



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ALOES

SUMMARY REPORT

1. Aloes (Barbados, Cape) is the dried yellow latex-like juice obtained from the cells just beneath the epidermis of the leaves of various species of *Aloe* (family: *Liliaceae*). Aloes is a potent laxative, the anthranoids contained therein being the main active principle. The main species of origin are: Barbados aloes: *Aloe barbadensis* (*Aloe vera*, *Aloe vulgaris*) containing not less than 28% of anthranoids calculated as anhydrous barbaloin (synonym: aloin); Cape aloes: *Aloe ferox* and its hybrids containing not less than 18% of anthranoids calculated as anhydrous barbaloin; *Aloe Perry Baker*, *Aloe socotrine* are also used. Aloes contains anthracene compounds in free and bound (anthraglycosides) form: aloin (18 to 25% barbaloin in *Aloe barbadensis*, 4.5 to 9% capaloin in *Aloe ferox*, 7.5 to 10% socaloin in *Aloe socotrine*), Aloe-emodin (0.05 to 0.5%), anthrone compounds (not well defined), hormone like substances and resins (16 to 63%). Preparations: such as standardised Aloe Dry Extract (European and British Pharmacopoeia) contain 19 to 21% of anthranoids, calculated as anhydrous barbaloin.

2. In veterinary medicine Aloes is used as a laxative. In cattle and sheep doses of 12.5 to 25 mg/kg bw are used, while horses receive 25 to 50 mg/kg bw. Aloes is diluted with water and given orally in 2 parts at a 30-minute interval. Aloes is also used as a tincture (1:5 in ethanol 60%).

Uses in humans include use as bitter tonic, eupeptic and cholagogic agent when given in small doses. At higher doses it acts as a laxative. Anthraquinone laxatives are used in human medicine at doses averaging 20 to 40 mg in adults. Topical use in dermatology and cosmetics is reported as well as parenteral administration of leave extracts to treat skin leishmaniosis. In some Member States the use of anthranoid purgatives has been restricted to short-term treatment of constipation, due to the carcinogenic effect of the anthracene derivative danthron in animal experiments.

Council Directive 88/388/EEC of 22 June 1988 sets the maximum contents of aloin (synonym: barbaloin) in foodstuffs for human consumption to 0.1 mg/kg in both foodstuffs and beverages, with the exception of alcoholic beverages, which may contain up to 50 mg/kg aloin. Aloin may not in itself be added to such foodstuffs but either be naturally present or following the addition of flavourings prepared from natural raw materials.

3. Aloes has purgative action due to the presence of various anthranoids, mostly glycosidic anthraquinones, the action of which depends on the liberation of 1,8-dihydroxyanthracene derivatives (aloe-emodin-9-anthrone being the main active metabolite) from its inactive precursor glycosides (barbaloin, a C-glycoside) by the intestinal microflora. Aloe-emodin-9-anthrone acts specifically on the colon by two different mechanisms of action: stimulation of colonic motility by stimulation of the myenteric plexuses, and stimulation of secretion processes (increased mucus and chloride secretion) resulting in enhanced fluid production. In humans defecation occurs after a delay of 6 to 12 hours, due to the time required for metabolism and transport to the colon. After prolonged use the myenteric plexuses will degenerate, resulting in a loss in motility.

Additional properties are reported for the dry extracts, which are reported to have shown antibiotic effects against staphylococci and *Mycobacterium tuberculosis*, and individual constituents of Aloes. Aloe-emodin inhibits the *in vitro* growth of *Bacillus subtilis* and *Staphylococcus aureus* and has certain antiviral properties against enveloped viruses as well as some antifungal properties against plant-pathogenic fungi.

4. Anthracene precursors, i.e. glycosides like barbaloin, are poorly absorbed after oral administration. Only after the hydrolysis of the sugar moiety in the colon (in the case of barbaloin to aloe-emodin) and reduction to the active anthrone compound (in the case of barbaloin to aloe-emodin-9-anthrone) this latter metabolite is absorbed to a moderate extent.

In humans, after oral administration of Aloes for 7 days at a dose equivalent to 16.4 mg anthranoids, the metabolite aloe-emodin was detected in plasma only sporadically (maximum concentration less than 2 ng/ml). After a single oral dose of Aloes, rhein as a metabolite was found at concentrations ranging from 6 to 29 ng/ml, the median $t_{\max}$ being 16 hours; administration for 7 days gave no indication of accumulation. The extent of the absorption of aloe-emodin-9-anthrone is not known.

Oral administration of a dose of 4.5 mg/kg bw free aloe-emodin (^{14}C -labelled) to rats leads to maximum plasma levels (248 ng equivalents/ml in males and 441 ng equivalents/ml in females) 1.5 to 3 hours after treatment. Due to its rapid oxidation to rhein, metabolism to an unidentified metabolite and conjugation, the bioavailability of free aloe-emodin is much lower. Less than 10% of the plasma radioactivity were attributable to free aloe-emodin. Concentrations of free rhein were consistently higher (range 14 to 30 ng/ml at 1.5 to 3 hours). Conjugates of aloe-emodin and rhein accounted for the majority of the plasma radioactivity up to 6 hours after treatment (45% in females and about 70% in males at 1.5 hours, about 60% in both sexes at 3 hours). The unidentified metabolite represented 10 to 25% of the plasma radioactivity up to 6 hours. The absolute bioavailability of aloe-emodin could not be determined as intravenous administration impracticable, but was estimated as 20 to 25% based on urinary excretion and due to the fact that biliary excretion can not be excluded.

Highest concentrations of aloe-emodin related residues were found in kidney (1400 to 1700, 700, and 600 μg equivalents/kg at time-points before 24, at 24 and at 48 hours, respectively), followed by liver (700, 90, 150 and 80 μg equivalents at 3, 24, 48 and 96 hours, respectively). Residues in liver consisted mainly of aloe-emodin (60% free substance, up to 80% including conjugated aloe-emodin). In kidney the total aloe-emodin content ranged from 23 to 42% (4 to 14% free aloe-emodin). The percentage of rhein was higher in kidney than in liver. Some sex differences in metabolism were apparent: female rat liver oxidised and conjugated aloe-emodin more efficiently and a higher percentage of rhein with corresponding lower concentrations of conjugated rhein were found in female kidneys. Maximum residues in muscle were around 20 μg equivalents/kg at 3 and 6 hours after administration, declining to 4 μg equivalents/kg at 48 hours.

About 20 to 25% of the dose of 4.5 mg/kg bw free aloe-emodin (^{14}C -labelled) are absorbed and excreted in urine. Most of the urinary radioactivity was excreted within 12 hours (18%). No qualitative but some quantitative sex-related differences in the urinary metabolite pattern were seen. The amount of free aloe-emodin decreased over time in both sexes, but the percentage was consistently higher in females. Males excreted more rhein conjugates, whereas the amount of free rhein was comparable in both sexes. An unidentified metabolite was found in low concentrations (0.6% unconjugated, 1.1 to 1.3% in total) in urine. More than 75% of the dose was recovered in faeces, the major part within the first 48 hours. Small amounts of rhein and the unidentified metabolite were found while conjugates of aloe-emodin or rhein were not detected. Of the faecal radioactivity only 20 to 40% was extractable with methanol and mainly due to free aloe-emodin. The nature of the non-extractable residues is not known.

Information on the excretion into milk is not available for Aloes, however excretion of rhein, a metabolite of aloe-emodin, into breast milk of humans has been reported (median value: 0.007% of the dose resulting from daily intake of 5 g of a standardised senna-based laxative).

5. No data on the acute toxicity of Aloes were provided. Acute toxic properties of Aloes are mainly an excessive laxative effect, nephritis and discoloration of the urine.

For aloin an LD₅₀ of 260 mg/kg bw, an LD₁₀₀ of 440 mg/kg bw and a maximum tolerated dose of 50 mg/kg bw were observed in mice after intramuscular administration. For emodin, an anthranoid constituent of numerous plant species of the genus *Rhamnus* and structurally similar to aloe-emodin, an intraperitoneal LD₅₀ of 35 mg/kg bw has been reported.

6. No data on the oral repeated dose toxicity of Aloes or its constituents were provided.

A summary of repeated dose toxicity studies with emodin, an anthranoid constituent of numerous plant species of the genus *Rhamnus* and structurally similar to aloe-emodin, in rats and mice conducted under the National Toxicology Program of the United States of America was available. Emodin was given in the feed for 16 days or 14 weeks. Observed effect included reduced feed consumption, body weight gain and final body weight, changed haematological parameters in the 14-week study at the high doses and macroscopic kidney and/or gallbladder lesions in the 16-day study. In rats, no effects were observed at dietary doses up to 2000 mg/kg feed (equivalent to approximately 160 mg/kg bw) for 16 days, while in the 14-week study effects were observed at all doses (hyaline droplets in kidney of all treated males, doses ranging from 312.5 to 5000 mg emodin/kg feed). In mice in the 16-day study no effects were recorded up to 2000 mg/kg feed (390 and 540 mg/kg bw in males and females respectively), while in the 14-week study no effects were reported at the lowest dose of 312.5 mg/kg feed (equivalent to 50 and 60 mg/kg bw, respectively).

7. No information on the mutagenicity and carcinogenicity of Aloes has been provided. However, anthraquinone and a number of its derivatives are genotoxic and carcinogenic. The anthranoid laxative danthron (1,8-dihydroxyanthraquinone) has been found to induce malignant tumours in several animal species.

Aloe-emodin was studied in a number of *in vitro* and *in vivo* test systems. Dose-dependent mutations were induced in *Salmonella typhimurium* strains TA 1537, TA 1538 and TA 98 in the absence of, and in TA1538 in the presence of metabolic activation, by a second research group also in TA 1537 in presence of metabolic activation. Metabolic activation reduced the effect observed in strains TA98, TA1538 and TA1978 without. Regarding the induction of gene mutations at the HPRT-locus in Chinese hamster V79 cells one group reports no gene mutations without metabolic activation up to cytotoxic concentrations (numerical values not given). However, in 1 experiment slightly higher mutation frequencies were observed at 10 µg/ml without metabolic activation and at 100 µg/ml with metabolic activation. The findings could not be reproduced in 2 other independent tests and thus overall aloe-emodin was considered not mutagenic in this test system. A second group reports gene mutations (2 to 3 times the background levels) in 2 experiments in the same test system at concentrations from 5 to 30 µg/ml. Aloe-emodin induced structural chromosome aberrations in Chinese hamster ovary (CHO) cells with (37.5 to 75 µg/ml) and without metabolic activation (18.75 to 75 µg/ml), the full range of concentrations not being stated. Aloe-emodin is a weak inducer of unscheduled DNA synthesis in primary rat hepatocytes (test concentrations 6.5 to 100 µg/ml). The mutagenic effects were however not expressed *in vivo* in a micronucleus test in mice (oral dose of 1500 mg/kg bw), in an *in vivo* cytogenetic analysis in the bone marrow of rats at oral doses up to 2000 mg/kg bw, in a mouse spot test at oral doses of 200 mg/kg bw and 2000 mg/kg bw and in a test for induction of unscheduled DNA synthesis in rat hepatocytes after oral doses of 100 and 1000 mg/kg bw. Blood levels of aloe-emodin in rodents after oral administration of 2000 mg/kg bw were in the same range as concentrations causing *in vitro* mutagenicity.

Emodin, an anthranoid constituent of numerous plant species of the genus *Rhamnus* and structurally similar to aloe-emodin, gave some positive results in genotoxicity tests. Emodin was mutagenic in *Salmonella typhimurium* strain TA 1537 after metabolic activation, but not in strains TA 98, TA 100, and TA 1535. In a second *Salmonella*-microsomal assay mutations were seen in strain TA 100 with metabolic activation, but not in strain TA 98 with and without or in TA 100 without metabolic activation. Chromosomal aberrations were observed in Chinese hamster ovary cells *in vitro* with and without metabolic activation. *In vivo* micronucleus tests in rat and mouse bone marrow and in mouse peripheral blood after intraperitoneal injection and in male mice in peripheral blood after oral administration were negative, while weakly positive results were seen in female mice after oral administration of the substance.

8. Limited information was available on the carcinogenic effects of aloin. Dietary administration of aloin at a concentration of 300 mg/kg feed for 20 weeks (dose per kilogram bodyweight not stated) did not promote dimethylhydrazine induced colorectal cancer in male mice.

The carcinogenicity of emodin, an anthranoid constituent of numerous plant species of the genus *Rhamnus* and structurally similar to aloe-emodin, was studied in F344/N rats and B6C3F1 mice after administration of the substance in the feed for 2 years under the National Toxicology Program of the United States of America. There was no evidence of carcinogenic activity of emodin in male rats exposed to 280, 830 or 2500 mg/kg feed, while in female rats the evidence was considered equivocal based on a marginal increase in the incidence of Zymbal's gland carcinoma. In male mice the evidence was also considered equivocal based on a low incidence of uncommon renal tubule neoplasms, while in female mice there was no evidence of carcinogenic effects of emodin up to the highest dose tested, i.e. at dietary concentrations of 312, 625 and 1250 mg/kg feed.

Aloe-emodin is reported to have anti-leucaemic properties in mice, however only an abstract of the report was available.

9. Despite the carcinogenic properties of danthron and the suspected carcinogenic activity of other 1,8-dihydroxy-anthraquinones, the use of the substances as laxatives in human medicine has not been prohibited, it was only restricted to short-term use. The reason was that no carcinogenic effects are expected after short-term administration. The *in vitro* mutagenic activity observed for aloe-emodin was not expressed in *in vivo* test systems. For emodin, a substance structurally and pharmacologically related to the main active principle of Aloes, aloe-emodin, no or equivocal evidence for carcinogenic activity was found in animal experiments. For both emodin and aloe-emodin also anti-leucaemic effects have been reported. Considering the very limited use of *Aloes* in veterinary medicine (very infrequent, short-term treatment of individual animals only) and the limited absorption of the active principles after oral administration, the risk from veterinary use of *Aloes* to the consumer of foodstuffs of animal origin is considered negligible.
10. No information on residues in edible tissues and their depletion following treatment of target animals was provided, however the data in rats show that the constituents of major toxicological concern undergo only limited absorption from the gastrointestinal tract after oral administration.

Conclusions and recommendation

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

- Aloes is used in a small number of individual animals only, for infrequent or non-regular treatments,
- the animals treated with Aloes are unlikely to be sent for slaughter during or immediately after treatment,
- the constituents of toxicological concern of Aloes, barbaloin, is poorly absorbed from the intestine,
- barbaloin, the major constituent of concern to the consumer, is to a limited degree naturally present in the human diet;

the Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for Aloes and recommends its inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Aloes, Barbados and Cape, their standardised dry extract and preparations thereof	All food producing species	