Poster # 1442

An in-vitro absorption screen with rosin/resins using everted rat intestinal sacs

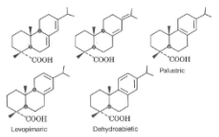
Larry G. Higgins[1], Robert P. Powrie[1], Mike Penman[2], Steve Green[3], Rob Lobbes[4], Leon Rodenburg[5] and Cliff R. Elcombe[1]
[1]CXR Biosciences Ltd, Dundee, UK., [2]Penman Consulting bvba, Brussels, Belgium,. [3]Eastman Chemical, Kingsport, TN, USA., [4]Arizona Chemical, Almere, The Netherlands,. [5]Eastman Chemical, Capelle aan den IJssel, The Netherlands,. [2,3,4,5]Hydrocarbon Resins & Rosin Resins REACH Consortium (H4R).

Introduction

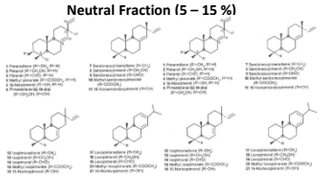
Rosin and Rosin derivatives are chemicals based upon rosin derived from pine trees and are used in a wide variety applications such as adhesives, pharmaceutical, electronics, paper and as chewing gum and paints.

Rosin and its derivatives are a regarded as UVCBs* in regulatory programmes and indeed the composition of rosin is complex and variable. No single constituent is present at a concentration > 10 %. When derivatives are made from rosin, the number of constituents increases significantly with thousands of potential component structures / isomers. Typical representative structures (not exhaustive) are given below for the acid and neutral fractions and for an ester derivative.

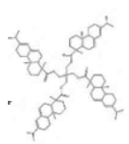
Acid Fraction (85 – 95 %, 20 acids)



Neutral Fraction (5 – 15 %)



Rosin Pentaerythritol ester



EU manufacturers and importers registered 39 rosin derivatives for REACH[1] through the Hydrocarbon Resins & Rosin Resins REACH Consortium (H4R - http://h4rconsortium.com/). To meet the information requirements of REACH a category approach was adopted[2] to facilitating data sharing and to potentially minimising the use of animals in higher tier tests. Four categories of rosin derivatives were developed.

1. Rosin, hydrogenated rosin and their salts *2. Rosin esters*
3. Rosin adducts and rosin adducts salts, *4. Rosin adduct esters*

When developing these categories, one of the hypotheses was that the more "bulky" derivatives would be absorbed to a lesser degree than rosin itself, and therefore read across from rosin to other low molecular weight derivatives was valid. It was appreciated when developing this argument that supplementary information would be required in support of the hypothesis to provide confidence in the approach[3]. If this could be demonstrated, then the necessity for extensive higher tier testing, such as OECD 443 one generation study and further developmental toxicity testing may be fulfilled by read across.

The approach used to test the hypothesis was the gut sac model described below using rosin, hydrogenated rosin and the bulky pentaerythritol rosin ester. In addition to looking into gut absorption, metabolism in the intestine was also examined, to see if it may have influence on absorption of rosin and derivatives. The techniques used were semi quantitative and designed to give an indication of ranking of materials rather than absolute values.

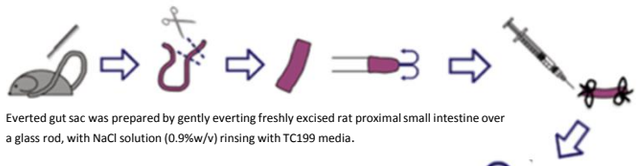
Experimental - Male Han Wistar rats (approx. 8-12 weeks old) were supplied by Harlan UK . Rosin derivatives were supplied by H4R members. Stability of selected test items were confirmed in water and Fed-state simulated intestinal fluid media (FeSSIF) with and without oxygenation

Metabolism. Proximal small intestine microsomes and cytoplasm was prepared by centrifugation and incubated with test substances in the presence and absence of cofactors. Midazolam was used as control to confirm metabolic potential of prepared microsomes. 4-nitrophenyl acetate and 4-nitrophenyl palmitate was used to confirm esterase and lipase activity of prepared cytosol.

Analytical – Rosin incubated media samples were extracted using iso-octane, centrifuged and an aliquot of supernatant removed for analysis by time-of-flight mass spectrometry. Samples scanned in negative ion mode from 100-2000 mass to charge ratio (m/z).

*Unknown or Variable composition, Complex reaction products or Biological materials

Method[4,5,6]



Everted gut sac was prepared by gently everting freshly excised rat proximal small intestine over a glass rod, with NaCl solution (0.9%w/v) rinsing with TC199 media.

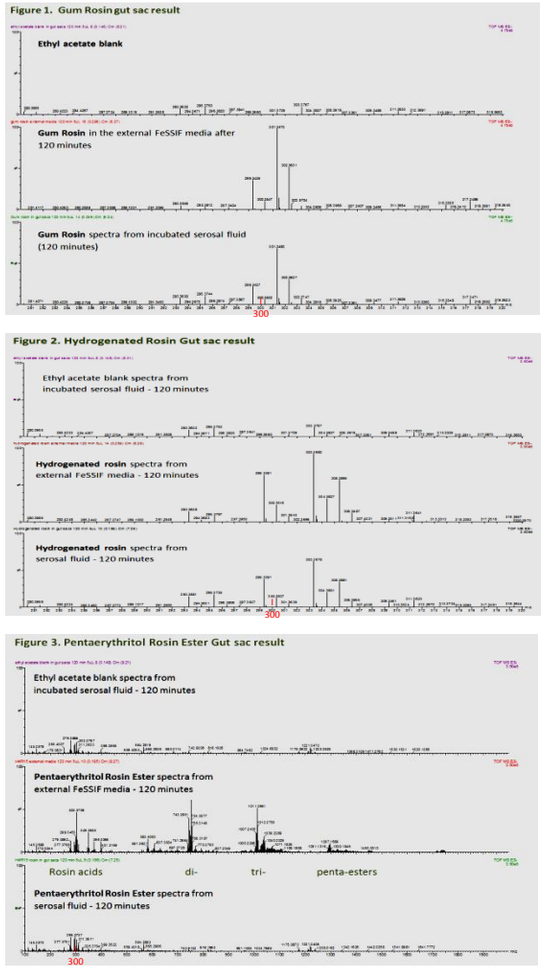
≈2.5 cm of gut tied off and filled with oxygenated Fed State simulated intestinal fluid (FeSSIF).

Paced in a flask containing ≈10ml pre-gassed FeSSIF medium (95% 02, 5% CO2) at 37˚C prior to incubation.

After incubation sacs removed, washed in TC199 and blotted dry. Sacs cut open and serosal fluid collected.
Sacs weighed before and after fluid collection to accurately determine the volume of the medium in the sac.

Adapted from Carvalho,FC *et al* (2010)[6]

Results - Gut Sac Absorption

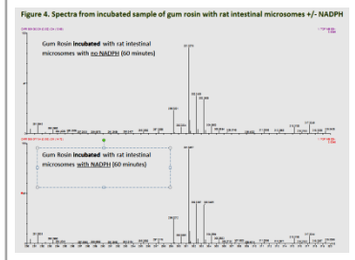


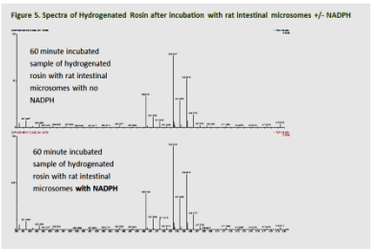
1. Gum Rosin gut sac result - rosin acid components present in the serosal fluid at approximately 70% of the concentration in the external media.

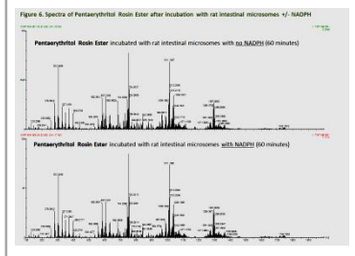
2. Hydrogenated Rosin gut sac result - rosin acid components present in the serosal fluid at approximately 60% of the concentration in the external media.

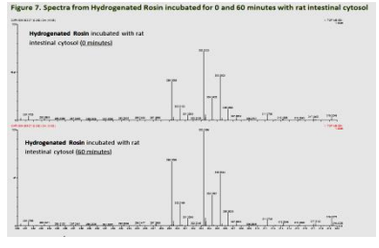
3. Pentaerythritol Rosin Ester gut sac result - none of the higher molecular weight components of the rosin ester are present the serosal fluid

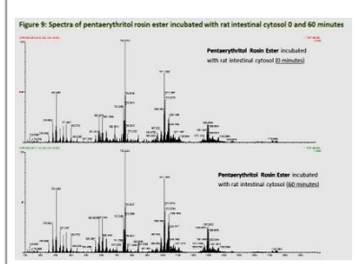
Results – Intestinal Metabolism

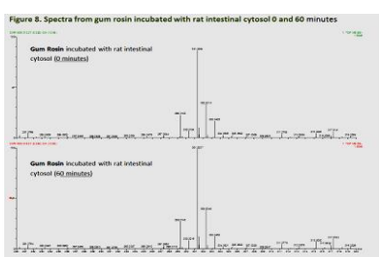












Incubation of the three rosin derivatives investigated with proximal intestinal microsomes or cytosol did not show any detectable metabolite formation. Microsomal enzyme activity was confirmed using midazolam as a test substrate (rate of loss of parent calculated as 217 $pmol^{-1}$ min^{-1} mg^{-1} protein) and 4-nitrophenyl acetate and 4-nitrophenyl palmitate was used to confirm that cytosolic esterase and lipase activity was intact.

Conclusions

No metabolism of Rosin, hydrogenated rosin or pentaerythritol rosin ester was observed by intestinal microsomes or cytosol. So, it is unlikely that metabolism influences absorption in the test system. No oxidation of the test materials was observed. There was absorption of acid fraction rosin constituents across the rat gut in the test system at a rate of 70-80%.

For pentaerythritol rosin ester, residual, unreacted resin acids, that are also present in rosin, were absorbed. The di-, tri- and tetra-esters were not (mono-ester is not present in this product), suggesting that it is appropriate to read across from rosin and other lower molecular derivatives to the ester.

Such studies can be used to help select the most appropriate substances to test within a category and to give confidence in read across hypotheses, reducing the number of animals used in regulatory testing programmes.

References

1) EC (2006) Regulation No 1907/2006 Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)
2) OECD (2014) - Guidance on Grouping of Chemicals, second edition Series on Testing and Assessment No. 194, 2014
3) ECHA (2015) Read-across assessment framework http://echa.europa.eu/documents/10162/13628/raaf_en.pdf
4) Wilson and Wiseman. Metabolic activity of the small intestine of the rat and golden hamster (Mesocricetus Auratus, H. *J. Physiol*. (1954) i23, 126-i30
5) Alam MA, *et al* Everted gut sac model as a tool in pharmaceutical research: limitations and applications. *J Pharm Pharmacol*. 2012 Mar;64(3):326-36
6) Carvalho FC *et al* (2010) . Mucoadhesive drug delivery systems. *Braz. J. Pharm. Sci.* [online]. 2010, vol.46, n.1 pp. 1-17

H4R CONSORTIUM

CXR biosciences®
the investigative & exploratory toxicology company