



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

QUILLAIA SAPONINS

SUMMARY REPORT

1. Saponins are some of the most common constituents of plants and are found in over 500 plant genera. Saponins occur in some plants used as human foods, e.g. potatoes, tomatoes, onions, soya, beans, peas, tea, oats and some herbs and spices. They also occur in some animal forage crops, e.g. alfalfa. Chemically, saponins are glycosides with one or more mono- or oligo-saccharide groups in glycosidic links with a non-polar aglycone, usually of a steroid or triterpenoid structure.
2. Saponins are surface active agents which produce a soapy lather when they are shaken with water. Most commercially- produced "saponin" or "quillaia extract" is an aqueous extract of the dried inner bark of the tree *Quillaia saponaria* Molina. The bark extract is widely used in toiletry preparations and in the food and beverage industries, as an emulsifier and foaming agent. In soft drinks, quillaia extracts are used at concentrations of up to 200 mg/kg. Quillaia extracts are frequently included in cough mixtures for human use.
3. Pharmacopoeial monographs for *Quillaia* have been included in the British and French Pharmacopoeias.
4. *Quillaia* saponins have immunostimulant properties. In veterinary medicines, the main use is as a vaccine adjuvant. The adjuvant component is a purified form of quillaia extract, of uniform composition, usually denoted "Quil A". A typical parenteral dose of vaccine contains 350 µg of saponins.
5. Saponins possess a number of pharmacological properties of which the most important is their ability to lyse erythrocytes *in vivo*. Consequently saponins are very toxic after intravenous administration; death results from a massive production of erythrocyte debris together with reduced oxygen-carrying capacity of the blood. The exact nature of the molecular interactions is unclear though it is generally accepted that the haemolytic activity results from an increase in the permeability of the cell's plasma membrane. Saponins, incorporated in an ISCOM (immune stimulating complex) system, are used in veterinary medicine to ameliorate the haemolytic activity of vaccines. The ISCOMs are stable complexes of cholesterol, phospholipid and *Quillaia* saponins which are used as antigen carriers in subunit vaccines.
6. High concentrations of saponins in the diet of livestock and laboratory animals leads to growth retardation. Although the reasons are not completely understood, there is evidence that the availability of some essential nutrients is impaired. The palatability of the feed is also reduced leading to reduced feed intake. In chickens, it was shown that the effect could be overcome by addition of 1% cholesterol to the diet. Administration of saponins prevented the experimental induction of high plasma cholesterol concentrations in rabbits and monkeys.

7. Saponins are not significantly absorbed after oral administration. *In vitro* experiments showed that saponins were hydrolysed to sapogenins and sugars by the gut microflora. Groups of male Wistar rats, albino mice and Leghorn chicks (one-month old) were kept on diets containing soybean saponins. No saponins or sapogenins were detected in any of the blood samples taken. The animals were killed and residues of sapogenins (but not saponins) were found in the ceca and colon of all 3 species. In the small intestine, only residues of saponins were found. The failure of the haemolytic saponins to cross the gut mucosa has been attributed to the rapid elimination of permeabilised mucosal cells of the small intestine by the normal process of epithelial replacement. The low oral toxicity of orally-administered saponins could then be explained by the large surface area of the gastrointestinal tract of mammals in relation to the concentration of saponins.
8. According to a published report, the acute oral LD50 was 1625 mg/kg bw in the mouse. In the same publication, acute intravenous, intraperitoneal and subcutaneous LD50s of 275, 275 and 650 mg/kg bw respectively, were reported. However the origin of the material tested was unclear and other workers have reported much lower parenteral LD50's. For example, the acute subcutaneous LD50 of "Quil A", a purified form of *Quillaia* saponins, was reported to be in the region of 50 mg/kg bw.
9. In a repeated-dose study in rats, quillaia bark extract was administered in the diet at concentrations of 0, 0.6, 2 or 4% for 13 weeks. Bodyweight gain was reduced in the group receiving 4%. In the groups given 2% and 4%, relative liver weights were reduced in males and the relative stomach weight was increased in both sexes. There were no substance-related histopathological changes. The NOEL was 0.6% of the diet, equivalent to 400 mg/kg bw per day.
10. In cattle, sheep and pigs, a subcutaneous dose of 1 mg of Quil A was usually well-tolerated. The adverse effects were generally limited to a small soft swelling and a short rise in body temperature; both effects resolved within 2 days.
11. There was no information concerning the reproductive toxicity of *Quillaia* saponins. Daily intraperitoneal injections of 0 (untreated), 0 (saline), 5, 15 or 25 mg/kg bw per day of saponins extracted from *Xanthocephalum microcephala* (broomweed) were administered to pregnant Sprague-Dawley rats. Like the quillaia saponins, the broomweed saponins were reported to be triterpenoids. Maternal toxicity (reduced weight gain, behavioural changes) was observed in all the saponin-treated groups. There were no offspring in the 25 mg/kg bw group because all the litters were resorbed and several dams died. There was no evidence of teratogenicity at any dose level. Foetotoxicity (reduced foetal weight and length) was observed in the 15 mg/kg bw group but not in the group administered 5 mg/kg bw saponins. Other workers reported that the saponins from *Xanthocephalum microcephala* did not have any teratogenic effect in cattle but adversely affected the body weight of calves and caused abortions when administered parenterally.
12. Saponins are a normal component of the human diet and there have been no reports of adverse reproductive effects in humans arising from the consumption of commodities containing saponins.
13. The mutagenic potential of several purified saponins extracted from alfalfa was investigated in an *in vitro* bacterial assay for gene mutation using *S. typhimurium* TA97, TA98, TA100 and TA 102; there were no significant increases in revertants in either the presence or absence of metabolic activation. In another study, 13 saponins were isolated from various plants, identified, and their mutagenic potential was investigated using a modified liquid incubation technique of the *Salmonella*/microsomal assay. All the saponins were non-mutagenic.
14. Groups of TO strain mice were fed diets containing 0, 0.1, 0.5 or 1.5% quillaia bark extract for 84 weeks. There were no substance-related effects on survival or behaviour. Haematology values were measured during weeks 26 and 84; there were no consistent dose-related changes. Body weight gain was significantly reduced in male mice given 1.5%. Relative brain and stomach weights were increased in males (but not females) given 1.5%. There were no substance-related histopathological changes. The NOEL was 0.5%, equivalent to 700 mg/kg bw per day.

15. Groups of rats were fed diets containing 0, 0.3, 1.0 or 3.0% quillaia bark extract for 108 weeks. Body weight gain and food consumption were reduced in male rats given 3.0%. There were no substance-related effects on haematology or urinalysis values. There was no evidence of carcinogenicity.
16. Saponins extracted from *Quillaja saponaria* Molina were reviewed by JECFA in 1982 and in 1986. An Acceptable Daily Intake of 0 - 5 mg/kg bw per day was established.
17. There were no residues data resulting from the use of saponins in veterinary medicine. A typical injectable vaccine contains 350 µg of saponins per dose. Assuming a worst case scenario with all the material remaining at the injection site with no metabolism and excretion, the maximum possible daily ingestion of residues would be 0.35 mg per person per day, i.e. approximately 0.1% of the ADI calculated by JECFA.

Conclusions and recommendation

Having considered the criteria laid down by the Committee for the inclusion of substances into Annex II of Council Regulation (EEC) No 2377/90 and in particular that:

- Quillaia saponins are very poorly absorbed after oral administration,
- Their toxicity after oral administration was low,
- Quillaia saponins are a natural component of the diet.

The Committee considers that there is no need to establish an MRL for *Quillaia* saponins and recommends its inclusion into Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table :

Pharmacologically active substance(s)	Animal species	Other provisions
<i>Quillaia</i> saponins	All food producing species	