LLNA tests were conducted before formal publication of the guidelines, to generally accepted standards).

Contract research organisations (CROs) identified are:

Biogir SA, Gazinet, France

CTL – Central Toxicology Laboratory, Alderley Park, Cheshire, UK

Eurofins/Product Safety Laboratories, Dayton, NJ, USA

EVIC CEBA, Blanquefort, France

Harlan/Safepharm Laboratories, Derby, UK*

HRC - Huntingdon Research Centre, Huntingdon, UK*

Scantox Laboratories, Ejby, Denmark (now Citoxlab Scantox)

*These have now merged to form Envigo.

APPENDIX 3: Appendix Tables Referred to in the body of the report

Table A1. Cross reactivity of testing substance and oxidised gum rosin in the GPMT.

Test material	Report	Induction intradermal	Induction topical	Challenge	Responders	Classification	
Gum rosin, naturally oxidised	CTL/P/5532	3% olive oil	75% olive oil	20%	Test: 10/10	Positive	(Not statistically significant, thus report says negative, but (10-6)/10 >30%).
					Control: 3/5		
Colophony	CTL/P/5532	3% olive oil*	75% olive oil	20%	Test: 10/10	Positive	
(Chemotechnique Diagnostics)					Control: 2/5		
Colophony	CTL/P/5532	3% olive oil*	75% olive oil	20%	Test: 5/10	Positive	
(Bio Diagnostics)					Control: 1/5		
Olive oil		3% olive oil*	75% olive oil	Olive oil	Test:1/10	Negative	
					Control: 0/5		
Induction was with oxidised gum rosin; control was with the omission of test substance in induction phase. Challenge was with the colophony samples.							

TABLE A2 : GPMT AND OTHER ADJUVANT BASED STUDIES WITH ROSIN, OXIDISED ROSIN, HYDROGENATED ROSIN AND DISPROPORTIONATED

GPMT								
CAS no	Test material	Test reference	Induction	Induction	Challenge	Result	EU	
		(Year test reported/signed)	I.d.	topical		(responders)	Classification	
8050-09-7	Rosin							
	Gum rosin	CTL/F/147 (ECHA11)	3% corn oil/DMF	30% corn oil/DMF	3%, 10%, 30% corn oil/DMF	Test: 0/19; 0/19; 0/19	negative	
		-1997				Control: 0/10; 0/10; 0/10		
			10% corn oil/DMF	30% corn oil/DMF	0%, 3%, 10%, 30% corn oil/DMF	Test:1/20; 0/20; 0/20; 0/20	negative	
						Control: 0/10; 0/10; 0/10		
	Gum rosin	CTL/P/4921 (ECHA3)	10% olive oil	75% paraffin wax	0%, 10%, 30%, 75% paraffin wax	Test: 0/20; 0/20; 1/20; 2/20	negative	
		-1997				Control: 0/10; 0/10; 0/10; 0/10		
	Gum rosin	Scantox 25212/A	0.3% coconut oil	50% coconut oil	50% coconut oil	Test: 1/20	negative	

		-1997				Control: 0/10		
	Gum rosin	EVIC-CEBA (ECHA9)	3% olive oil	25% vaseline	0%, 0.1% (rest invalid - skin irritation)	Test 0/20; 0/20	negative	
		-1994				Controls: (vehicle induction and challenge)2/10; 2/10		
						controls: (vehicle only induction) 3/10, 2/10		
	Gum rosin	EVIC CEBA 3572 (ECHA7)	3% olive oil	25% vaseline	0%, 0.1%; 0.5% (rest invalid - skin irritation)	Test: 0/20; 1/20	negative	
		-1994				Controls (vehicle induction and challenge): 0/10; 0/10		
						Controls (vehicle induction only): 0/10; 0/10		

	Tall oil rosin	CTL/E/0186	10% olive oil	40% olive oil	3%, 10%, 30%, 40% olive oil	Test: 0/20; 0/20; 1/20; 1/20	negative	
		-1996				Control:		

	Wood rosin	Hill Top 96-8098-21 (ECHA5)	10% diethylphthalate	40% diethylphthalate	3%, 10%, 30%, 40% diethylphthalate	Test: 0/20; 1/20	negative	
		-1996				Controls (vehicle induction and challenge): 0/10; 0/10		
						Controls (vehicle induction only): 0/10; 0/10		
	Wood rosin	CTL/P/5234 (ECHA8)	10% olive oil	30% olive oil	10%, 30% olive oil	Test: 0/20; 1/20	negative	
		-1997				Control: 0/10; 0/10		
	Wood rosin	CTL/P/5224 (ECHA6)	10% olive oil	40% olive oil	25%, 40% olive oil	25% Test:1/20, 1/20; 40% Test 1/20, 1/20 (24 and 48 h readings)	negative	(different animals at 24 and 48 h, same animals in 25% and 40% group)
		-1997				Control: 0/10; 0/10		

	Oxidised rosin							
	Gum rosin, naturally oxidised	CTL/P/5415	3% olive oil	75% olive oil	3%, 10%, 25%, 50% olive oil	Test: 2/20; 6/20; 14/20; 19/20	Positive	
		-1997				Control: 0/10; 0/10; 2/10; 4/10		
		CTL/P/5532	3% olive oil	75% olive oil	20% olive oil	Test 10/10	Positive	Controls for the colophony diagnostic samples; >30%responders (positive) but report says negative as not statistically significant.
		-1997				Control 6/10		
	Gum rosin, naturally oxidised	Scantox 25212/B	0.3% coconut oil	50% coconut oil	50% coconut oil	Test: 20/20	Positive	
		-1997				Control: 3/10		
	Wood rosin, naturally oxidised	CTL/E/0183	10% olive oil	40% olive oil	3%, 10%, 30%, 40% olive oil	Test: 0/20; 0/20; 3/20; 8/20	Positive	

		-1997				Control: 0/9; 0/9; 0/9; 0/9		
	Other							
	Disproportionated gum rosin	CTL/E/0185 (ECHA10)	10% olive oil	40% olive oil	3%, 10%, 30%, 40% olive oil	Test: 0/20; 0/20; 0/20; 0/20	Negative	
		-1997				Control: 0/10; 0/10; 0/10; 0/10		
		EVIC-CEBA T124/3651	3% olive oil	25% vaseline	0.5%, 1%, 2% vaseline	Test: 0/20, 9/20, 19/20	Negative	vehicle inadequate (Klimisch 3)
		-1994				Control: 0/10, 7/10 , 9/10		
		EVIC-CEBA T125/3651	3% olive oil	25% vaseline	0.5%, 1%, 2% in vaseline	Test: 1/20, 7/20, 14/20	Negative	
		-1994				Control: 0/10, 5/10,9/10		
	'Superoxidised' gum rosin	CTL/P/4920	10% olive oil	75% olive oil	1%, 3%, 10%, 30% olive oil	Test: 0/19; 0/19; 0/19; 0/19	Negative	
		-1997				Control: 0/10; 0/10; 0/10;		

						0/10		
65997-06-0	Hydrogenated gum rosin	CTL/E/0184 (ECHA1)	10% olive oil	40% olive oil	3%, 10%, 30%, 40% olive oil	Test: 0/20; 0/20; 0/20; 0/20	Negative	
		-1997				Control: 0/10; 0/10; 0/10; 0/10		
OTHER RELEVANT ADJUVANT BASED TESTS								
65997-06-0	Hydrogenated gum rosin	Biogir SMK93.1058 (ECHA2)	FCA i.d., test substance	0.5 mL undiluted	25%, 50% in acetone	Test: challenge 1: 20/20; Control: 10/10	Negative	
		-1993	topically 0.5 ml undiluted	(SLS treated skin)	rechallenge 12.5%, 25%, 50% in alcohol	Rechallenge: Test 1/16; 0/16; Control 1/12; 0/12		
	Hydrogenated gum rosin	Biogir SMK93.11 (ECHA3)	FCA i.d., test substance	0.5 g undiluted	25%, 50% in paraffin oil	Test: 0/14; 0/14	Negative	
		-1993	topically 0.5 g	(SLS and petroleum		Control: 0/14; 0/14		
				jelly treated skin)				

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

	ECHA refers to ECHA registration entry number for skin sensitisation tests.							
2008	All the studies on rosin except that from Hill Top were reported in Botham et al,.							
	In all cases the positive controls were satisfactory.							
	FCA - Freunds Complete Adjuvant							
	SLS - sodium lauryl sulphate							

TABLE A3 : LLNA WITH ROSIN, ROSIN HYDROGENATED ROSIN SALTS AND OXIDISED ROSIN

CAS no	Test material	Test reference	Dose levels		SI	EU	
			Induction			Classification	
8050-09-7	Rosin	CTL (ECHA2)	0.25%, 2.5%, 25%	butanone	0.85, 1.33, 2.88	Negative	
		-2002					
61790-50-9	Resin acids and rosin acids, potassium salt	Harlan 2916/0040 (ECHA)	5%, 10%, 25%	dimethylformamide	1.06, 2.38, 1.84	Negative	
		-2010					
9007-13-0	Resin acids and rosin acids, calcium salt	Harlan 2916/0053 (ECHA)	5%, 10%, 25%	dimethylformamide	1.47, 1.44, 1.46	Negative	
		-2010					
	Oxidised rosin	CTL/GM 7446	0.5%, 1%, 2.5%, 5%, 10%, 25%	acetone-olive oil (3:1)	1.0, 1.01, 0.97, 1.58, 1.26, 2.87	Negative	
		-2001					
		(Botham et al., 2008)					

68990-01-2	Resin acids and rosin acids, hydrogenated,	Safepharm 674/100	5%, 10%, 25%	ethanol-water (4:1)	0.99, 1.86, 3.69	Positive	Klimisch grade 3 Laboratory preparation by a Company not placing it on the market and
	potassium salt	-2005					unjustified use of non-standard solvent

TABLE A4: BUEHLER ASSAYS WITH ROSIN AND OXIDISED ROSIN

CAS no	Test material	Test reference	Induction topical		Challenge	Result (responders)	EU Classification	
8050-09-7	Rosin	CTL/F/148 (ECHA4)	10%, 30%	3%DMF in corn oil	3%, 10%, 30%	10% gp: Test - 0/19; 0/19; Control: 0/10, 0/10	Negative	24, 48 h, 3% results not recorded.
		Botham et al, 2008				30% gp: Test - 0/20; 0/20; Control - 0/10, 0/10	Negative	
		-1998						
	Oxidised rosin	Eurofins/PSL 20436 Botham et al, 2008	moistened	olive oil	75%	Test - 0/20; Control - 0/10	Negative	

TABLE A5: GPMT STUDIES WITH ROSIN AND HYDROGENATED ROSIN ESTERS

CAS no	Test material	Test reference	Induction		Challenge	Result (responders)	EU Classification	
		(Year test reported/signed)	I.d.	topical			Classification	
GPMT								
Rosin based esters								
8050-25-7	Resin acids and rosin acids, esters with triethylene glycol	HRC 920173D/BGV1/10 (ECHA)	7.5% in Alembicol D	neat	100%, 50%, 20%, 10% in Alembicol D	Test: 11/20. 6/20/ 1/20, 1/20 Control: 17, 10/20, 4/20, 2/20	Negative	read at 24, 48 and 72h.
		-1992						
8050-26-8	Resin acids and rosin acids, esters with pentaerythritol	Scantox 25212/C (ECHA2)	10% coconut oil	50% in coconut oil	25%, 50% in coconut oil	0/20; 0/20	Negative	
		-1997						
	Resin acids and rosin acids, esters with pentaerythritol	CTL/P/6022	10% coconut oil	50% in coconut oil	50%, 75% in coconut oil	Test 5/20, 0/20; Control 1/10, 0/10	Negative	Not in ECHA!

		-1999						
	Resin acids and rosin acids, esters with pentaerythritol	CTL/P/5197	1% in olive oil	65% in olive oil	10%, 25%, 50%, 65% in olive oil	Test: 1/20, 0/20, 11/20, 15/20 Control: 0/10, 0/10	Given as positive, but actually Negative	Not on ECHA! (Improper sample preparation involved heating; Klimisch 3). NB (15-6)/20 is <30%, therefore result is negative.
		-1997				1/10, 3/10		
	Resin and rosin acids, esters with pentaerythritol	CTL/P/5553	10% in olive oil	75% in white petrolatum	10%, 25% 50% in white petrolatum	0	Positive	Not on ECHA! (Improper sample preparation involved dissolution in n-hexane before addition of the olive oil/petrolatum). Klimisch 3.
		-1997		petrolatum				
	Resin acids and rosin acids, esters with pentaerythritol	HRC 92017D/BGV5/SS (ECHA3)	7.5% liquid paraffin	76.9% liquid paraffin	76, 38, 20, 10% in liquid paraffin	Test 0/20, 0/20, 0/20 Control: 0/20, 0/20, 0/20	Negative	20% and 10% read together
		-1992						
	Resin acids and rosin acids, esters with pentaerythritol	HRC 90695/BGV 3/55 (ECHA4)	7.5% in 5% acetone-95% Alembicol D	60% in acetone	60%, 30% in acetone	Test: 0/20, 0/20; Control 0/18, 0/18	Negative	read at 24, 48 and 72h.

		-1990						
8050-31-5	Resin acids and rosin acids, esters with glycerol	CTL/P/6021 (ECHA2)	10% in coconut oil	75% in coconut oil	50%, 75% in coconut oil	Test 9/20, 2/20; Control 5/10, 0/10	Negative	
		-1999						
	Resin acids and rosin acids, esters with glycerol	CTL/P/5552 (ECHA4)	10% in olive oil	75% in white petrolatum	50%, 20%, 10%, 0% in white petrolatum	Test 13/20,10/20, 4/20, 0/20; Control 4/10, 2/10, 0/10, 0/10	Negative	
		-1997						
	Resin acids and rosin acids, esters with glycerol	CTL/P/5196	10% in olive oil	75% in olive	10, 25, 50, 75% in olive oil	Test: 0/20, 0/20, 4/20, 11/20; Control 0/10, 0/10,	Negative	Not in ECHA (Sample heated - Klimisch 3). Although top dose 35% responders this was not statistically significant
		-1997		oil		0/10, 2/10		
	Resin acids and rosin acids, esters with glycerol	Scantox 25212/E	10% in coconut oil	50% in coconut oil	25% 50% in coconut oil	Test: 1/20; 1/20; Control 0/10, 0/10	Negative	Not on ECHA - named in report as Bevipale 85
		-1997						

	Resin acids and rosin acids, esters with glycerol	HRC 920114D/BGV 5/SS (ECHA3)	7.5% in Alembicol D	83% in Alembicol D	83%, 41% in Alembicol Dn	83% Test 15/20, 3/20, 0/20, Control 12/20, 3/20, 0/20; 41%- Test 0/20, 0/20; control 0/20, 0/20	Negative	83% examined at 24, 48 and 72 h
		-1992						
Hydrogenated rosin esters								

8050-15-5	Resin acids and rosin acids, hydrogenated, methyl esters	R-00017, T97-0237	10% in corn oil	100%	25% in petrolatum	Test 0/10, Control 0/10	Negative	
		Consumer Product Testing, Fairfield, NJ (ECHA1)						
		-1997						
	Resin acids and rosin acids, hydrogenated, methyl esters	NOTOX 0305/385 (ECHA2)	5% in corn oil	100%	0%, 10%, 50%, 100% in corn oil	Test 0/19, Control 0/10	Negative	10 test, 5 control animals
		-1986						
	Resin acids and rosin acids, hydrogenated, methyl esters	NOTOX 0304/384 (ECHA3)	5% in corn oil	100%	0%, 10%, 50%, 100% in corn oil	Test 0/20; Control 0/8	Negative	
		-1986						
65997-13-9	Resin acids and rosin acids, hydrogenated, esters with glycerol	R-0057, T 97-0055 (ECHA1)	10% in corn oil	100%	25% in petrolatum	Test 3/10, 0/10; Control 1/10, 0/10	Negative	
		Consumer Product Testing, Fairfield, NJ						

		(ECHA1)						
		-1997						
OTHER RELEVANT ADJUVANT BASED TESTS								
Rosin based esters		-1993						
8050-31-5	Resin acids and rosin acids, esters with glycerol	Biogir SMK93.10 (ECHA5)	FCA i.d., test substance	0.5 mL undiluted	12.5% and 6.25% in vaseline	Test: 0/20, 0/20, Control: 0/20, 0/20	Negative	
		-1993	topically 0.5 ml undiluted	(SLS treated skin)				
65997-13-9	Resin acids and rosin acids, hydrogenated, esters with glycerol	Biogir SMK 93.12 (ECHA2)	FCA i.d., test substance	0.5 mL undiluted	100%, 50%, 25%, 12.5%	Test 0/15, 0/15 Control 0/15, 0/15	Negative	
		-1993	topically 0.5 ml undiluted	(SLS treated skin)				

TABLE A6 : LLNA WITH ROSIN ESTERS

CAS no	Test material	Test reference	Dose levels induction		SI	EU classification
8050-26-8	Resin acids and rosin acids, esters with pentaerythritol	Safepharm 1586/001 (ECHA1)	0%, 0.1%, 1%, 10%	acetone: olive oil (4:1)	1.4, 0.79, 2.33	Negative
		Illing et al, 2009				
		-2002				
8050-31-5	Resin acids and rosin acids, esters with glycerol	Safepharm 1586/004 (ECHA1)	0%, 0.5%, 5%, 50%	acetone: olive oil (4:1)	1.4, 1.3, 2.2	Negative
		Illing et al, 2009				
		-2002				

TABLE A7: HUMAN REPEAT INSULT PATCH TESTS WITH ROSIN AND HYDROGENATES ROSIN, ESTERS

			No of participants	Exposures	Frequency	Challenge	Reactors	Conclusion
8050-31-5	Resin acids and rosin acids, esters with glycerol	ECHA	50	15	Alternate	After 14 days	*1	Negative
		-1954			days			
8050-15-5	Resin and rosin acids, hydrogenated, methyl ester	ECHA	209	9	3 times/week	After 14 days	*0	Negative
		-1998			for 3 weeks			
64365-17-9	Resin and rosin acids, hydrogenated, esters with pentaerythritol	ECHA	55	9	3 times/week	After 14 days	*0	Negative
		-1960			for 3 weeks			
65997-13-9	Resin and rosin acids, hydrogenated, esters with glycerol	ECHA	202	9	3 times/week	After 14 days	*0	Negative
		-1997			for 3 weeks			

TABLE A8: GPMT STUDIES WITH ROSIN ADDUCTS

CAS no	Test material	Test reference	Induction	Induction	Challenge	Result	EU Classification	
		(Year test reported/signed)	I.d.	topical		(responders)		
Group A - forming an acid anhydride ring								
8050-28-0	Rosin, maleated (10% maleation)	CTL/E/182 (ECHA2)	0.003% in olive oil	30% in olive oil	30%, 10% in olive oil	30% Test 19/19, Control 7/10; 10% Test 15/19, Control 0/10	Positive	
		-1997						
	Rosin, maleated (5% maleation)	CTL/E/181	0.01% in olive oil	30% in olive oil	3%, 1% in olive oil	3% Test 12/20, Control 0.10; 1% Test 4/20, Control 0/10	Positive	(not in ECHA registration)
		-1997						
Group B - Other adducts								
91081-53-7	Rosin, reaction products with formaldehyde	CTL/P/5199 (ECHA2)	10% in olive oil	75% in olive oil	10%, 25%, 50%, 75% in olive oil	75% and 50% Test 2/20, 25% and 10% Test 0/20, Controls 0/10	Negative	
		-1997						

TABLE A9: LLNA WITH ROSIN ADDUCTS

CAS no	Test material	Test reference	Dose levels induction		SI	EU classification	
8050-28-0	Rosin, maleated	Safepharm 1586/002 (ECHA1)	0.5%, 5%, 50%	acetone:olive oil (4:1)	1.91, 22.8, 19.4	Positive	EC3 0.74%
		-2002					
		(Illing et al, 2009)					
65997-04-8	Rosin, fumarated	Safepharm 1586/011 (ECHA1)	0.5%, 5%, 50%	acetone:olive oil (4:1)	1.2, 7.0, 12.8	Positive	EC3 1.9%
		-2002					
		(Illing et al, 2009)					
95009-65-7	Rosin, fumarated, reaction product with formaldehyde	Safepharm 1586/015 (ECHA1)	0.5%, 5%, 50%	acetone:olive oil (4:1)	0.9, 3.0, 7.2	Positive	EC3 5%
		-2003					
		(Illing et al, 20098)					

TABLE A10: BUEHLER ASSAYS WITH ROSIN ADDUCTS

CAS no	Test material	Test reference	Induction topical		Challenge	Result (responders)	EU Classification	
Group A - forming an acid anhydride ring								
8050-28-0	Rosin, maleated	Eurofins/PSL 21612	80%	olive oil	5% olive oil	Test 16/20, Control 0/10	Positive	
		(Illing et al., 2009)						
		-2007						
	Induction: Rosin, maleinated	Eurofins/PSL 21612	80%	olive oil	75% olive oil	Test 0/20, control 0/10	Negative	No cross-reaction
	Challenge : Oxidised gum rosin	(Illing et al., 2009)						
		-2007						
Group B - Other adducts								
91081-53-7	Rosin, reaction product with formaldehyde	CTL /E/120 (ECHA1)	30%	corn oil	3%, 10%	30% Test 12/20, Control 7/10	Negative	
		-1995			corn oil	3% Test 1/20, Control 0/10		

TABLE A11: GPMT STUDIES WITH ROSIN ADDUCT ESTERS

CAS no	Test material	Test reference	Induction I.d.	Induction topical	Challenge	Result (responders)	EU Classification	
		(Year test reported/signed)						
94581-16-5	Resin acids and rosin acids, maleated, esters with glycerol	CTL/P/5177	0.3% in	50% in	1%, 3%, 10%, 25%	Test: 11/19, 9/19, 16/19, 19/19	Positive	Substance not yet registered, sample named 45-B-347.
	(16% maleation)	-1997	olive oil	olive oil	in olive oil	Control: 0/10, 0/10, 1/10, 2/10		
	Resin acids and rosin acids, maleated, esters with glycerol	CTL/P/5168	0.3% in	65% in	3%, 10%, 25%, 50%	Test 0/20, 0/20, 0/20, 10/20	Negative	Substance not yet registered, sample named 4A PS960041 synthetic resin.
	(8% maleation)	-1997	olive oil	olive oil	in olive oil	Control: 0/10, 0/10, 0/10, 4/10		

94581-17-6	Resin acids and rosin acids, maleated, esters with pentaerythritol	CTL/P/5512	0.3% in	75% in	3%, 10%, 25% 50%	Test: 18/19; 19/19, 19/19, 19/19	Positive	Substance not yet registered, sample named 45-B-348.
	(16% maleation)		olive oil	white petrolatum	in white petrolatum	Control: 1/10, 1/10, 6/10, 6/10		
	Resin acids and rosin acids, maleated, esters with pentaerythritol	CTL/P/5191	0.3% in	50% in	3%, 10%, 25%, 50%	Test: 0/20, 0/20, 0/20, 18/20	Positive	Substance not yet registered, sample named 5A PS960042 synthetic resin
	(8% maleation)	-1997	olive oil	olive oil	in olive oil	Control: 0/10, 0/10, 0/10, 2/10		
94581-15-4	Resin acids and rosin acids, fumarated, esters with pentaeythrito	HRC 920112D/BGV 5/SS (ECHA2)	7.5% in	83.3% in	83.3%, 41.6% in	0/20, Control 0/20	Negative	

	I							
		-1992	Alembicol D	Alembicol D	Alembicol D			

TABLE A12: LLNA WITH ROSIN ADDUCT ESTERS

CAS no	Test material	Test reference	Dose levels Induction		SI	EU Classification	
94581-15-4	Resin acids and rosin acids, fumarated, esters with pentaeythritol	Harlan 2916/005 (ECHA1)	0%, 10%, 25%, 50%	dimethylformamide	1.80, 2.52, 4.24	Positive	EC3 32%
		-2010					
	Resin acids and rosin acids, fumarated, esters with pentaeythritol	Safepharm 1078/097 (ECHA3)	0%, 10%, 25%, 50%	butanone	2.63, 4.07, 3.65	Positive	EC3 13.5%
		-2003					
94581-17-6	Resin acids and rosin acids, maleated, esters with pentaerythritol	Safepharm 1586/013 (ECHA5)	0%, 0.25%, 2.5%, 25%	acetone:olive oil (4:1)	1.25, 1.36, 4.17	Positive	EC3 16%
	(acid value 35)	-2003					
		(Illing et al, 2009)					
	Resin acids and rosin acids, maleated, esters with pentaerythritol	Safepharm 1586/012 (ECHA6)	0%, 0.25%, 2.5%, 25%	butanone	1.34, 1.41, 3.83	Positive	EC3 17%
	(acid value 13)	-2003					
		(Illing et al, 2009)					
	Resin acids and rosin acids, maleated, esters with pentaerythritol	Safepharm 1586/006 (ECHA7)	0%, 0.25%, 2.5%, 25%	butanone	0.39, 1.34, 3.3	Positive	EC3 22%

	(acid value 24.5)	-2002					
		(Illing et al, 2009)					
92202-14-7	Resin acids and rosin acids, fumarated, esters with	Safepharm 1078/098 (ECHA)	0%, 10%, 25%, 50%	butanone	4.37, 4.37, 4.73	Positive	Not calculable but ~10%
	glycerol and pentaerythritol	-2003					

TABLE A13: LLNA with OTHER MODIFIED ROSINS

CAS no	Test material	Test reference	Dose levels Induction	SI	EU Classification		
8050-18-8	Decarboxylated rosin	Safepharm 1586/003	0.5%, 5%, 50%	1.3, 1.8, 2.7	Negative	Not registered	
		Illing et al, 2009					
		-2002					
	Triethanolamine salt of dehydrogenated rosin	Safepharm 1586/009	0.5%, 5%, 50%	1.4,1.8, 4.5	Positive	EC3 25%	dark brown viscous liquid
		Illing et al, 2009					
		-2002					
67700-45-2	Phenolic reaction product of rosin	Safepharm 1586/14	0.5%, 5%, 50%	1.16,0.68, 2.56	Negative	Not registered	
	(polymer)	Illing et al, 2009					
		-2003					
68152-62-5	Gum rosin, fumarated or maleated, reacted	Safepharm 1586/005	0.25%, 2.5%, 25%	0.85, 1.33, 2.88	Negative	Not registered	
	with formaldehyde and nonylphenol, and esterified.	Illing et al, 2009					
		-2002					