

22 November 2016 EMA/HMPC/759585/2015 Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Aloe barbadensis* Mill. and on *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	Aloe barbadensis Mill. (barbados aloes) Aloe [various species, mainly Aloe ferox Mill. and its hybrids] (cape aloes), folii succus siccatus (dried juice of the leaves)
Herbal preparation(s)	Dry extract (DER 1-3:1), extraction solvent: water, standardised to contain 28.6 -36.6% hydroxyanthracene derivatives, calculated as aloin (photometric method)
Pharmaceutical form(s)	Herbal substance for oral preparation
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Based on Article 10a of Directive 2001/83/EC (well-established use)



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Table of Contents

Table of Contents	. 2
1. Introduction	.4
1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof.	.4
1.2. Search and assessment methodology	.5
2. Data on medicinal use	.6
2.1. Information about products on the market	.6
2.1.1. Information about products on the market in the EU/EEA Member States	.6
2.2. Overall conclusions on medicinal use	10
3 Non-Clinical Data	11
3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal	••
preparation(s) and relevant constituents thereof	11
3.1.1. Primary pharmacodynamics	11
3.1.2. Secondary pharmacodynamics	18
3.1.3. Safety pharmacology	20
3.1.4. Pharmacodynamic interactions	20
3.2. Overview of available pharmakokinetic data regarding the herbal substance(s), herbal	
preparation(s) and relevant constituents thereof	21
3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal	າາ
3 3 1 Single dose toxicity	23 23
3.3.2. Reneat dose toxicity	23
3.3.3 Genotoxicity	25
3.3.4. Carcinogenicity	28
3.3.5. Reproductive and developmental toxicity	32
3.3.6. Local tolerance	34
3.3.7. Other special studies	34
3.3.8. Conclusions	34
3.4. Overall conclusions on non-clinical data	34
4. Clinical Data	35
4.1. Clinical pharmacology	35
4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s including data on relevant constituents	s) 35
4.1.2. Overview of pharmakokinetic data regarding the herbal substance(s)/preparation(s)	
including data on relevant constituents	35
4.2. Clinical efficacy	36
4.2.1. Dose response studies	36
4.2.2. Clinical studies (case studies and clinical trials)	37
4.3. Clinical studies in special populations (e.g. elderly and children)	48
4.4. Overall conclusions on clinical pharmacology and efficacy	48
5. Clinical Safety/Pharmacovigilance	49
5.1. Overview of toxicological/safety data from clinical trials in humans	49
5.2. Patient exposure	51
5.3. Adverse events, serious adverse events and deaths	51

5.4. Laboratory findings	. 51
5.5. Safety in special populations and situations	. 51
5.5.1. Use in children and adolescents	. 52
5.5.2. Contraindications	. 52
5.5.3. Special warnings and precautions for use	. 52
5.5.4. Drug interactions and other forms of interaction	. 53
5.5.5. Fertility, pregnancy and lactation	. 53
5.5.6. Overdose	. 53
5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability	. 54
5.5.8. Safety in other special situations	. 54
5.6. Overall conclusions on clinical safety	. 55
6. Overall conclusions (benefit-risk assessment)	55
Annex	56
List of references	. 56

1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

Herbal substance(s)

This assessment report reviews the scientific data available for the dried juice of the leaves of *Aloe vera* (L.) Burm. f., known also as *Aloe barbadensis* Mill., (barbados aloes), or of *Aloe ferox* Mill. and its hybrids (cape aloes).

The word "Aloe" in pharmacopoeias and formularies refers to the herbal substance derived from the dried leaf juice. This has always created confusion due to the fact that the leaves are the source of two products "aloe dried juice" and "aloe gel", which are quite different in their chemical composition and their therapeutic properties.

This assessment report does not cover aloe gel, for which a medicinal use is not considered evident (Reynolds and Dweck, 1999; Radha and Laxmipriya, 2014).

Herbal preparations are used worldwide and it is belonging to the most popular medicinal plants of the world. Accordingly, aloe has been included into collections of monographs. The most important amongst them are:

European Pharmacopeia – Aloes, Barbados

Barbados aloes consists of the concentrated and dried juice of the leaves of *Aloe barbadensis* Mill. It contains not less than 28% of hydroxyanthracene derivatives (HAD) expressed as barbaloin $(C_{21}H_{22}O_9; Mr 418.4)$ and calculated with reference to the dried herbal substance. The material complies with the European Pharmacopoeia monograph "Aloes, Barbados" (0257).

European Pharmacopeia – Aloes, Cape

Cape aloes consists of the concentrated and dried juice of the leaves of various species of Aloe, mainly *Aloe ferox* Mill. and its hybrids. It contains not less than 18% hydroxyanthracene derivatives, expressed as barbaloin ($C_{21}H_{22}O_9$; Mr 418.4) and calculated with reference to the dried herbal substance. The material complies with the European Pharmacopoeia monograph "Aloes, Cape" (0258).

European Pharmacopeia – Aloes dry extract, standardised

Aloes dry extract, standardised, is prepared from Barbados aloes (0257) or Cape aloes (0258), or a mixture of both. The content of hydroxyanthracene derivatives is 19.0 % to 21.0 %, expressed as barbaloin ($C_{21}H_{22}O_9$; M_r 418.4), adjusted if necessary (dried extract). The extract is produced from the herbal drug by a suitable procedure using boiling water.

Further monographs established on Aloe preparations:

United States Pharmacopeia: Aloe, USP 38, NF 33, Vol 2, p 2096

Japanese Pharmacopeia XV: Powdered Aloe, p 1255

WHO Monographs on Selected Medicinal Plants, 1999

- Aloe, Vol 1

Assessment report on *Aloe barbadensis* Mill. and on *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus EMA/HMPC/759585/2015

- Aloe Vera Gel Vol 1

German commission E, BAnZ 133 since 21.7.1993

ESCOP Monographs, 2. Ed., Supplement 2009, p 6

- Aloe barbadensis

British Herbal Pharmacopeia, 1990, Vol 1

- Aloes, barbados; Aloes, cape

British Herbal Compendium, 1992, Vol 1

- Aloes, Barbados; Aloes, cape

The constituents with known therapeutic activity of barbados aloes are anthrone-10-C-glycosides viz. a mixture of aloin A (10S,1'S) and aloin B (10R,1'S), named barbaloin and their 6'-0-p-coumaroylesters, a mixture of 7-hydroxyaloins A (10S) and B (10R) and their 6'-0-p-coumaroylesters and a mixture of 8-0-methyl-7-hydroxyaloins A (10S) and B (10R) and their 6'-0-cinnamoylesters.

The constituents with known therapeutic activity of cape aloes are 10-C-glycosides viz. a mixture of aloins A (10S) and B (10R), named barbaloin, and 5-hydroxyaloin A (10S) besides 10-C-11-0-diglycosides viz. aloinosides A and B (11-0- α -L-rhamnosides of aloins A and B).

There are also small quantities in both aloes of the aglyka, aloe-emodin and chrysophanol, and 2alkylchromones named aloeresins (WHO monograph Aloe, 1999; Blaschek *et al.*, 2003).

• Herbal preparation(s)

The plant material of interest here is aloe dried juice, which is prepared by cutting transversely the leaf near the base and taking it inclined so that the juice contained in the specialised pericyclic cells and sometimes in the adjacent parenchyma flow out in about 6 h. The juice is allowed to dry with or without the aid of heat.

Aloe gel is a colourless mucilaginous gel obtained from the parenchymatous cells of the leaves. The mucilaginous parenchymous tissue is excised from fresh leaves. Therefore the leaves are "filleted", that means that the external green parts of the leaves are peeled. Depending on whether the anthranoid-containing cells beneath are also removed, the gel is free of anthranoids or not. The fillets are immediately utilised for preparations or lyophilised and kept dry until use.

This assessment refers to the dried juice (Aloes folii succus siccatus) but does not cover Aloe gel.

 Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable

1.2. Search and assessment methodology

Literature search was done via PubMed, DIMDI and SciFinder in medical and scientific databases as MEDLINE, National Centre for Biotechnology Information (NCBI), Cochrane Database of Systematic Reviews TOXLINE (date of search: March 2015). For the unqualified terms (Aloe, Aloe Barbadensis

clinical trials; aloe and in vitro; anthroquinones and in vitro, aloe-emodin and in vitro, aloe and in vivo; anthroquinones and in vivo, aloe-emodin and in vivo, aloe and preclin*; aloe, aloe-emodin hydroxyanthracen* and safety; aloe, aloe-emodin hydroxyanthracen* and the different indications) as text words in the title, abstract, and full journal article. The search strategy included the terms for aloe, and terms for the specific diseases or conditions derived from its traditional use and current indications, supplemented with those expected from non-clinical studies with aloe. In addition to the PubMed and SciFinder literature search, bibliographies of review articles and eligible articles were examined in an effort to identify all available literature that may not have been identified by the database research. The search was limited to English, French, Spanish and German language papers. Randomised studies that used combination products with aloe as one of its ingredients are not included.

Search engines used: Google

Scientific databases: PubMed, DIMDI, SciFinder

Medical databases: MEDLine, Cochrane Database of Systematic Reviews, EMBASE, BioMed Central

Toxicological databases: ToxLine

Pharmacovigilance resources: Vigilance central

Data from EU and non-EU regulatory authorities: WHO Monograph; NTP Technical Reports on Aloe vera whole leaf extract and Emodin

Other resources: Historical literature according to list of references

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of date	ta obtained from marketed	medicinal products	
Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status
Dry extract from aloe	For short-term use in	coated tablet	WEU since 1978, GER
(1.8-2.2:1) corresponding 15 mg hydroxyanthracene derivatives (HAD), calculated as aloin; extraction solvent water	constipation	41.25 – 52.5 mg dry extract/tablet oral use	
Dry extract from aloe	For short-term use in	coated tablet	WEU since 2010, GER

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status
(1.8-2.2:1) corresponding 10 mg HAD, calculated as aloin, extraction solvent water	constipation occurring occasionally.	27.5 – 35.0 mg dry extract/tablet oral use	
Dry extract from aloe (1-3:1) corresponding 10 mg HAD, calculated as aloin; extraction solvent water	For short-term use in constipation occurring occasionally.	granulate 2.5 g containing 50 mg dry extract oral use	WEU since 2013, GER

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Information on relevant combination medicinal products marketed in the EU/EEA

Not applicable

Information on other products marketed in the EU/EEA (where relevant)

Not applicable

2.1.2. Information on products on the market outside the EU/EEA

Assessed

2.2. Information on documented medicinal use and historical data from literature

In the available data on traditional use, it is not always possible to differentiate the various aloe preparations that are mentioned because of insufficient description/declaration of active ingredients. It is therefore not possible to attribute special traditional indications to defined aloe preparations, although it seems to be likely that aloe gel or similar preparations and not aloe dried juice were externally used.

Because of this lack of information, this section covers all traditional indications reported for "aloe" altogether.

Aloe has a long history as a medicine and skin care aid. For over 6,000 years aloe was used for a wide range of ailments. The ancient Egyptians used aloe to heal battle wounds and cure infections. The early Greeks used it for relieving blisters, burns and leg ulcers as well as bowel and stomach disorders. Legend is that Aristotle persuaded Alexander the Great to conquer the Isle of Socroto to secure enough *Aloe vera* to heal his soldiers' wounds. In England aloe was already used in the 10th century. In the 12th century aloe was brought to Germany by Albertus Magnus (Madaus, 1938).

Assessment report on *Aloe barbadensis* Mill. and on *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus EMA/HMPC/759585/2015

Dioskurides (translation: Berendes, 1902) already mentioned aloe in his materia medica 50-70 A.D.: Aloe is a purgative for the stomach and abdomen and in jaundice. It represses haemoptysis. Aloe administered externally heals wounds and ulcers as well as diseases of the eyes. It is good for headache administered with vinegar and rose ointment on the forehead and temples. Together with vine it slows up hair loss. For inflammation of the mouth and tonsils, it is mixed with honey and vine.

The British Pharmaceutical Codex (1911) mentions aloe as a purgative: because the action of aloe on the large intestine induces some pelvic congestion, it is therefore employed as an emmenagogue in amenorrhoea, generally with iron. The recommended dose is 1 to 3 decigrams (2 to 5 grains).

In the Dispensatory of the United States of America 1918 aloe is mentioned as a cathartic (purgative). It is also described that it was formerly almost universally believed that aloe possessed emmenagogue properties and it was accordingly largely used in the treatment of various forms of amenorrhea, but that it is extremely doubtful whether aloe exercises any action upon the pelvic organs which is not attributable to its cathartic effects (Remington & Wood, 1918).

In his "Manual of Materia Medica and Pharmacology", Culbreth (1927) mentions as indications constipation, atonic dyspepsia, jaundice, non-active haemorrhoids, amenorrhea, ascarides; for the two last may be given by enema.

Hager (1925) mentions the use as an appetising agent in low doses (0.05 - 0.1 g) and as a cathartic in higher doses (0.2 - 1.0 g). The use in menstruation, pregnancy and bleeding haemorrhoids is not recommended because of congestion of the pelvis organs. The use as a clysma and as an eye ointment or powder is not very common (Frerichs et al. 1925).

Madaus (1938) gives a review of the use of aloe as discribed above. Paracelsus already indicated the use as a purgative and as vesicant for an abscess. Lonicerus (1564) described the purgative, emmenagogue and expectorant effects. Aloe was used for haemoptysis, jaundice, hydrops and as a vermifuge. Externally aloe was used for headache, wounds, abscess and bleeding haemorrhoids.

Matthiolus (1626) added the use against hair loss. Hecker (1814) confirmed the indications mentioned above. Additionally he described the use in gout and external use in inflammations of the eyes. In Estonia aloe was sometimes used for tuberculosis.

Koenen (1977) described the use of "*Aloe hereroensis* Engler" in South Africa. An aqueous extract was used for complains of digestion and for pectoral complains.

Furthermore the South Africans used aloe for syphilis and for inflammations of the eyes (Watt *et al.*, 1962).

The WHO monograph "Aloe" mentions the following uses described in folk medicine, not supported by experimental or clinical data: treatment of seborrhoeic dermatitis, peptic ulcers, tuberculosis, fungal infections, and for reduction of blood sugar levels. The references given are not original sources and date from 1991 and 1995.

The accepted historical use of "Aloe" led to the establishment of German Commission E monograph and the European Scientific Cooperative on Phytotherapy (ESCOP) monograph "Aloe capensis (Cape Aloes)" (ESCOP, 2003). German pharmacovigilance actions have been published for anthranoidcontaining laxatives of 21 June 1996, which were intended to set a framework for safe use of hydroxyl anthracene derivatives containing herbal medicinal products (BfArM, 1996).

Table 2: Overview of historical data

Assessment report on *Aloe barbadensis* Mill. and on *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus EMA/HMPC/759585/2015

Herbal preparation	Documented use / Traditional use	Pharmaceutical form, Strength, Posology Duration of use	Reference
Curacao-Aloe, from concentrated dried juice of the leaves of Aloe barbadensis Miller, as well as their preparations in efficient posology. Extracts with water Aqueous-ethanolic dry extract -, Concentrated - and fluid extract as well as methanolic dry extracts	Obstipation	20-30 mg HAD /day, calculated as non- aqueous aloin The correct individual dose is the smallest amount required to produce a soft-formed stool.	European Commission monograph Aloe BAnz Nr 133, 21.7.1993
Cape aloes consists of the concentrated dried juice of the leaves various species of Aloe, mainly of <i>Aloe ferox</i> Mill. and its hybrids. It contains not less than 18% of HAD expressed as barbaloin and calculated with reference to the dried drug.	Short-term use of occasional constipation	The correct individual dose is the smallest amount required to produce a soft-formed stool. Adults and children over 10 years: preparations equivalent to 10-30 mg HAD to be taken once daily at night. The pharmaceutical form must allow lower dosages.	ESCOP Monograph Aloe Capensis, 2003
Aloe is the dried juice of the leaves of <i>Aloe vera</i> (L.) Burm. ferox or of <i>Aloe</i> <i>ferox</i> Mill. and its hybrids with <i>Aloe africana</i> Mill. and <i>Aloe spicata</i> Baker (Liliaceae).	Short-term treatment of occasional constipation	The correct individual dose is the smallest amount required to produce a soft-formed stool. As a laxative for adults and children over 10 years old, 0.04–0.11 g (Curacao or Barbados Aloe) or 0.06–0.17 g (Cape Aloe) of the dried juice, corresponding to 10–30mg hydroxyanthraqui- nones per day, or 0.1 g as a single dose in the evening.	WHO Monographs on Selected Medicinal Plants - Volume 1, 1999

Assessment report on *Aloe barbadensis* Mill. and on *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus EMA/HMPC/759585/2015

2.3. Overall conclusions on medicinal use

For over 6,000 years aloe was used for a wide range of ailments.

Besides the most important use as a laxative, the use as an emmenagogue and the external use for wounds and abscess are described in most references mentioned above, however the preparations used are not well defined.

As already mentioned in the Dispensatory of the United States of America 1918, it is extremely doubtful whether aloe exercises any action upon the pelvic organs which is not attributable to its cathartic effects (Remington & Wood, 1918). There are no plausible pharmacological data for this indication, nor for haemoptysis, jaundice or gout, etc.

Concerning the external use, the references do not describe exactly the preparations used.

Hydroxyanthracene derivatives are a group of natural constituents the pharmacologic action of which is accepted in literature. They are substantially contributing to the therapeutic activity as laxative medicinal products. Accordingly, herbal medicinal products have to be standardized to the content of hydroxyanthracene derivatives, for aloe preparations content is usually standardized to barbaloin. Based on existing marketing authorizations and with respect to an acceptable level of safety (see later sections) the well-established use can be attributed.

For other medicinal uses mentioned the requirements of well-established use are not fulfilled. With respect to traditional use there are no defined preparation for external use, in other cases the traditional use is not plausible (see later sections).

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
Dry extract from aloe (1.8-2.2:1) corresponding 15 mg HAD, calculated as aloin extraction solvent water	Herbal medicinal product for short- term use in cases of constipation.	Adults and children > 12 years: 1-2 coated tablets containing 41.25 – 52.5 mg dry extract (corresponding to 15-30 mg HAD calculated as aloin)	WEU since 1978, GER
Dry extract from aloe (1.8-2.2:1) corresponding 10 mg HAD, calculated as aloin extraction solvent water	Herbal medicinal product for short- term use in cases of occasional constipation.	Adults and children > 12 years: 1-3 coated tablets containing 27.5- 35.0 mg dry extract (corresponding to 10-30 mg HAD calculated as aloin)	WEU since 2010, GER
Dry extract from aloe	Herbal medicinal product for short-	Adults and children > 12 years:	WEU since 2013, GER

Table 3: Overview of evidence on period of medicinal use

Assessment report on *Aloe barbadensis* Mill. and on *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus EMA/HMPC/759585/2015

Herbal preparation	Indication	Posology,	Period of medicinal
Pharmaceutical form		Strength	use
(1-3:1) corresponding 10 mg HAD, calculated as aloin extraction solvent water	term use in cases of occasional constipation.	 ½ - 1 1/2 dosing spoons of granulate 2-3 times a week, 50.0 mg dry extract (corresponding to 10 mg HAD) 	

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

3.1.1. Primary pharmacodynamics

Data on the herbal substance

Nine hours after oral administration to rats, aloe produced diarrhoea in 20% rats at doses of 5 g/kg bw and in 100% rats at doses of 20 g/kg bw. Lower doses of aloe (0.1 and 1.0 g/kg) did not produce a diarrhoeal response. Pre-treatment (i.p.) of rats with a NO synthase inhibitor (NG-nitro-L-arginine methyl ester) reduced the diarrhoea induced by aloe (20 g/kg)) 9 h after its oral administration. The increase in faecal water excretion was also reduced. L-arginine administered to rats pre-treated with the NO synthase inhibitor, drastically reduced the effect of this inhibitor. Given alone, L-arginine did not modify aloe-induced diarrhoea. Basal $Ca^{2\pm}$ dependent NO synthase activity in the rat colon was dose-dependently inhibited by aloe (0.1 – 20.0 g/kg bw) and by aloin (0.1 – 1.0 g/kg bw), the active ingredient of aloe. The authors concluded that these results suggest that endogenous NO modulates the diarrhoeal effect of aloe (Izzo *et al.*, 1999).

Data on herbal preparations

Investigations of Izzo *et al.* suggested that nitric oxide (NO) is a possible mediator for the laxatives effect of anthranoid-containing products; in 1996 (Izzo *et al.*, 1996) and 1997 (Izzo *et al.*, 1997) they investigated the role of NO in senna- and cascara-induced diarrhoea in the rat, and in 1999 (Izzo *et al.*, 1999) the role of NO in aloe-induced diarrhoea in the rat.

In the study of Wintola *et al.* (2010), the efficacy of an aqueous total leaf extract of *Aloe ferox* Mill. the dried leaves were grinded into powder and 100 g of the material was extracted by shaking for 24 h in 1000 ml of distilled water, filtered freeze dried and reconstituted in water (ratio not defined) was studied against loperamide-induced constipation in Wistar rats. Constipation was induced by oral administration of loperamide (3 mg/kg body weight) while the control rats received normal saline. The constipated rats were treated with 50, 100 and 200 mg/kg body weight/day of the extract for 7 days during which the feeding characteristics, body weight, faecal properties and gastrointestinal transit ratio were monitored. The extract improved intestinal motility, increased faecal volume and normalized body weight in the constipated rats.

The study of Ashafa *et al.* (2011) evaluated the effect of an ethanolic total leaf extract of *Aloe vera* against loperamide-induced constipation in rats. The leaves of *Aloe vera* were thoroughly washed with

Assessment report on *Aloe barbadensis* Mill. and on *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus EMA/HMPC/759585/2015

distilled water, cut into thin slices, and air-dried at room temperature to a constant weight. The dried leaves were grinded into a powder and 40 g of the material was extracted by shaking it for 24 h in 500 ml of ethanol. The extract obtained was filtered through Whatman no. 1 (70 mm) filter paper and concentrated on a water bath at 45°C to give a yield of 5.2 g (hydroxyanthracene content (HAD) unknown). This was reconstituted in distilled water to give the required doses of 50, 100, and 200 mg/kg body weight for the experiment (relation not known). Rats were constipation induced by the oral administration of loperamide (3 mg/kg body weight) while the control animals received normal saline. Constipated rats were treated with 50, 100, and 200 mg/kg body weight, faecal properties, and gastrointestinal transit ratio were monitored. Treatment of constipated rats with the extract at 50, 100, and 200 mg/kg body weight for 7 days improved intestinal motility, increased faecal volume, and normalized body weight in the constipated rats. These are indications of the laxative property of the herb with the 200 mg/kg body weight of the extract showing the best effect.

Assessor 's Comment:

Data on the hydroxyanthracene content are not reported. Extracts were obtained from total leaves. Nevertheless, data demonstrate a dose dependent pharmacodynamic effect in rats.

Data on hydroxyanthracene derivatives

Hydroxyanthracene derivatives are considered as prodrugs which are degraded to the therapeutically active form during intestine passage. A broad set of literature data is describing details of the generally acknowledged mode of action, which is explaining the therapeutic usage as laxatives.

Ishii *et al.* (1990) investigated the mechanism of action of aloe-emodin-9-anthrone in causing a significant increase in the water content of the rat large intestine. Aloe-emodin-9-anthrone inhibited rat colonic sodium/potassium-adenosine triphosphatase (Na+/K+-ATPase) *in vitro*, and increased the para-cellular permeability across the rat colonic mucosa *in vivo*. Therefore, it seemed that the increase in water content of the rat large intestine produced by aloe-emodin-9-anthrone was due to both inhibition of absorption and stimulation of secretion without stimulation of peristalsis. Since, pre-treatment with loperamide completely prevented the increase of para-cellular permeability induced by aloe-emodin-9-anthrone, but did not completely reduce the concomitant increase in residual fluid volume, other multiple mechanisms of action might be involved in the increase of water content in the rat large intestine.

Hoenig *et al.* (1992) and Rauwald *et al.* (1992) studied the influence of 23 anthraquinones and anthrones on the regulatory volume decrease (RVD) which is effected in Ehrlichs ascites tumour cells by activation of Cl⁻-channels. They showed that the inhibition of the Cl⁻-channels' activity was the strongest by aloe-emodin-anthrone and aloe-emodin. These anthraquinones reduce the Cl- permeability of the cells, this influence being sometimes more pronounced than that of the Cl- channel blocker 130B. In contrast to the investigations of Ishii Y. *et al.* (1990) both substances showed no pronounced inhibition activity of the Na⁺/K⁺-ATPase. Rhein, frangula-emodin and other anthraquinones with an additional phenolic hydroxyl group showed inhibition.

Results of investigations of Capasso *et al.* (1983) in rat isolated colon suggest that the laxative properties of aloin and 1,8-dioxyanthraquinone may depend, at least in part, on increased prostaglandin synthesis by the intestinal tissue.

Tavares *et al.* (1996) compared the effects of rhein and aloe-emodin with that of ricinoleic acid and calcium ionophore A23187 on platelet-activating factor (PAF) release by human gastrointestinal mucosal pieces *in vitro*. Ricinoleic acid and calcium ionophore stimulated release of PAF from human

Assessment report on *Aloe barbadensis* Mill. and on *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus EMA/HMPC/759585/2015

stomach, ileum or colon mucosa. Aloe-emodin (100 μ g/ml) stimulated a small release of PAF in ileum and colon mucosa. Rhein had no effect. 5-aminosalicylic acid (100 μ g/ml) inhibited PAF release induced by the drugs. The authors concluded that rhein exerted its laxative effects by a mechanism that did not involve PAF release, and that aloe-emodin may act partly via PAF release.

Izzo *et al.* (1998) reviewed the key features of the involvement of NO and PAF in the action of laxatives. PAF is a phospholipid mediator of inflammation and stimulates anion secretion in animals and in isolated preparations of human colon. NO, synthesised from the amino acid L-arginine, is an important enteric inhibitory neurotransmitter. In addition, NO-donating compounds stimulate anion secretion in rat and guinea-pig colon.

Oral administration of sennosides (20-30 mg/kg) to fasted dogs has been shown to induce a strong and long-lasting inhibition of myoelectric colon activity which was evident after a delay of 6-10 h corresponding to oro-caecal transit and colonic metabolism and was accompanied by abundant diarrhoea. When sennosides were given 1 h before a meal, the postprandial increase in colon motility failed to appear. Recent studies with strain gage transducers confirm the inhibition of colonic motility after oral sennosides but, in addition, 3-10 'giant contractions' with a high amplitude appeared during the period of inhibition. Most of these single contractions were propagated over the second half of the colon at a velocity of 0.5-2 cm/min. Elimination of liquid faeces was always associated with giant contractions. These giant contractions have also been described with other stimuli (i.v. guanethidine or neostigmine, oral castor oil, intraluminal hypertonic glucose) and are therefore not specific for sennosides (Fioramonti *et al.*, 1988)

Longo (2002) referred in his monograph "Aloe today" (part four) to recent investigations that produced a better explanation of the mechanism of the action of stimulus on the colon. The inhibition of Na+/K+-ATPase and the release of NO are relevant to stimulate the secretion of electrolytes and the relaxation of smooth intestinal muscles. Furthermore, investigations outlined that NO plays a new physiopathological role regarding PAF, which causes the contraction of the smooth musculature.

The authors summarized that biological factors such as PAF and NO may play a role on the action of the stimulus on the colon, but it should be considered that the investigations were only experimental ones.

Ishii *et al.* (1994) measured simultaneously in the same rat charcoal transport, as an indicator of the degree of peristalsis, and water content in the large intestine after intracaecal administration of barbaloin. Charcoal transport was significantly accelerated at both 3.5 and 6.5 hours after the administration of barbaloin. At 6.5 h, diarrhoea instead of normal faeces was observed. Moreover, at 1 h before the acceleration of charcoal transport, a marked increase in water content of the large intestine was observed: It appeared that the increase in water content of the large induced by barbaloin preceded the stimulation of peristalsis, attended by diarrhoea. The authors therefore suggested that the increase in water content is a more important factor than the stimulation of peristalsis in the diarrhoea induced by barbaloin.

Aloe-emodin anthrone and rhein anthrone, and their equimolar mixture, induced excretion of an approximately equal number of faeces by intracaecal administration at a dose of 23.2 mumol kg-1 in mice (= 1.0 standard dose). The number of wet faeces induced by aloe-emodin anthrone was less than those of rhein anthrone and the mixture. At the same dose, rhein anthrone and the mixture significantly stimulated large intestinal propulsion, though aloe-emodin anthrone had little stimulatory effect. Aloe-emodin anthrone and rhein anthrone decreased net water absorption but could not reverse it to the net secretion at 1/2 dose. The mixture significantly decreased net water absorption and reversed it to the net secretion at this dose. These anthrones did not stimulate mucus secretion in the

Assessment report on *Aloe barbadensis* Mill. and on *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus EMA/HMPC/759585/2015

colon at 1/2 dose. The authors concluded that the synergistic purgative effect of aloe-emodin anthrone and rhein anthrone in mice results from synergistic stimulation of large intestinal transit and large intestinal water secretion (Yagi *et al.*, 1997).

The exact mechanism of action is still unknown. Besides a direct influence of the motility leading to a reduced transit time, an influence on secretion processes by two concomitant mechanisms is assumed Especially inhibition of absorption of water and electrolytes (Na^+, CI^-) into the colonic epithelial cells (antiabsorptive effect) and increase of the leakiness of the tight junctions and stimulation of secretion of water and electrolytes into the lumen of the colon. The fluid absorption is reduced. This may lead to an increase of the intestine content and the intraluminal pressure, which indirectly stimulate the peristalsis.

Preclinical pharmacokinetic data on hydroxyanthracene derivatives

Some pharmacokinetic data are presented here to support the pharmacodynamic concept and metabolism for the laxative effect. For more details see section 3.2.

To determine the intestinal uptake and metabolism of physiologically active aloe components using *in vitro* intestinal absorption model was the objective of the following study. The Caco-2 cell monolayer and the everted gut sac were incubated with 5-50 microM of aloin, aloe-emodin, and aloesin. The basolateral appearance of test compounds and their glucuronosyl or sulfated forms were quantified using HPLC. The % absorption of aloin, aloe-emodin, and aloesin was ranged from 5.51% to 6.60%, 6.60% to 11.32%, and 7.61% to 13.64%, respectively. Up to 18.15%, 18.18%, and 38.86% of aloin, aloe-emodin, and aloesin, respectively, was absorbed as glucuronidated or sulfated form. These results suggest that a significant amount is transformed during absorption. The absorption rate of test compounds except aloesin was similar in two models; more aloesin was absorbed in the everted gut sac than in the Caco-2 monolayer. These results provide information to establish adequate intake level of aloe to maintain effective plasma level (Park *et al.*, 2009)

Pharmakokinetic studies by Lang (1993) after oral administration of ¹⁴ C-aloe-emodin to male and female rats showed that 20-30% of the dose was excreted in the urine and the rest was excreted in the faeces as rhein and conjugates. 10% of the radioactivity was identified as free aloe-emodin in the plasma with a maximum peak at 1.5-3.0 h after the administration. Only the liver, kidney and gastrointestinal tract showed higher concentrations than the plasma. The terminal half-life of the radioactivity in the blood was 50 hours.

In contrast Ishii *et al.*, showed that aloe-emodin-9-anthrone was produced in the rat large intestine (Ishii *et al.*, 1994, Ishii *et al.*, 1999). Aloe-emodin-9-anthrone is the main active metabolite, which acts specifically on the colon (Ishii *et al.*, 1990).

Administration of emodin to rabbits by i.v. bolus resulted in a serum profile which could be well described by a two-compartment model. The AUC of emodin was 518 micrograms min/ml, clearance was 72.3 ml/min, and elimination half-life was 227 min which was much longer than that reported in a previous study. Oral administration of emodin resulted in a very low serum concentration. Protein binding of emodin was investigated by the equilibrium dialysis method. Emodin was found to be highly bound (99.6%) to serum protein (Liang *et al.*, 1995)

Assessment report on *Aloe barbadensis* Mill. and on *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus EMA/HMPC/759585/2015

Table 4: Overview of the main non-clinical data/conclusions

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
	Comparable/similar prepa	rations to preparation	s of the monograph	1
Aqueous leaf extract of <i>Aloe</i> <i>ferox</i> Mill not standardized	50, 100 and 200 mg/kg body weight/day of the extract	Loperamide-induced constipation in Wistar rats	Wintola <i>et al.,</i> 2010	The extract improved intestinal motility, increased faecal volume and normalized body weight in the constipated rats.
	Ot	her preparations		
Aloe	0.1 and 1 g/kg; 5 g/kg; 20 g/kg oral administration	Rat	Izzo <i>et al.,</i> 1999	Pre-treatment (i.p.) of rats with a NO synthase inhibitor (NG- nitro-L-arginine methyl ester) reduced the diarrhoea induced by aloe (20 g/kg)
Ethanolic leaf extract of <i>Aloe vera</i>	50, 100, and 200 mg/kg bw	Loperamide-induced constipation in rats	Ashafa <i>et al.,</i> 2011	50, 100, and 200 mg/kg body weight for 7 days improved intestinal motility, increased faecal volume, and normalized body weight
	Hydroxy	anthracene derivative	S	•
Aloe-emodin-9-anthrone		In vitro In vivo Rat	Ishii <i>et al.</i> ,1990	Aloe-emodin-9-anthrone inhibited rat colonic sodium/potassium-adenosine triphosphatase (Na+/K+- ATPase) <i>in vitro</i> , and increased the paracellular permeability across the rat colonic mucosa <i>in vivo</i>

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Emodin	i.v. and oral	<i>In vivo</i> ; rabbit	Liang <i>et al.,</i> 1995	Oral administration of emodin resulted in a very low serum concentration
23 anthraquinones and anthrones		In vitro Regulatory volume decrease RVD reduction in Ehrlich Aszites Cells by activation of CI- channels	Hoenig <i>et al.</i> , 1992	The inhibition of the Cl- channels' activity was the strongest by aloe-emodin- anthrone and aloe-emodin
Barbaloin	Intracaecal	Rat charcoal transport, as an indicator of the degree of peristalsis, and water content in the large intestine after intracaecal administration of barbaloin	Ishii, 1994	Charcoal transport was significantly accelerated at both 3.5 and 6.5 h after the administration of barbaloin
¹⁴ C-aloe-emodin		Male and female rats	Lang <i>et al.</i> , 1993	20-30% of the dose was excreted in the urine and the rest was excreted in the faeces as rhein and conjugates
Sennosides	Oral	Fasted dogs	Fioramonti <i>et al.,</i> 1988	Inhibition of colonic motility after oral sennosides but, in

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
				addition, 3-10 'giant contractions' with a high amplitude appeared during the period of inhibition over the second half of the colon at a velocity of 0.5-2 cm/min.
Aloe-emodin anthrone and Rhein anthrone	Intracecal	Mice	Yagi <i>et al.,</i> 1997	Rhein anthrone and the mixture significantly stimulated large intestinal propulsion, though aloe-emodin anthrone had little stimulatory effect.

Assessor's Comment:

In vitro and in vivo studies in rats, mice and rabbits demonstrated that aloe-emodin-9-anthrone disturbed the equilibrium between the absorption of water from the intestinal lumen via inhibition of active sodium/potassium adenosine triphosphatase and increased the para-cellular permeability across the colonic mucosa (Ishii et al., 1990). It stimulated peristaltic activity in the large intestine, stimulated mucus secretion (Ishii et al., 1994) and led to secretion of water into the lumen by a prostaglandin-dependent mechanism (Capasso et al., 1983). The result was a net reduction in water absorption and more frequent stools with softer consistency. Studies with strain gage transducers confirmed the inhibition of colonic motility after oral sennosides but, in addition, 3-10 'giant contractions' with a high amplitude appeared during the period of inhibition (Fioramonti et al., 1988).

3.1.2. Secondary pharmacodynamics

There are many experimental investigations, which study several effects of different ingredients of aloe. The part of the plant, from which the ingredients were isolated, has not always been defined exactly. Investigations of the gel or of substances isolated from the gel are not mentioned in the assessment report.

Cytotoxic effects of aloe-emodin

Data on hydroxyanthracene derivatives

Grimaudo *et al.* (1997) studied the antitumour effects of 5 purified compounds from the plant *Aloe vera* on human K562 leukaemia cells and on the multidrug resistant (MDR) variant cell line, K562/R. The glycosides aloin A and B, aloesin and aloeresin were devoid of antitumour activity up to 200 μ M concentrations. Only the aglycon aloe-emodin produced reproducible cytotoxic effects. Aloe-emodin caused mainly cytostasis and accumulation of the cells in the S and G2-M phases of the cell cycle during the first 48 h of treatment. Thereafter, massive cell death ensued.

Fenig *et al.* (2004) conducted a study to determinate if members of the anthraquinone family could be used as adjuncts to increase the growth inhibiting effect of anticancer agents in Merkel cell carcinoma (MCC). An adherent variant of MCC was derived from a previously established MCC cell line suspension. Emodin and aloe-emodin inhibited proliferation of the adherent MCC cells, with a slight advantage of aloe-emodin over emodin. Aloin had no effect on cell proliferation. The chemotherapeutic agents, cis-platinol (abiplastin), doxorubicin (adriablastin), and 5-fluorouracil, and the tyrosine kinase inhibitor STI 571, all independently inhibited the proliferation of adherent MCC cells. The addition of aloe-emodin potentiated their inhibitory effect, especially when low concentrations of the anticancer compounds were used.

Chen *et al.* (2004) evaluated the chemo preventive role of aloe-emodin in human promyelocytic leukaemia HL-60 cells *in vitro* by studying the regulation of proliferation, cell cycle and apoptosis. The authors concluded that aloe-emodin appears to exert its anti-carcinogenesis properties by inhibiting proliferation and inducing cell cycle arrest, and apoptosis underwent activation of caspase-3 in human leukemia HL-60 cells.

Lee *et al.* (2001, 2005) demonstrated that aloe-emodin induced a significant change in the expression of lung cancer cell apoptosis-related proteins compared to those of control cells.

In order to assess the role of oxidative stress in the cytotoxicity of natural hydroxyanthraquinones, the authors compared rhein, emodin, danthron, chrysophanol, and carminic acid, and a series of model quinones with available values of single-electron reduction midpoint potential at pH 7.0 (E(1)7), with respect to their reactivity in the single-electron enzymatic reduction, and their mammalian cell toxicity.

The toxicity of model quinones to the bovine leukemia virus-transformed lamb kidney fibroblasts (line FLK), and HL-60, a human promyelocytic leukemia cell line, increased with an increase in their E(1)7. A close parallelism was found between the reactivity of hydroxyanthraquinones and model quinones with single-electron transferring flavoenzymes ferredoxin: NADP+ reductase and NADPH: cytochrome P450 reductase, and their cytotoxicity. This points to the importance of oxidative stress in the toxicity of hydroxyanthraquinones in these cell lines, which was further evidenced by the protective effects of desferrioxamine and the antioxidant N,N'-diphenyl-p-phenylene diamine, by the potentiating effects of 1,3-bis-(2-chloroethyl)-1-nitrosourea, and an increase in lipid peroxidation (Nemeikaite-Ceniene *et al.*, 2002).

Anti-inflammatory effect

In investigations using the contact hypersensitivity response Yagi *et al.* (2003) showed a preventive effect of aloesin, isolated from Aloe species, on the UV-B-induced immune suppression. Furthermore, aloesin inhibited tyrosine hydroxylase and dihydroxyphenylalanine (DOPA) oxidase activities of tyrosinase from normal human melanocyte cell lysates. Therefore the authors regard this substance as a positive pigment-altering agent.

Antibacterial effect

Data on hydroxyanthracene derivatives

The phenolics and aloins of *Aloe vera* were found to have dose-dependent non-competitive inhibitory effects on *Clostridium histolyticum* metalloproteinases and collagenases (Barrantes and Guinea, 2003).

Data on herbal preparations

Lorenzetti *et al.* (1964) showed a bacteriostatic effect of the "juice of the leaves" on *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium xerose* and *Salmonella paratyphi*. Aloe-emodin and chrysophanol were ineffective.

Another investigation by Liu *et al.* (1996) showed potent antibacterial activity of aloe-emodin (source not mentioned) against methicillin-resistant *Staphylococcus aureus*.

Antifungal effect

Data on herbal preparations

Shamim *et al.* (2004) evaluated the activity of *Aloe barbadensis* Miller against some common fungal species associated with superficial mycoses. The ethanol and aqueous extracts it were tested to establish the antimycological effects against dermatophytes, saprophytes, and Candida species isolated from infected hospitalised patients. The *in vitro* antifungal activity was established by observing and measuring the zones of inhibition formed on selective nutrient media. Zones of inhibition were categorised as very high (41-50 mm), high (31-40 mm), medium (21-30 mm), and low (11-20 mm). High zones of inhibition were noted with the ethanol extract.

Modulation of immune response

Data on herbal preparations

In a study the specific and non-specific immune response of BALB/c mice, healthy and immunosuppressed with murine lymphoma L5178Y, treated with bitter yellow juice (extract) of *Aloe vera* (L) were investigated. It was observed that the immunosuppressed mice, treated with the whole extract of the bitter yellow juice achieved restoration of immunological parameters in cellular immune response and phagocytosis. On the other hand, the humoral immunity was not restored. Also, in the

healthy rodents treated with the extract, it caused the stimulation of specific and non-specific responses, the results had significant differences with the obtained ones in untreated mice (Oronzo-Barocio *et al.*, 1999).

3.1.3. Safety pharmacology

Data on herbal preparations

Wintola *et al.* evaluated in 2011 the toxicological effect of aqueous leaf extract of the herb at 50, 100 and 200 mg/kg body weight for 7 days on the haematological parameters as well as liver and kidney function indices in loperamide-induced constipated rats. The extract did not cause any significant (p > 0.05) effect on the kidney and liver-body weight ratio as well as the kidney function indices including serum levels of creatinine, uric acid, urea, calcium and potassium ions at all the dosages investigated. Whereas the serum levels of total protein, albumin, bilirubin and gamma glutamyl transferase (GGT) were not affected, the elevated activities of alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) in the untreated constipated animals were normalized following treatment with extract. The data obtained with respect to the haematological analysis indicated that the extracts had no significant (p > 0.05) effect on the haematological parameters with the exception of lymphocyte count which was increased in the untreated constipated rats. This was attenuated after administering the herb.

Aloe has also been reported to have an effect on liver function. The cytoprotective effect of aloe extract against 1,4-naphthoguinone-induced hepatotoxicity was evaluated in primary cultured rat hepatocytes. After exposure to 1,4-naphthoquinone (100 microM), a decrease in cell viability measured as >60% lactate dehydrogenase depletion was induced. Cellular glutathione (GSH) and protein-SH levels were also significantly decreased in a time-dependent manner. 1 g Aloe powder (a mixture A. ferox Miller, A. africana Miller, A. spicata Baker) mixed with 20 parts of 50% ethanol for 24 h; centrifugation 11,400xg for 10 min; ethanol evaporated; residue freeze dried; then 10 parts of water were added; mixing for 3 h; centrifugation 11,400xg for 10 min; supernatant freeze dried to yield the 50% ethanol fraction; the 50% ethanol fraction suspended in water, extracted with chloroform; the residual water fraction extracted with ethyl acetate, followed by butanol). However addition of aloe extract resulted in a dosedependent improvement of these effects. This cytoprotective effect of aloe could be attributed to its inhibition of GSH and protein-SH depletions. The effects of aloe extracts were also dose-dependent. Addition of diethyl maleate (1 mM), a cellular glutathione-depleting agent, to hepatocytes treated with both 1,4-naphthoquinone and aloe extract, induced depletion of GSH, but did not affect protein-SH or lactate dehydrogenase. These results suggest that the 1,4-naphthoquinone-induced toxicity in rat hepatocytes was inhibited by aloe extract, and that this protective effect was due to the maintenance of cellular thiols, especially protein-SH (Norikura et al., 2002).

3.1.4. Pharmacodynamic interactions

Chronic use or abuse of aloe dried juice preparations may lead to hypokalaemia. This hypokalaemia and the increased loss of potassium may increase the activity of cardiac glycosides and interfere with the action of antiarrhythmic agents (interaction with antiarrhythmic medicinal products, which induce reversion to sinus rhythm, e.g. quinidine) and medicinal products inducing QT-prolongation (Haverkamp, 2002). Concomitant use with medicinal products inducing hypokalaemia (e.g. diuretics, adrenocorticosteroids and liquorice root) may aggravate electrolyte imbalance.

Chung *et al.* (1996) investigated the influence of aloe on the ethanol metabolism in rats based on reports indicating that an extract of aloe enhances ethanol metabolism. Aloe contains aloin, a C-glycoside of anthraquinone. Quinones in general have a functional role in elevating the ethanol

metabolism rate *in vivo*. Upon oral administration of aloin (300 mg/kg) 12 h before ethanol administration, the area under the curve of blood ethanol significantly decreased by 40%, while the slope of elimination and rate of disappearance from the body increased by 60% and 64%, respectively.

3.2. Overview of available pharmakokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Data on hydroxyanthracene derivatives

Aloins A and B, hydroxyaloins and the aloinosides A and B are not absorbed in the upper gut. In humans, they pass into the colon unmodified after oral ingestion. Human intestinal flora are able to break down O-glycosides easily but only to some extent C-glycosides of most anthranoids. A strictly anaerobic bacterium, *Eubacterium* sp. BAR, was isolated from human faeces as one of the intestinal bacteria capable of metabolising barbaloin to aloe-emodin anthrone (Hattori *et al.*, 1988; Che *et al.*, 1991). Barbaloin was transformed to aloe-emodin anthrone in the faeces from gnotobiotic rats mono-associated with the *Eubacterium* sp. BAR, but not in faeces from conventional rats or the gnotobiotic rats mono-associated with *Peptostreptococcus intermedius*, a human intestinal anaerobic bacterium capable of reducing sennidins to rhein anthrone. Only in gnotobiotic rats mono-associated with the *Eubacterium* sp. BAR administration of barbaloin causes severe diarrhea. The faecal water content was significantly increased (Akao *et al.*, 1996). In contrast Ishii *et al.*, 1998). Aloe-emodin-9-anthrone was produced in the rat large intestine (Ishii *et al.*, 1994; Ishii *et al.*, 1998). Aloe-emodin-9-anthrone is the main active metabolite, which acts specifically on the colon (Ishii *et al.*, 1990).

It is not known to what extent aloe-emodin-9-anthrone is absorbed. In the case of senna, animal experiments with radio-labeled rhein anthrone administered directly into the caecum demonstrated absorption < 10% (De Witte and Lemli, 1988).

After oral administration of 4.5 mg/kg 14C-aloe-emodin to rats 20 – 30% of the dose was excreted in urine and the rest in faeces. Aloe-emodin was quickly metabolised to rhein, to an unknown metabolite and to conjugates of all three. In the plasma about 10% of 14C-activity was identified as free aloe-emodin. Maximum plasma values were reached 1.5 – 3 h p.a. with 248 (male) and 441 (female) ng equivalents aleo-emodin/ml. Maximum concentrations in plasma were about 3 times higher than those in ovaries and 10 times higher than those in testes. Because of the low activity concentrations in the reproductive organs TLC analysis was not possible. But if the metabolic profile of these organs is assumed to be the same as in plasma, concentrations of free aloe-emodin can be calculated to be maximally about 2-4 ng/g in testes and 8-10 ng/g in ovaries after an oral dose of 4.5 mg/kg. Only liver, kidney and intestinal tract showed higher concentrations than plasma. Terminal half-life (for radioactivity) in blood was about 50 h (Lang, 1993).

The absorbed rhein anthrone is glucuronidised in the liver. One part of the glucuronides is excreted via the urine and cause the yellow or red-brown discolouration of the urine. The other part is excreted via the bile (Lemli *et al.*, 1980).

The % absorption of aloin, aloe-emodin, and aloesin ranged from 5.51% to 6.60%, 6.60% to 11.32%, and 7.61% to 13.64%, respectively. Up to 18.15%, 18.18%, and 38.86% of aloin, aloe-emodin, and aloesin, respectively, was absorbed as glucuronidated or sulfated form. These results suggest that a significant amount is transformed during absorption. These results provide information to establish an adequate intake level of aloe to maintain an effective plasma level (Park *et al.*, 2009).

Pharmacokinetic studies by Lang (1993) after oral administration of ¹⁴ C-aloe-emodin to male and female rats showed that 20-30% of the dose was excreted in the urine and the rest was excreted in the faeces as rhein and conjugates. 10% of the radioactivity was identified as free aloe-emodin in the

plasma with a maximum peak at 1.5-3.0 h after the administration. Only the liver, kidney and gastrointestinal tract showed higher concentrations than the plasma. The terminal half-life of the radioactivity in the blood was 50 hours.

Administration of emodin to rabbits by i.v. bolus resulted in a serum profile which could be well described by a two-compartment model. The AUC of emodin was 518 micrograms. min/ml, clearance was 72.3 ml/min, and elimination half-life was 227 min which was much longer than that reported in a previous study. Oral administration of emodin resulted in a very low serum concentration. Protein binding of emodin was investigated by the equilibrium dialysis method. Emodin was found to be highly bound (99.6%) to serum protein (Liang *et al.*, 1995).

The metabolism of emodin (1, 3, 8-trihydroxy-6-methylanthraquinone), a compound present in pharmaceutical preparations was studied in vitro. With rat liver microsomes, the formation of two emodin metabolites, omega-hydroxyemodin and 2-hydroxyemodin, was observed. The rates of formation of omega-hydroxyemodin were not different with microsomes from rats that had been pretreated with inducers for different cytochrome P450 enzymes. Thus, the formation of omegahydroxyemodin seemed to be catalysed by several cytochrome P450 enzymes at low rates. The formation of 2-hydroxyemodin was increased in liver microsomes from 3-methylcholanthrenepretreated rats and was inhibited by alpha-naphthoflavone, by an anti-rat cytochrome P450 1A1/2 antibody, and, to a lesser degree, by an anti-rat cytochrome P450 1A1 antibody. The authors concluded the involvement of cytochrome P450 1A2 in the formation of this metabolite. However, other cytochrome P450 enzymes also seemed to catalyse this reaction. The anthraquinone chrysophanol (1,8-dihydroxy-3-methylanthraquinone) is transformed, in a cytochrome P450dependent oxidation, to aloe-emodin (1, 8-dihydroxy-3-hydroxymethylanthraquinone) as the major product formed. The mutagenicity of the parent dihydroxyanthraguinones and their metabolites was compared in the in vitro micronucleus test in mouse lymphoma L5178Y cells. 2-Hydroxyemodin induced much higher micronucleus frequencies, compared with emodin. omega-Hydroxyemodin induced lower micronucleus frequencies, compared with emodin. Aloe-emodin induced significantly higher micronucleus frequencies than did chrysophanol (Müller et al, .1998).

A study by Wang et al. (2001) was performed to determine the effects of emodin on cytochrome P450 (P450)-dependent monooxygenases of human lung adenocarcinoma CL5 cells. Treatment of CL5 cells with 100 microM emodin for 24 h induced benzo[a]pyrene hydroxylation, 7-ethoxyresorufin Odeethylation, and 7-ethoxycoumarin O-deethylation activities of S9 fractions. Immunoblot analysis of CL5 S9 proteins revealed that emodin induced proteins immunorelated to P450s 1A1 and 1B1. Northern blot analysis of total cellular RNA showed that emodin induced P450s 1A1 and 1B1 mRNA levels in CL5 cells. These inductive effects on P450 monooxygenase activity, protein, and mRNA were concentration- and time-dependent. Addition of emodin to CL5 cell microM S9 inhibited its 7ethoxycoumarin O-deethylation activity. Treatment of CL5 cells with 10 microM 3-methylcholanthrene for 24 h induced monooxygenase activity and P450s 1A1 and 1B1 proteins and mRNA levels. Treatment of the lung cells with 100 microM emodin or purpurin (1,2,4-trihydroxyanthraquinone) for 24 h produced greater induction of P450s 1A1 and 1B1 mRNA than did anthraflavic acid (2,6dihydroxyanthraquinone) or anthraquinone. The emodin treatment induced P450s 1A1 and 1B1 mRNA in human lung carcinoma NCI-H322 and breast cancer MCF-7 cells. Emodin induced P450 1A1, but not 1B1, mRNA in human hepatoma HepG2 cells. The authors concluded that emodin is an inducer of P450s 1A1 and 1B1 protein and mRNA in human lung adenocarcinoma CL5 cells. Modulation of P450 by emodin may be an important factor affecting metabolism and toxicity of the hydroxyanthraquinone in humans (Wang et al., 2001).

Assessor's conclusions on pharmacokinetic data:

Aloe-emodin is quickly oxidized to rhein and an unknown metabolite or it is conjugated. In rats less than 10% of the orally administered total dose was recovered as unconjugated aloe-emodin. Concentrations found in gonads were below 10 ng/g ovary and below 5 ng/g testis after oral application of 4.5 mg/kg bw.

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

Aloe-emodin and emodin are naturally occurring anthraquinones present in the roots of aloe. Anthraquinone glycosides are poorly absorbed from the gastrointestinal tract but are cleaved by gut bacteria to produce aglyka (such as emodin) that are more readily absorbed and are responsible for the purgative properties of these preparations. There is extensive exposure to emodin and other anthraquinones resulting from the use of herb-based stimulant laxatives. Extracts from the roots, bark, and/or dried leaves of buckthorn, senna, cascara, aloe, frangula, rhubarb and rhamnus have been used as laxatives since ancient times and currently are widely used in the preparation of herbal laxative preparations. Reports that 1,8-dihydroxyanthraquinone, a commonly used laxative ingredient, caused tumours in the gastrointestinal tract of rats raised the possibility of an association between colorectal cancer and the use of laxatives containing anthraquinones. Because emodin, a hydroxyanthraquinone structurally similar to 1,8-dihydroxyanthraquinone, is present in herbal laxatives and was reported to be mutagenic in bacteria, it was considered a potential carcinogen and was selected for in-depth evaluation.

3.3.1. Single dose toxicity

Data on herbal preparations

Dosed water studies with aloe latex (HAD content unknown) in mice revealed no acute toxicity in the leaf pulp at 500 mg/kg bw. At higher doses a decrease of central nervous system activity was observed (Shah *et al.*,1989)

3.3.2. Repeat dose toxicity

Data on herbal preparations

The following study was designed to examine the effects of long-term (1.5 and 5.5 months) *Aloe vera* (*Aloe barbadensis*) ingestion on the growth, food intake and serum chemistry of Fischer 344 male rats. *Aloe vera* powders, produced by two different methods, were mixed with rat chow at selected concentrations. Process A aloe was prepared from skinned aloe filets by homogenization followed by lyophilisation and grinding to a fine powder; Process B aloe was prepared similarly except that the homogenate was charcoal filtered prior to lyophilisation. Ingestion of Process A aloe at concentrations greater than 1% was associated with diarrhoea and a decrease in weight gain. Ingestion of 1% Process A and both 1% and 10% Process B aloe had no adverse effect on body weight gain, food intake, gastrointestinal transit time and gross pathology. Serum chemistry was minimally affected. The rats ingesting 10% Process B aloe exhibited a slight, but significant increase in fluid intake. The results indicate that, although high concentrations of aloe should be avoided, ingestion of moderate levels (1%) of aloe from either process causes no apparent adverse effects in the rat (Herlihy *et al.*, 1998a).

The aim of the next study was to examine the effects of long-term (1.5 and 5.5 months) *Aloe vera (Aloe barbadensis)* ingestion on a variety of metabolic parameters of Fischer 344 male rats. Rats were fed *Aloe vera* powders produced by two different methods and mixed with rat chow at various concentrations. Process A *Aloe* was prepared from skinned *Aloe* filets by homogenization followed by

lyophilisation and included in the rat chow at the 1% level. Process B *Aloe* was prepared similarly except the homogenate was charcoal filtered prior to lyophilisation and mixed with chow at both 1% and 10% levels. Plasma concentrations of parathyroid hormone (PTH) and calcitonin were lower in the older (7 month) *Aloe* fed than in the control rats of the same age. No statistically significant effects were observed at the younger age (3 month). *Aloe* feeding did not alter the plasma glucose and insulin levels at any age and had only minor effects on the plasma corticosterone concentrations in the older rats. Serum lipid peroxides were decreased by *Aloe* feeding, but the malondialdehyde production of cardiac and hepatic mitochondrial and microsomal membranes were unaffected. No alteration in protein turnover was observed in the *Aloe* fed rats (Herlihy *et al.*, 1998b).

A one year study was conducted to evaluate the chronic toxicity of *Aloe arborescens* Miller var. *natalensis* Berger (ALOE) whole leaf powder in the diet at doses of 4.0%, 0.8% or 0.16% to groups of male and female Wistar Hannover rats. No deaths occurred at any dose level throughout the treatment period. Both sexes receiving 4.0% showed diarrhoea, with a reduced body weight gain. Increase of white blood cells in the male 4.0% group, decrease of haemoglobin in the female 4.0% and 0.8% groups, decrease of inorganic phosphate in the male 4.0% and 0.8% groups and female 4.0% group, and decrease of Calcium and alanine aminotransferase in the female 4.0% group were observed. Relative kidney weight showed increase in the female 4.0% group and relative heart and brain weights were decreased in the female 4.0% and 0.8% groups. Histopathologically, both sexes receiving 4.0% showed severe sinus dilatation of ileocecal lymph nodes, and yellowish pigmentation of ileocecal lymph nodes and renal tubules. The no observed adverse effect level (NOAEL) for ALOE was the 0.16% in diet, which is equivalent to 87.7 and 109.7 mg/kg/day in males and females, respectively (Matsuda *et al.*, 2008).

Data on hydroxyanthracene derivatives

Male and female F344/N rats and B6C3F1 mice were exposed to emodin (at least 94% pure) in feed for 16 days and 14 weeks in the NTP Toxicology Programme (NTP, 2001).

16-day study in rats: Groups of five male and five female rats were fed diets containing 0, 600, 2,000, 5,500, 17,000, or 50,000 ppm emodin (equivalent to average daily doses of approximately 50, 170, 480, 1,400, or 3,700 mg emodin/kg body weight to males and 50, 160, 460, 1,250, or 2,000 mg/kg to females). Three female rats died before the end of the study. Mean body weights of males and females exposed to 5,500 ppm or greater were significantly less than those of the controls. Feed consumption by males and females receiving 17,000 or 50,000 ppm was decreased throughout the study. Macroscopic lesions were present in the kidney of rats exposed to 17,000 or 50,000 ppm.

16-day study in mice: Groups of five male and five female mice were fed diets containing 0, 600, 2,000, 5,500, 17,000, or 50,000 ppm emodin (equivalent to average daily doses of approximately 120, 400, 1,200, or 3,800 mg/kg to males and 140, 530, 1,600, or 5,000 mg/kg to females; 50,000 ppm equivalents were not calculated due to high mortality). All mice exposed to 50,000 ppm died before the end of the study. Mice in the 17,000 ppm groups lost weight during the study. Feed consumption by 5,500 ppm females was greater than that by the controls throughout the study. Macroscopic lesions were present in the gallbladder and kidney of mice exposed to 17,000 ppm.

14-week study in rats: Groups of 10 male and 10 female rats were fed diets containing 0, 312.5, 625, 1,250, 2,500, or 5,000 ppm emodin (equivalent to average daily doses of approximately 20, 40, 80, 170, or 300 mg/kg to males and females). Mean body weights of males exposed to 2,500 ppm or greater and females exposed to 1,250 ppm or greater were significantly less than those of the controls. During the first week of the study, feed consumption by males exposed to 2,500 or 5,000 ppm and females exposed to 5,000 ppm was less than that by the controls. Feed consumption by these groups

was similar to that by the controls for the remainder of the study. In rats exposed to 2,500 or 5,000 ppm, there were increases in platelet counts in males and females and segmented neutrophil counts in females. Total serum protein and albumin concentrations were decreased in females exposed to 2,500 or 5,000 ppm. Relative kidney weights of rats exposed to 1,250 ppm or greater and relative lung weights of rats exposed to 625 ppm or greater were significantly increased compared to the control groups. Relative liver weights were increased in females exposed to 625 ppm or greater. The estrous cycle length was significantly increased in females exposed to 1,250 or 5,000 ppm. All male rats exposed to 1,250 ppm or greater and all exposed female rats had pigment in the renal tubules; and the severity of pigmentation generally increased with increasing exposure concentration. The incidences of hyaline droplets in the cortical epithelial cytoplasm were increased in all groups of exposed males and in females exposed to 312.5, 625, or 1,250 ppm.

14-week study in mice: Groups of 10 male and 10 female mice were fed diets containing 0, 312.5, 625, 1,250, 2,500, or 5,000 ppm emodin (equivalent to average daily doses of approximately 50, 100, 190, 400, or 800 mg/kg to males and 60, 130, 240, 500, or 1,100 mg/kg to females). All mice survived to the end of the study. Mean body weights of males exposed to 2,500 or 5,000 ppm were significantly less than those of the controls. Feed consumption by exposed groups was generally similar to that by the controls. Relative kidney weights of male mice exposed to 1,250 ppm or greater, relative lung weights of males exposed to 625 ppm or greater, and relative liver weights of female mice exposed to 625 ppm or greater were increased. The incidences and severities of nephropathy were increased in males and females exposed to 1,250 ppm or greater. The incidences of renal tubule pigmentation were significantly increased in males exposed to 625 ppm or greater.

During subchronic 90 day studies increased mortality, decreased red blood cell count and significant sperm damage were noted (Shah *et al.*, 1989).

3.3.3. Genotoxicity

Data on herbal preparations

Morimoto *et al.* (1982) reported the results of the Ames test and the rec-assay for aloe crude extracts. The investigators found neither water nor methanol extracts (no further information available) of aloe to have mutagenic activity in *Salmonella typhimurium* strains TA98 or TA100. A water extract of aloe was reported to produce a positive effect in the rec-assay using *Bacillus subtilis*.

Marquardt *et al.* (1987) (cited in the unpublished report of Brusick (1994)) conducted a more thorough evaluation of aloe-extract in the Ames test. A wide range of mutant strains (TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538) was included, and "faecalase" (gut flora enzymes) was employed in order to breakdown any potentially active glycosides. The results of this investigation were negative with the maximum test concentration set at 3,000 μ g/plate. Barbaloin was also reported negative in this study.

In 1992 Cytotest Cell Research GmbH & Co. conducted a series of genetic tests using a batch of commercial aloe-extract (also cited in the unpublished report of Brusick, 1994). The Ames test (employing TA1535, TA1537, TA1538, TA98, and TA100) only produced a mutagenic effect in strain TA1537 at 5,000 µg/plate without and with S9 mix but not at the next lower concentration of 1,000 µg/plate.

A mammalian cell assay for gene mutation conducted in V79 cells showed no evidence of mutagenicity with aloe extract in concentrations up to 1,000 μ g/ml without S9 mix and up to 5,000 μ g/ml with S9 mix.

Aloe-extract was shown to be clastogenic in CHO cells. In the absence of S9 mix, aloe-extract induce significant increases in chromosome breakage at concentration of 3,000 μ g/ml (30-hour harvest) and 4,000 μ g/ml (24-hour harvest). No clastogenicity was observed with S9 mix at concentrations up to 4,750 μ g/ml.

However, an *in vivo* test for clastogenicity with aloe-extract (Bootman *et al.* 1987a, cited in the unpublished report of Brusick, 1994) produced no evidence of a response in the mouse micronucleus test at a maximum applicable dose of 1.5 mg/kg (orally).

Data on hydroxyanthracene derivatives

In vitro

A variety of structurally related hydroxyanthraquinone derivatives (HAD) were investigated in a test battery for the evaluation of mutagenicity and cell-transforming activity. The tests were: *Salmonella typhimurium* mutagenicity assay, V79-HGPRT mutagenicity assay, DNA-repair induction assay in primary rat hepatocytes and the *in vitro* transformation of C3H/M2 mouse fibroblasts. In *Salmonella*, most of the tested compounds were mutagenic in strain TA1537, but only a few were active in other strains. Among these were HAD with a hydroxymethyl group, such as lucidin and aloe-emodin. In V79 cells, only HAD with 2 hydroxy groups in the 1.3 positions (1,3-DHA, purpurin, emodin) or with a hydroxymethyl sidechain (lucidin and aloe-emodin) were mutagenic. The compounds found to be active in V79 cells were also active in the DNA-repair assay and in the C3H/M2 transformation assay. Thus, it appears that the genotoxicity of HAD is dependent on certain structural requirements. (Westendorf *et al.*, 1990)

Studies were undertaken to elucidate further the mechanism by which emodin was converted into a direct-acting mutagen to *Salmonella typhimurium* TA1537 by the hepatic microsomes and the reconstituted cytochrome P-450 system. Emodin was activated into a mutagenic principle(s) in the reconstituted cytochrome P-450 system, and its mutagenicity was significantly higher with the fraction II (P-448 type) than the fraction I (P-450 type) derived from the hepatic microsomes of PCB-induced rats. Thin-layer chromatographic analysis revealed that the purified cytochrome II-a (maximal CO-differential spectrum at 448.0 nm and high-spin form) activity converted emodin into 2-hydroxy-emodin, a direct-acting mutagen (Tanaka *et al.*, 1987).

The hepatic microsomes derived from various animal species transformed emodin (1,3,8-trihydroxy-6methylanthraquinone) into an unidentified anthraquinone h, along with 2-hydroxy-, 4-hydroxy- and 7hydroxyemodins. TLC, UV, MS and NMR clarified this unidentified major metabolite as omega-hydroxyemodin (1,3,8-trihydroxy-6-hydroxymethylanthraquinone). Among 7 animal species, the highest activity to produce this omega-hydroxyemodin was observed in the hepatic microsomes of guinea pig and rat, followed by mouse and rabbit. The microsomal activity to convert emodin into omegahydroxyemodin was accelerated by the pre-treatment of animals with phenobarbital, and inhibited by SKF 525A. The microsomal hydroxylation reactions of the methyl residue and the anthraquinoid nucleus of emodin were presumed to be catalysed regiospecifically by multiple forms of cytochrome P-450. Omega-hydroxyemodin was not mutagenic to *Salmonella typhimurium* in the absence of S9, but exhibited mutagenicity in the presence of an activating system. This genotoxic potential was comparable to 2-hydroxyemodin, a direct-acting mutagen (Murakami *et al.*, 1987).

Emodin, danthron and aloe-emodin were tested in a number of *in vitro* assay systems. All three compounds induced tk-mutations in mouse lymphoma L5178Y cells. Induction of micronuclei also occurred in the same cell line, and was dose-dependent, with the potency ranking being danthron > aloe-emodin > emodin. In a DNA decatenation assay with a network of mitochondrial DNA of *C. fasciulata*, all three test compounds inhibited the topoisomerase II-mediated decatenation. Danthron

and aloe-emodin, but not emodin, increased the fraction of DNA moving into comet tails when tested at concentrations around 50 microM in single-cell gel-electrophoresis assays (SCGE; comet assay). Comet assays were also used in modified form to determine whether pre-treatment of the cells with the test compounds would reduce the effects of etoposide, a potent topoisomerase II inhibitor. All three test chemicals were effective in this pre-treatment protocol, with danthron again being the most potent (Müller *et al.*, 1996).

Genetic toxicology studies were conducted with emodin (at least 94% pure) in *Salmonella typhimurium* and cultured Chinese hamster ovary cells (NTP, 2001). Emodin was mutagenic in *Salmonella typhimurium* strain TA100 in the presence of S9 activation; no mutagenicity was detected in strain TA98, with or without S9. Chromosomal aberrations were induced in cultured Chinese hamster ovary cells treated with emodin, with and without S9. All three tested anthraquinones, emodin, aloe-emodin, and danthron, showed capabilities to inhibit the non-covalent binding of bisbenzimide Hoechst 33342 to isolated DNA and in mouse lymphoma L5178Y cells comparable to the topoisomerase II inhibitor and intercalator m-amsacrine. In a cell-free decatenation assay, emodin exerted a stronger, danthron a similar and aloe-emodin a weaker inhibition of topoisomerase II activity than m-amsacrine. Analysis of the chromosomal extent of DNA damage induced by these anthraquinones was performed in mouse lymphoma L5178Y cells clones showed similar chromosomal lesions when compared to the topoisomerase II inhibitors etoposide and m-amsacrine, but were different from mutants induced by the DNA alkylator ethyl methanesulfonate (Müller, Stopper, Dekant, 1998).

Brown and Dietrich (1979) reported that aloe-emodin was mutagenic in *Salmonella typhimurium* strain TA1537. The mutagenicity was observed in the absence of S9 mix (terms of revertants per nmol test agent: 0.22; terms of revertants per plate less background for a given quantity (μ g) of test agent: 22 (50 μ g) without S9 activation, 13 (50 μ g) with S9 activation; 61 (100 μ g) without S9 activation, 14 (100 μ g) with S9 activation; 12 (250 μ g) without S9 activation, 15 (250 μ g) with S9 activation.

Westendorf *et al.* (1990) reported on the genotoxicity of aloe-emodin in a broad spectrum of *in vitro* assays. Positive results were obtained in the Ames test with *Salmonella typhimurium* strains TA1537, TA1538, TA98 and TA1978. In the HPRT test, no reproducible induction of mutations was obtained, while unscheduled DNA synthesis (UDS) and cell transformation was induced. These results led to the conclusion that aloe-emodin interacts with bacterial and mammalian DNA under certain *in vitro* conditions.

Heidemann *et al.* (1996) undertook *in vitro* experiments to clarify the genotoxic potential of the hydroxyanthraquinone aloe-emodin. The results confirmed that aloe-emodin is able to induce mutagenic effects *in vitro*.

In vivo:

Toxicological data from *in vitro* investigations (Westendorf *et al.*, 1990) indicated that the hydroxyanthraquinones, emodin and aloe-emodin, might represent a genotoxic risk. However, results from *in vivo* investigations (Heidemann *et al.*, 1996) did not indicate a genotoxic potential.

A study by Mengs *et al.* (1997) was performed to investigate the potential of emodin (1,3,8trihydroxy-6-methylanthra-quinone) to induce micronuclei in polychromatic erythrocytes (PCEs). Mice of both genders received a single oral dose of 2,000 mg emodin/kg and were killed 24 and 48 h later. Bone marrow cells were collected from 5 males and 5 females and 2000 PCEs per animal were scored for the presence of micronuclei. There was no enhancement in the frequency of micronuclei at both preparation intervals when compared to the negative controls. Blood level examinations confirmed the systemic availability of emodin. Plasma levels of up to 190 micrograms emodin/ml represented concentrations being in the concentration range that induced positive responses in several genotoxicity cell culture assays (Mengs *et al.*, 1997).

Genetic toxicology studies were conducted with emodin (at least 94% pure) in rat and mouse bone marrow cells and mouse peripheral blood erythrocytes (NTP, 2001). Three separate *in vivo* micronucleus tests were performed with emodin. A male rat bone marrow micronucleus test, with emodin administered by three intraperitoneal injections, gave negative results. Results of acute exposure (intraperitoneal injection) micronucleus tests in bone marrow and peripheral blood erythrocytes of male and female mice were negative. In a peripheral blood micronucleus test on mice from a 14-week study, negative results were seen in male mice, but a weakly positive response was observed in similarly exposed females.

Heidemann *et al.* (1996) undertook *in vivo* experiments (micronucleus assay in bone marrow cells of NMRI mice; chromosome aberration assay in bone marrow cells of Wistar rats; mouse spot test [DBA/2J x NMRI]) to clarify the genotoxic potential of the hydroxyanthraquinone aloe-emodin. No indication of a mutagenic activity of aloe-emodin was found. Information about a possible reaction of aloe-emodin with DNA was derived from an *in vivo* UDS assay. Hepatocytes of aloe-emodin treated male Wistar rats did not show DNA damage via repair synthesis. These available data suggest that aloe-emodin is able to interact with DNA under certain *in vitro* conditions. However, *in vivo* the results did not indicate a genotoxic potential. Therefore the authors assume that a genotoxic risk for man might be unlikely.

3.3.4. Carcinogenicity

Data on herbal preparations

A 2 years carcinogenicity study of Aloe, Aloe arborescens Miller var. natalensis Berger, a food additive was conducted for assessment of toxicity and carcinogenic potential in the diet at doses of 4% or 0.8% in groups of male and female Wistar Hannover rats. The whole leaf powder of Aloe arborescens, the same grade used as a food additive was mixed at concentrations of 0.0% (Control), 0.8%, and 4.0% into powdered basal diet and pelleted. The concentrations of aloenin and aloin (barbaloin and isobarbaloin) in the whole leaf powder of Aloe arborescens and the pelleted diet were measured and evaluated using high-performance liquid chromatography. The concentrations of aloin and aloenin in the whole leaf powder after storage for 2 weeks were 0.83% and 1.91%, respectively. The concentrations of aloin and aloenin in the pelleted diet after 2 weeks of storage at room temperature were 0.0009% and 0.0022% for the 0.8% diet and 0.0179% and 0.0663% for the 4.0% diet. Both sexes receiving 4% showed diarrhoea, with loss of body weight gain. The survival rate in the 4% female group was significantly increased compared with control females after 2 years. Haematological and biochemical examination showed increase of RBC, Hb, and Alb in the 4% males. The cause of these increases could conceivably have been dehydration through diarrhoea. AST and Na were significantly decreased in the males receiving 4%, and CI was significantly decreased in both 4% and 0.8% males. A/G was significantly increased in the 4% females, and CI was significantly decreased (0.8%) in the female group. Histopathologically, both sexes receiving 4% showed severe sinus dilatation of ileocecal lymph nodes, and yellowish pigmentation of ileocecal lymph nodes and renal tubules. Adenomas or adenocarcinomas in the cecum, colon, and rectum were observed in 4% males but not in the 0.8% and control male groups. Similarly, in females, adenomas in the colon were also observed in the 4% but not 0.8% and control groups. In conclusion, Aloe, used as a food additive, exerted equivocal carcinogenic potential at 4% high-dose level on colon in the 2 years carcinogenicity study in rats. The authors concluded that aloe is not carcinogenic at nontoxic-dose levels and that

carcinogenic potential in at 4% high-dose level on colon is probably due to irritation of the intestinal tract by diarrhoea (Yokohira *et al.*, 2009)

The National Center for Toxicological Research (NCTR) conducted 14-day, 13-week, and 2 years carcinogenesis studies on the leaf extracts of Aloe vera plants. The Aloe vera plant extracts used in these studies were obtained from freshly harvested Aloe barbadensis Miller plants and were freezedried (6% moisture) and gamma-irradiated to preserve quality. No other additives were used in their preparation. Solutions of non-decolorized extracts of Aloe vera leaves (DER and HAD content unknown) were added to the drinking water to groups of rats and mice for 2 years. Groups of 48 rats received solutions containing 0.5% (= 0.62 g Aloe vera whole-leaf extract per kg bw per day, 1% or 1.5% of Aloe vera extract in the drinking water, and groups of mice received solutions containing 1%, 2%, or 3% = 11.8 g Aloe leaf extract/kg bw/day of whole leaf *Aloe vera* extract. Similar groups of animals were given plain drinking water and served as the control groups. At the end of the study tissues from more than 40 sites were examined for every animal. In all groups of rats and mice receiving the Aloe vera extract, the rates of hyperplasia in the large intestine were markedly increased compared to the control animals. There were also increases in hyperplasia in the small intestine in rats receiving the Aloe vera extract, increases in hyperplasia of the stomach in male and female rats and female mice receiving the Aloe vera extract, and increases in hyperplasia of the mesenteric lymph nodes in male and female rats and male mice receiving the Aloe vera extract. In addition, cancers of the large intestine occurred in male and female rats given the Aloe vera extract, though none had been seen in the control groups of rats for this and other studies at this laboratory. The authors concluded that nondecolorized Aloe vera caused cancers of the large intestine in male and female rats and also caused hyperplasia of the large intestine, small intestine, stomach, and lymph nodes in male and female rats. Aloe vera extract also caused hyperplasia of the large intestine in male and female mice and hyperplasia of the mesenteric lymph node in male mice and hyperplasia of the stomach in female mice (Boudreau et al., 2013).

Assessor 's Comment regarding Boudreau et al., 2013:

The studies are not unambiguously transferable to humans, due to lacking extract specifications. A dose effect relation is therefore debatable since the HAD content of the whole leaf aloe extract, its composition and the posology are unknown. In this 2 years rat study, treatment (max. dose 11.8 g Aloe vera leaves extract/kg bw/day) related neoplasms were limited to the large intestine and occurred as adenomas and carcinomas in the ileocecal junction, cecum, and the ascending and transverse colons of the male and female rats. The neoplasms were confined within the mucosal wall of the large intestine and did not metastasize to regional mesenteric lymph nodes or more distant sites. However, in association with the whole set of existing data they contribute to the concern about genotoxicity and carcinogenicity of anthraquinones.

The effects of long-term *Aloe vera* ingestion on age-related diseases were investigated using male specific pathogen-free (SPF) Fischer 344 rats. Experimental animals were divided into four groups: Group A, the control rats fed a semi-synthetic diet without *Aloe vera*; Group B, rats fed a diet containing 1% freeze-dried *Aloe vera* blended filet; Group C, rats fed a diet containing 1% charcoal-processed, freeze-dried *Aloe vera* filet; and Group D, rats fed the control diet and given whole leaf charcoal-processed *Aloe vera* (0.02%) in the drinking water. This study demonstrates that life-long *Aloe vera* ingestion produced neither harmful effects nor deleterious changes. In addition, *Aloe vera* ingestion appeared to be associated with some beneficial effects on age-related diseases. Groups B exhibited significantly less occurrence of multiple causes of death, and a slightly lower incidence of fatal chronic nephropathy compared with Group A rats. Groups B and C rats showed the trend, slightly lower incidences of thrombosis in the cardiac atrium than Group A rats. Therefore, these findings

suggest that life-long *Aloe vera* ingestion does not cause any obvious harmful and deleterious side effects (Ikeno *et al.*, 2002).

Data on hydroxyanthracene derivatives

In a model of dimethylhydrazine(DMH)-induced colorectal tumours in male mice neither aloin- nor sennoside-enriched diets (0.03% corresponding to 100 mg aloin or sennoside/kg/day) promoted incidence and growth of adenomas and carcinomas after 20 weeks as evidenced by different endpoint parameters, based on a macroscopic evaluation and microscopic examination. In the DMH-induced tumour model a tumour incidence appeared which allowed an increasing or decreasing effect to be detected after additional treatment, i.e. a 50% incidence of tumour-bearing animals. With regard to hepatotoxic and nephrotoxic effects, DMH itself enhanced plasma levels of GPT and SDH which were further significantly increased by co administration of aloin. The anthranoids alone had no effect. No effects on serum electrolyte concentrations were observed after any of the treatments (Siegers *et al.*, 1993).

A free-floating cell line has been established from a metastatic lesion of a Merkel cell carcinoma (MCC) patient. The cell line was characterized by immunocytochemical reactions with antibodies against the epithelial and neuroendocrine antigens: cytokeratin 20, neuron-specific enolase, chromogranin A, neurofilament protein, synaptophysin, and calcitonin. Karyotype analysis of the MCC cells showed deletion in chromosomes 3 and 7, loss of chromosome 10, and several translocations in other chromosomes. No mutation was detected in the TP53 gene, after analysing the complete coding region. Growth factors such as basic fibroblast growth factor, transforming growth factor-beta, and nerve and epidermal growth factors had no effect on the proliferation of the cells. The differentiation-inducing agents sodium butyrate and dimethyl sulfoxide, especially the former, markedly inhibited the proliferation of the MCC cells. Aloe-emodin, significantly inhibited the growth of MCC cells. Aloe-emodin has been reported to be nontoxic for normal cells but to possess specific toxicity for neuroectodermal tumor cells (Wasserman *et al.*, 2002).

In 2001 the National Toxicology Program (NTP) of the U.S. Department of Health and Human Services published a technical report on toxicology and carcinogenesis studies of emodin.

2-years study in rats: Groups of 65 male and 65 female rats were fed diets containing 0, 280, 830, or 2,500 ppm emodin (equivalent to average daily doses of approximately 110, 320, or 1,000 mg/kg to males and 120, 370, or 1,100 mg/kg to females). Ten male and ten female rats from each group were necropsied at 6 months. Blood samples from five male and five female rats in each group were evaluated at 3, 6, and 12 months for plasma emodin concentrations; these rats were necropsied at 12 months. Survival, body weights, and feed consumption: Survival of exposed males and females was similar to that of the controls. The mean body weights of rats in the 2,500 ppm groups were less than those of the controls beginning at week 2 of the study. Feed consumption by exposed groups was similar to that by the controls throughout the study. Pathology findings: Three Zymbal's gland carcinomas were observed in female rats exposed to 2,500 ppm. This incidence exceeded the range observed for current historical controls and was considered an equivocal finding. At the 6- and 12month interim evaluations and at 2 years, emodin-related increases in the incidences of renal tubule hyaline droplets occurred in all exposed groups. The incidences of renal tubule pigmentation were significantly increased in all exposed groups of males at 2 years. There were negative trends in the incidences of mononuclear cell leukaemia in male and female rats, and the incidences in the 2,500 ppm groups were significantly decreased. In females exposed to 2,500 ppm, the incidence was below the historical control range; the incidence in males exposed to 2,500 ppm was at the lower end of the historical control range.

2-years study in mice: Groups of 60 male mice were fed diets containing 0, 160, 312, or 625 ppm emodin (equivalent to average daily doses of approximately 15, 35, or 70 mg/kg). Groups of 60 female mice were fed diets containing 0, 312, 625, or 1,250 ppm emodin (equivalent to average daily doses of approximately 30, 60, or 120 mg/kg) for 105 weeks. Ten male and ten female mice from each group were necropsied at 12 months. Survival, Body Weights, and Feed Consumption Survival and mean body weights of exposed males and females were similar to those of the controls. No differences in feed consumption were noted between exposed and control groups. Pathology Findings: Low incidences of renal tubule adenoma and carcinoma occurred in exposed male mice; these incidences included one carcinoma each in the 312 and 625 ppm groups. Renal tubule neoplasms are rare in male mice, and their presence in these groups suggested a possible association with emodin exposure. At the 12-month interim evaluation, the severity of nephropathy was slightly increased in males exposed to 625 ppm. Also at 12 months, the severity of nephropathy increased from minimal in the lower exposure groups to mild in females exposed to 1,250 ppm; the incidence in this group was significantly increased compared to the control group. At 2 years, the severities of nephropathy were slightly increased in males exposed to 625 ppm and females exposed to 1,250 ppm. The incidences of nephropathy were significantly increased in all exposed groups of females. At the 12-month interim evaluation, the incidences of renal tubule pigmentation were significantly increased in all exposed groups of males and in females exposed to 625 or 1,250 ppm. The severities increased with increasing exposure concentration. At 2 years, the incidences of renal tubule pigmentation were significantly increased in all exposed groups; severities increased with increasing exposure concentration.

Conclusion by the "National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee":

- The studies give no evidence of carcinogenic activity of emodin in male rats and female mice, and equivocal evidence in female rats and male mice.
- In view of conflicting results on genotoxicity, it was noted the first pass effect and need for metabolic activation suggesting a metabolite as the genotoxic form. The metabolite 2hydroxyemodin acts as the genotoxin (NTP, 2001).

The occurrence of intestinal tumours in rats has been reported by Mori *et al.* (1985) following the dietary administration of chrysazin (1,8-dihydroxy-9, 10-anthracenedione = danthrone) for 16 months at the concentration of 1%. Twelve out of 18 rats survived more than one year. Of these, 7 rats developed intestinal tumours of the colon or caecum, adenomas or adenocarcinomas. Besides these neoplasms, focal hyperplastic lesions of the glandular epithelium of the colon and caecum were frequently encountered in treated animals both with and without intestinal tumours. In the liver of several rats, some histological changes such as focal necrosis, fibrosis, cystic lesions and bile duct proliferation were seen.

Another carcinogenicity testing of chrysazin was performed in 1986 (Mori *et al.*) in C3H/HeN mice by dietary administration for 540 days at a concentration of 0.2% since mice did not tolerate larger doses (1% and 0.5%). All the mice that were given the chemical and survived more than 500 days, developed adenomatous hyperplasia with cystic glands of the caecum and colon. These lesions were not seen in animals of the control group. Some of the hyperplastic lesions were difficult to distinguish from true neoplasms. Similar hyperplastic lesions were also recognised together with the carcinomas in rats. Carcinogen-induced hyperplastic lesions have been regarded as an important precursor change for malignancies in various experimental models. The authors therefore stated that the lesions obtained here appear to indicate a certain carcinogenic potency of chrysazin in mice, and mouse intestine may be less sensitive than rat intestine to the carcinogenic action of the chemical. The incidence of hepatocellular carcinoma of mice given chrysazin (4/17) was significantly higher than that

of the controls (0/19). However, benign hepatocellular neoplasms were also seen in the animals of the control group. The authors therefore concluded that chrysazin enhanced the progression of spontaneously occurring hepatocarcinogenesis.

Danthron and 8 other hydroxyanthraquinones were comparatively investigated by Wölfle *et al.* (1990) for activities associated with tumour promotion, such as stimulation of cell proliferation and enhancement of malignant transformation. The *in vivo* treatment of primary rat hepatocytes with danthron, aloe-emodin, chrysophanol, and rhein resulted in a 2-3-fold increase of DNA synthesis; lucidin and purpurin were less active, and emodin, purpuroxanthin, and alizarin were essentially inactive. In addition, danthron, rhein, and chrysophanol, but not alizarin, enhanced transformation of C3H/M2 mouse fibroblasts initiated by N-methyl-N'-nitro-N-nitrosoguanidine or 3-methylcholanthrene. The results of these *in vitro* studies suggest that hydroxyanthraquinones, possessing 2 hydroxy groups in 1,8-positions, e.g. danthron, rhein, and chrysophanol, may have tumour-promoting activities.

The aim of the study of Schörkhuber et al. (1998) was to demonstrate the effect of the 1,8dihydroxyanthraquinone (DHA)-laxatives, danthrone, rhein, aloe-emodin and sennidine, on colorectal tumour cells, because available information on their implication in colon carcinogenesis was still inconclusive. In SW480 carcinoma cultures, dose-dependent induction of urokinase secretion into the medium was the predominant effect. Simultaneously, cell numbers were decreased by DHA-aglyka, but not by sennoside, nor the biphenylic laxative bisacodyl. DNA synthesis was not similarly reduced: 0.4-4 µM danthrone and sennidine even stimulated 5-bromo-2'-desoxyuridine (BrdU) uptake into DNA. When uptake was normalised to cell number, danthrone and sennidine doubled BrdU uptake/106 cells, 18 µM rhein and 0.7 µM aloe-emodin induced increases of 37 and 50%, respectively. This may at least partially be due to selective resistance of S-phase cells to DHA-caused cell loss. In VACO235 adenoma cells, sennidine and aloe-emodin did not affect urokinase secretion, but stimulated growth. Both cell numbers and DNA synthesis were increased. In contrast to SW480 carcinoma cells, VACO235 cells were also sensitive to sennoside and bisacodyl. No effects of DHA were observed in normal colorectal epithelial cells. The biological effects were preceded by specific phosphorylation of cellular proteins with molecular weights of 110, 78, 63, 57 kDa, indicating the specific induction of a cellular signalling cascade by the laxatives.

3.3.5. Reproductive and developmental toxicity

Data on herbal preparations

No teratogenic or foetotoxic effects were seen in rats after oral treatment with aloe extract (up to 1000 mg/kg) or aloin A (up to 200 mg/kg) (WHO, 1999). The pregnant rats were treated between the 10th and 13th day of the gestational period. A caesarean section was done on the 21st day post conception.

The gastrointestinal tract of neonates is sensitive to dietary manipulations. The effects of orally administered extracts of *Aloe vera* (ethanolic extract and aqueous extract; DER and HAD unknown) in unweaned rats were investigated. The aqueous and alcohol (ethanol) extracts were prepared from fresh *Aloe vera* leaves. Briefly, 370 g of freshly cut leaves of *Aloe vera* were mixed with either 100 ml of 70% ethanol or 100 ml of distilled water and crushed in a blender, agitated on a shaker incubator for 12 hours, filtered through filter paper and then lyophilized in a lyophiliser. The yield for alcohol extract was 1.5% and for aqueous extracts it was 1.2%. Six day old Sprague-Dawley rats were gavaged with aqueous or alcohol extracts of *Aloe vera* (low dose 50mg/kg or high dose 500mg/kg) daily for eight days. All data were expressed as mean \pm SD and analysed by one way ANOVA. Pups receiving high doses of either extract had a significantly higher body mass gain than the group receiving lower dose (p < 0.05). Tibial length was significantly increased in the high dose aqueous extract group (15-26%). The differences in growth could not be attributed to circulating insulin-like

growth factor-1 as the levels were not significantly different. The caecum was significantly enlarged in the rats that received the high doses of both extracts. Although, there was no significant difference in the non-fasting plasma concentration of glucose and triglycerides, the hepatic lipid and glycogen content were significantly higher (p < 0.001) for the high dose aqueous extract group. The plasma alanine transaminase was not affected by the treatments, however the high doses of the extracts significantly increased plasma alkaline phosphatase activity. Short term administration of *Aloe vera* extracts resulted in growth promotion, enhanced hepatic storage of metabolic substrates, increased ALP possibly in relation to bone growth and caused hypertrophy of the caecum of neonatal rats. (Beya *et al., 2012*).

Data on hydroxyanthracene derivatives

After intragastric administration of sennosides at a dose of 2, 10, 20, 50, 100 mg/kg to pregnant rats and rabbits there was no evidence of any embryolethal, teratogenic or fetotoxic action. Furthermore, sennosides had no effect on the postnatal development of young animals, on the rearing behaviour of mother animals or on male or female fertility (Mengs, 1986).

The effects of sennosides on uterine motility were evaluated by electromyography in healthy adult ewes between day 70 and 120 of pregnancy to assess possible disturbances of the physiological pattern of contractility and eventual risks in pregnancy maintenance. At this stage of pregnancy, the ovine genital tract presented motility episodes of 6-8 min duration occurring at approximately hourly intervals. A dose-range study (10-160 mg/kg intracolonically) in 2 ewes showed that diarrhoea was systematically obtained with doses greater than 20 mg/kg and was connected with a marked depression of both ileum and spiral colon motility. A standard dose of 60 mg/kg administered intracolonically 1-3 times at 7- to 10-day intervals to 12 ewes was used in the uterus studies. The experiments showed that sennosides did not stimulate uterine motility in the pregnant ewe, but slightly depressed it in some ewes. Cervix motility was never influenced. Intolerance of the drug was observed in half of the animals resulting mainly in anorexia or weakness and confirming a specific toxicity of senna in ruminants which is not known from other species. These effects were not related to uterine motility and pregnancy maintenance was normal in all ewes (Garcia-Villar *et al.*, 1988).

Animal experiments demonstrated that placental passage of rhein is small. Aloe-emodin is quickly oxidised to rhein and an unknown metabolite, or conjugated.

Emodin was administered in feed to timed-mated Sprague-Dawley (CD) rats (0, 425, 850, and 1700 ppm; gestational day [GD] 6-20), and Swiss Albino (CD-1) mice (0, 600, 2500 or 6000 ppm; GD 6-17). Ingested dose was 0, 31, 57, and approximately 80-144 mg emodin/kg/day (rats) and 0, 94, 391, and 1005 mg emodin/kg/day (mice). Timed-mated animals (23-25/group) were monitored for body weight, feed/water consumption, and clinical signs. At termination (rats: GD 20; mice: GD 17), confirmed pregnant dams (21-25/group) were evaluated for clinical signs: body, liver, kidney, and gravid uterine weights, uterine contents, and number of corpora lutea. Fetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations/variations. There were no maternal deaths. In rats, maternal body weight, weight gain during treatment, and corrected weight gain exhibited a decreasing trend. Maternal body weight gain during treatment was significantly reduced at the high dose. In mice, maternal body weight and weight gain was decreased at the high dose. Prenatal mortality, live litter size, fetal sex ratio, and morphological development were unaffected in both rats and mice. At the high dose, rat average fetal body weight per litter was unaffected, but was significantly reduced in mice. The rat maternal lowest observed adverse effect level (LOAEL) was 1700 ppm; the no observed adverse effect level (NOAEL) was 850 ppm. The rat developmental toxicity NOAEL was > or =1700 ppm. A LOAEL was not established. In mice, the maternal toxicity LOAEL was

6000 ppm and the NOAEL was 2500 ppm. The developmental toxicity LOAEL was 6000 ppm (reduced fetal body weight) and the NOAEL was 2500 ppm (Jahnke *et al.*, 2004).

Based on the evaluation of developmental toxicity of emodin in rats and mice, the following values have been calculated (Jahnke *et al.*, 2004):

Mice maternal toxicity	LOAEL= 1005 mg/kg/day	NOAEL=391 mg/kg/day
Mice developmental toxicity	LOAEL= 1005 mg/kg/day	NOAEL=391 mg/kg/day
Rats maternal toxicity	LOAEL= 80-144 mg/kg/day	NOAEL=57 mg/kg/day
Rats developmental toxicity		NOAEL> 80-144 mg/kg/day

3.3.6. Local tolerance

Not applicable

3.3.7. Other special studies

Not applicable

3.3.8. Conclusions

Overall, available data are insufficient and the results of available investigations are not consistent. However, data are not appropriate to fully exclude the concern on genotoxic and carcinogenic potential. Therefore, use during pregnancy and lactation is contraindicated, although no irreversible changes or risks have been observed regarding reproduction toxicity *in vivo* when using the normal recommended doses. Other treatments like behavioural modification, dietary changes and use of bulk forming agents should be the first actions taken during pregnancy and lactation to treat constipation. Secondly, it is regarded appropriate to restrict the duration of use to a maximum of one week.

Use during lactation is contraindicated as there are insufficient data on the excretion of metabolites in breast milk, too. Investigations with a "standardised senna laxative", which also contains Plantago ovata seeds/husks as bulk substances, showed that small amounts of active metabolites (rhein) are excreted in breast milk. No laxative effect in breast fed babies has been reported (Faber P. and Strenge-Hesse A., 1988). Aloe-emodin is quickly oxidised to rhein and an unknown metabolite or conjugated. Data on excretion of aloe-emodin and emodin via milk on any species are lacking.

3.4. Overall conclusions on non-clinical data

Pharmacodynamic data regarding the *Aloe vera* latex containing preparations support their short term well-established use as a laxative. NTP Toxicology programme did not identify any cancerogenicity in recommended doses for emodin. The metabolite 2-hydroxyemodin acts as the genotoxin. The corresponding NTP programme on an *Aloe vera* whole leaf extract (without extract specification, HAD content unknown) showed in high posologies intestinal carcinomata. The relevance for humans is unclear.

As there is limited information on reproductive and developmental toxicity, but a potential genotoxicity and carcinogenicity the use during pregnancy and lactation is contraindicated. The duration of use is limited to a maximum of one week.

4. Clinical Data

4.1. Clinical pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Laxative effect

Constipation is said to be present when passed stools are of hard consistency and when evacuation of faeces is too difficult, too infrequent and irregular. The physiological range for frequency of bowel movements is wide, extending from three times daily to once every 2 to 3 days. In the pathogenesis of constipation the colon plays a key role because this is where the contents of the gut remain for 24 – 48 hours. During this period the liquid contents from the small intestine are converted into faeces by absorption of water and electrolytes in response to the action of bacteria. These functions are dependent on the interplay of peristaltic processes which mix the contents and the normal coordination of the anorectal muscles during defaecation. A disturbance involving any of these individual areas may lead to constipation. In this context, functional disturbances are far more common than those of an organic origin. In addition, assessment is problematic because the symptoms are perceived differently by the individuals affected (Ewe 1994, Gabler 1994), due to different concepts of what normal bowel habits are.

Aloe dried juice belongs to the stimulant laxatives. Aloe-emodin-9-anthrone is the main active metabolite, which acts specifically on the colon (Ishii *et al.* 1990).

Prolonged use of aloe containing preparations is associated with watery diarrhoea leading to electrolyte imbalance and the increased loss of potassium can lead to hypokalaemia. The increased loss of potassium is largely the result of compensatory reaction to the excessive loss of sodium, which induces a compensatory production of aldosterone that can exacerbate the hypokalemic condition and increase renin production (Mascolo *et al.*, 2004).

4.1.2. Overview of pharmakokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

The ESCOP monograph mentioned an unpublished research report of a human pharmacokinetic study in 6 healthy volunteers (ref. 39 in 3). After oral administration of aloes (equivalent to 16.4 mg of hydroxyanthracene derivates) for 7 days, aloe-emodin was detected as a metabolite in the plasma only sporadically and with maximum concentrations of less than 2 ng/ml. In the same study rhein was detected in the plasma in concentrations ranging from 6-28 ng/ml after single dose administration. In 7-day administration there was no evidence of accumulation of rhein.

In the pharmakokinetic study by Krumbiegel and Schulz (1993) therapeutic doses of two laxatives (Agiolax and Sennatin) were repeatedly administered to 10 healthy volunteers (18-32 years, male) in a two-way change-over design.4 single doses of Agiolax (6.3 g granulate containing 13,23 mg total anthronoids, including 7,62 mg potential rhein and 0,378 mg aloe-emodin) or Sennatin (2 tablets containing 20,36 mg total anthronoids including 13,06 mg potential rhein 0,4 mg potential aloe-emodin) were given to the volunteers at 24 h intervals. Blood samples were collected up to 96 h after the first dose, and plasma levels of total aloe-emodin and rhein were determined simultaneously with a sensitive (lower limit of quantification: 0.5 ng aloe-emodin and 2.5 ng rhein per millilitre plasma) and specific fluorometric HPLC method. Aloe-emodin was not detectable in any plasma sample of any subject. Rhein concentration time courses showed highest levels of 150-160 ng/ml and peak maxima

at 3-5 h and 10-11 h after dosing probably according to absorption of free rhein and rhein released from prodrugs (e.g. sennosides) by bacterial metabolism, respectively.

The absorbed rhein anthrone is glucuronidised in the liver. One part of the glucuronides is excreted via the urine and cause the yellow or red-brown discolouration of the urine. The other part is excreted via the bile (Lemli *et al.* 1980; Stolk and Hoogtanders 1999).

4.2. Clinical efficacy

Laxative effect

Constipation is said to be present when passed stools are of hard consistency and when evacuation of faeces is too difficult, too infrequent and irregular. The physiological range for frequency of bowel movements is wide, extending from three times daily to once every 2 to 3 days. In the pathogenesis of constipation the colon plays a key role because this is where the contents of the gut remain for 24 – 48 hours. During this period the liquid contents from the small intestine are converted into faeces by absorption of water and electrolytes in response to the action of bacteria. These functions are dependent on the interplay of peristaltic processes which mix the contents and the normal coordination of the anorectal muscles during defaecation. A disturbance involving any of these individual areas may lead to constipation. In this context, functional disturbances are far more common than those of an organic origin. In addition, assessment is problematic because the symptoms are perceived differently by the individuals affected (Ewe 1994; Gabler 1994), due to different concepts of what normal bowel habits are.

The prevalence of constipation in the worldwide general population ranged from 0.7% to 79% (median 16%). The epidemiology of constipation in children was investigated and prevalence rate was between 0.7% and 29.6% (median 12%) (Mugie *et al.* 2011).

Aloe dried juice belongs to the stimulant laxatives. Aloe-emodin-9-anthrone is the main active metabolite, which acts specifically on the colon (Ishii *et al.* 1990).

4.2.1. Dose response studies

There are no dose-finding studies available.

The recommended dosage as a laxative for adults, elderly and adolescents over 12 years (10 - 30 mg hydroxyanthracene derivatives only once daily at night) is supported by experts' opinions and by clinical investigations with other anthranoid-containing laxatives like senna preparations. The German Commission E monograph indicates a daily dose of 20 - 30 mg hydroxyanthracene derivatives calculated as aloin but recommends that the pharmaceutical form must allow lower dosages than the usual daily dose.

The ESCOP monograph and the WHO monograph recommend 10 – 30 mg hydroxyanthracene derivates.

This dosage recommendation is also given in consideration of the toxicological data, which were evaluated and led to pharmacovigilance actions in Germany for anthranoid-containing laxatives in 1996.

Through the individual product information (especially the package leaflet), patients should be informed that the correct individual dose is the smallest required to produce a comfortable soft-formed motion.

It is normally sufficient to take an anthranoid-containing laxative up to two to three times a week (Hitzenberger *et al.* 1999).

4.2.2. Clinical studies (case studies and clinical trials)

There are no clinical studies, which evaluate the efficacy of barbados aloes or cape aloes in patients with occasional constipation.

Koch (1995) evaluated the laxative effect of aloin in experiments on herself. Neither a dose of 20 mg aloin nor an increase to 60 mg aloin caused a laxative effect. Aloin was found in the faeces. The author also studied the use of aloe as a laxative in 3 patients given 50 mg aloin in a gelatine capsule in the evening at 8 p.m. Test person A (female) fed upon vegetable and animal products, test person E (female) predominantly fed upon fish and meat and test person H (male) was a vegetarian. Test person A experienced soft stools once at day 1 and 2 and normal stools at day 3. Test person E experienced soft stools at day 1 for four times. Test person H experienced soft stools once at day 1, 2 and 3. These different results corresponded to the cleavage of aloin and appearance of aloe-emodin in the faeces. Test person E consumed an oral ferric product additionally. This product seemed to support the cleavage of aloin. This was confirmed when test person A received a ferric product too. The author concluded that the laxative effect depends on the cleavage of aloin in aloe-emodin.

Odes et al. (1991) evaluated the effect of a laxative preparation, composed of celandine, Aloe vera and psyllium in patients with chronic constipation i.e. requiring laxative treatment for at least 2 years. The aloe preparation in this combination product derived from the leaves of Socotrine Aloes and also contained barbaloin and other anthraquinone derivatives. Capsules of 500 mg were made up to contain the active ingredients celandine, Aloe vera and psyllium in the ratio 6:3:1. Thirty five men and women were randomised to receive capsules containing celandine-Aloe vera-psyllium, or placebo, in a doubleblind trial including a 14-day basal period and a 28-day treatment period. Twenty one of these had simple constipation, and the others suffered from irritable bowel syndrome with constipation. Organic causes for constipation were excluded. Nineteen patients on celandine-Aloe vera-psyllium and 13 on placebo successfully completed the study. The initial dose was 1 capsule per day, taken with water at bedtime, and increasing to 3 capsules per day depending on the response. The patients kept a daily diary card during the basal and treatment periods and recorded: date, number of capsules taken, number of bowel actions, stool consistency, abdominal pain and distension, heartburn, other medications and fibre supplements taken to relieve constipation, medicinal products taken for other conditions, and fluid intake. Symptoms of the last 2 weeks of the treatment period were compared to those in the 14-day basal period. Patients on celandine-Aloe vera-psyllium took 10.1 +/- 4.1 capsules per week and patients on placebo 15.8 + - 6.9 capsules (p=0.02). The mean number of bowel actions per week in patients on celandine-Aloe vera-psyllium increased from 4.6 +/- 2.4 to 7.9 +/- 3.9 (p<0.002) and in the placebo group from 3.9 +/- 1.6 to 4.3 +/- 2.1 (p-value not mentioned). The stool consistency score decreased significantly in the verum group (p<0.002), while the placebo group demonstrated no changes. Subjects on celandine-Aloe vera-psyllium group had a higher basal pain score than those receiving placebo (p<0.005), and there was no statistically significant improvement in either this or the placebo group during the trial. Overall, 16 of 19 patients on celandine-Aloe verapsyllium regarded themselves as improved as compared with only 4 of the 13 patients on placebo (p<0.05). No subjects developed any side-effects from the treatment.

Assessor's Comment:

This investigation of a combination product of three herbal substances cannot establish the contribution of aloe to the observed effects. Furthermore, the herbal substance is "Socotrine Aloes", which does not correspond to barbados aloes and cape aloes described in this assessment report and

derives from a different species. This species however contains also barbaloin and other anthraquinone derivatives (Blaschek et al., 2003).

Kopp (1979) tested in an open study Chol-Kugeletten® for 10 days in a combined treatment of a total of 18 cholecystectomised patients or patients with gallstones, in comparison with a placebo (physiological saline solution) as to its choleretic properties and tolerability. Chol-Kugeletten® contains amongst others 25 mg aloe extract, 7 mg celandine extract, 5 mg curcuma root extract, 30 mg Fel Tauri depurat. sicc. (ox bile), 2.5 mg bisacodyl and 2.5 mg peppermint oil. Twelve patients received Chol-Kugeletten® and 6 patients placebo. Three patients of the verum group were excluded because of incomplete data. Before the therapy begun, and after 5 and 10 days of oral administration of one tablet three times a day, the secretion capacity of the individual patients was measured by means of an intraduodenal tube by Bartelheimer's method. At these dates the secretion capacity was measured before and hourly after intraduodenal application of 3.33 ml Chol-Kugeletten® suspension (corresponding to 1 Chol-Kugeletten® tablet) or placebo for five times. The choleresis could be raised significantly for hours. No further increase of the quantity of the secretion was obtained after 5 or 10 days of administration. The author concluded that there is a complete development of the action of the preparation that sets in immediately. Both the systemic and the local tolerability of Chol-Kugeletten® were good; the upper abdomen symptoms could be alleviated in both groups, but more with Chol-Kugeletten®. No details are given in the publication.

Assessor's Comment:

This investigation of a combination product of several agents cannot exactly show the contribution of aloe to the observed effects.

Siegers *et al.* (1993) reported about a retrospective study of 3,049 patients, who underwent diagnostic colorectal endoscopy. The incidence of pseudomelanosis coli was 3.13% in patients without pathological changes. In those with colorectal adenomas, the incidence increased to 8.64% (p<0.01), and in those with colorectal carcinomas it was 3.29%. This lower rate was probably caused by incomplete documentation of pseudomelanosis coli in those with carcinoma. In a prospective study of 1,095 patients, the incidence of pseudomelanosis coli was 6.9% for patients with no abnormality seen on endoscopy, 9.8% (p=0.068) for patients with adenomas and 18.6% for patients with colorectal carcinomas. From these data a relative risk of 3.04 (1.18, 4.9; 95% confidence interval) can be calculated for colorectal cancer as a result of chronic anthranoid laxative abuse.

Kune *et al.* (1988) and Kune (1993) reported about the "Melbourne Colorectal Cancer Study". Commercial laxative use as a risk factor in colorectal cancer was investigated as one part of this large population based epidemiological study of colorectal incidence, aetiology and survival. Commercial laxative use was similar in 685 colorectal cancer patients and 723 age/sex matched European Union based controls. Also, when laxatives were subdivided into various groups containing anthraquinones, phenolphthalein, mineral salts and others, previous laxative intake was similar between cases and controls. Previous use of anthraquinone laxatives and of phenolphthalein containing laxatives was not associated with the risk of colorectal cancer. Furthermore the results of this study suggest that chronic constipation, diarrhoea, and the frequency and consistency of bowel motions are unlikely to be etiologic factors in the development of colorectal cancer. They indicate that it is the diet and not the constipation that is associated with the risk of large-bowel cancer. Additionally, a highly statistically significant association (p=0.02) with the risk of colorectal cancer was found in those who reported constipation and also had a high fat intake.

In a retrospective study a cohort of 2,277 patients was defined by colonoscopy. Among other factors Nusko *et al.* (1993) tested whether in these patients laxative use or the endoscopically diagnosed

presence of melanosis coli were risk factors related to colorectal neoplasm. In comparison to patients taking no laxatives there was no significant increase in colorectal cancer rate either in laxatives users or in patients with melanosis coli. However, there was a statistically significant association between the occurrence of colorectal adenomas and laxative use (relative risk of all patients exposed to laxatives = 1.72; of patients exposed to laxatives without melanosis coli = 1.47). The relative risk of adenoma development in patients with melanosis coli was 2.19. Taking into account that polyps can be diagnosed in the dark mucosa of melanosis coli patients more easily, the authors concluded that even this relative risk of 2.19 seems to be related to a generally enhanced risk of laxative intake rather than to a special group of (anthranoid-containing) laxatives.

Sonnenberg *et al.* (1993) performed a meta-analysis, since individual case control studies failed to resolve the question whether constipation and use of cathartics (purgatives) represent significant risk factors of colorectal cancer. The analysis of 14 previously published (from 1954 to 1988) case control studies revealed statistically significant risks for colorectal cancer associated with both constipation and use of cathartics, the pooled odds ratios (OR) and their 95 percent confidence intervals being 1.48 (1.32-1.66) and 1.46 (1.33-1.61), respectively. The increased risk applied similarly to both sexes, it was higher in cancer of the colon than rectum. Since constipation and cathartics are associated with much lower odds ratios than various dietary components, such as fat, meat, alcohol, and low-vegetable or low-residue diets, the authors concluded that their risk reflects the confounding influence of underlying dietary habits.

Loew *et al.* (1994) conducted a comparative study involving 423 patients with colorectal neoplasms and 522 patients with benign proctologic disorders who were regular users of laxatives for bowel regulation. A pseudo melanosis coli (PMC) test was used as an indicator of exposure to anthranoidcontaining laxatives to determine if these preparations were potential colorectal carcinogenics. Results indicated no significant difference of the PMC rates between carcinoma (6.1%) and the control groups (4.2%) ($p \le 0.197$).

Jacobs and White (1998) examined the associations of colon cancer with constipation and use of commercial laxatives in a case-control study among men and women aged 30 - 62 years (424 incident cases and 414 random-digital-dial controls). Constipation was defined by "feeling constipated to the point of having to take something". The adjusted relative risk (RR) was 2.0 [95% confidence interval (CI) = 1.2-3.6] for constipation 12-51 times per year, and 4.4 (95%CI = 2.1-8.9) for constipation 52 or more times a year. Cumulative lifetime use of commercial laxatives was also associated with increased risk of colon cancer. When adjusted for constipation, commercial laxative use was no longer associated with increased risk (RR = 0.3, 95%CI = 0.1-0.9 for less than 350 uses; RR = 0.9, 95% CI = 0.4-2.3 for 350 or more uses). The association with constipation remained. In this study, no subject reported use of anthranoid-containing laxatives.

Nusko *et al.* (2000) performed a prospective case control study to investigate the risk of anthranoidcontaining laxative use for the development of colorectal adenomas or carcinomas. A total of 202 patients with newly diagnosed colorectal carcinomas, 114 patients with adenomatous polyp, and 238 patients (controls) with no colorectal neoplasm who had been referred for total colonoscopy were studied. The use of anthranoid preparations was assessed by standardised interview, and endoscopically visible or microscopic melanosis coli was studied by histopathological examination. There was no statistically significant risk of anthranoid use for the development of colorectal adenomas (unadjusted odds ratio 1.0; 95% CI 0.5-1.9) or carcinomas (unadjusted odds ratio 1.0; 95% CI 0.6-1.8). Even after adjustment for the risk factors age, sex, and blood in the stools by logistic regression analysis the odds ratio for adenomas was 0.84 (95% CI 0.4-1.7) and for carcinomas 0.93 (95% CI 0.5-1.7). Also, there were no differences between the patient and control groups for duration of intake. Macroscopic and high grade microscopic melanosis coli were not significant risk factors for the development of adenomas or carcinomas.

Willems *et al.* (2003) described a case of melanosis coli, which occurred in a 39 years old liver transplant patient, who took an over-the-counter product containing aloe, rheum and frangula. The typical brownish pigmentation of the colonic mucosa developed in a period of ten months. The anthranoid medication was stopped and follow-up colonoscopy one year later showed normal looking mucosa once more. However, in contrast to previous examinations, a sessile polypoid lesion was found in the transverse colon. Histology showed tubulovillous adenoma with extensive low-grade dysplasia. Since there had been preliminary reports suggesting a possible role of anthranoid-containing laxatives in the development of colorectal adenomas and cancer, the authors discouraged their use.

Roberts *et al.* (2003) conducted a population-based, case-control study with equal representation by white and black men and women aged 40 - 80 years. Constipation, defined as fewer than three reported bowel movements per week, was associated with a greater than two-fold risk of colon cancer (OR 2.36; 95% CI = 1.41-3.93) adjusted for age, race, sex, and relevant confounders. The OR for constipation was slightly higher for distal than for proximal colon cancers. There was no association with laxative use (OR 0.88; 95% CI = 0.69-1.11). The authors did not explicitly mention anthraquinone-containing laxatives. They mentioned the group "stimulants, fibers, natural remedies, stool softeners, oils, osmotic agents, enemas, suppositories, and unknown". They mention in particular phenolphthalein and magnesium.

Nilsson *et al.* (2004) examined the impact of constipation and laxative treatment on the blood levels of homocysteine, folate and cobalamine in a population-based sample of aged people. Elevated plasma homocysteine secondary to reduced supply of folate and cobalamine, might indicate an increased risk of cancer, and cardiovascular and neurological diseases. The homocysteine level depends on the supply of folate and cobalamine, which constipation and/or laxative treatment might compromise. The study was based on biochemical tests in 341 females and 183 males aged 82 years and older. The concentrations of homocysteine (plasma), folate, cobalamine and urea (serum) were measured in subjects with and without ongoing treatment with laxative products. Values were adjusted for age, gender and frailty, as well as for clinical diagnoses and medicinal product therapies known to affect homocysteine levels. Homocysteine levels were increased and those of folate reduced in aged subjects on laxatives. Homocysteine remained elevated after adjusting for frailty and various neurological disorders. There was no significant effect on homocysteine and folate in constipated subjects without laxatives.

Joo *et al.* (1998) investigated changes occurring on barium enema in patients ingesting stimulant laxatives. The study consisted of two parts. In part 1, a retrospective review of consecutive barium enemas performed on two groups of patients with chronic constipation (group 1, stimulant laxative use (n=29); group 2, no stimulant laxative use (n=26)) was presented to a radiologist who was blinded to the patient group. A data sheet containing classic descriptions of cathartic colon (historic term for the anatomic alteration of the colon secondary to chronic stimulant laxative use) was completed for each study. Chronic stimulant laxative use was defined as stimulant laxative ingestion more than three times per week for 1 year or longer. To confirm the findings of the retrospective study, 18 consecutive patients, who were chronic stimulant laxative users, underwent barium enema examination, and data sheets for cathartic colon were completed by another radiologist (part 2). Colonic redundancy (group 1, 34.5%; group 2, 19.2%) and dilatation (group 1, 44.8%; group 2, 23.1%) were frequent radiographic findings in both patient groups and were not significantly different in the two groups. Loss of haustral folds, however, was a common finding in group 1 (27.6%) but was not seen in group 2 (p<0.005). Loss of haustral markings occurred in 15 (40.5%) of the total stimulant laxative users in

the two parts of the study and was seen in the left colon of 6 (40%) patients, in the right colon of 2 (13.3%) patients, in the transverse colon of 5 (33.3%) patients, and in the entire colon of 2 (13.3%) patients. Loss of haustra was seen in patients chronically ingesting bisacodyl, phenolphthalein, senna, and casanthranol. The authors concluded that long-term stimulant laxative use results in anatomic changes in the colon characterised by loss of haustral folds, a finding that suggest neuronal injury or damage to colonic longitudinal musculature caused by these agents.

Add-on therapy of Aloe and different polychemotherapies in different metastatic tumortumour entities

A study was planned to include 240 patients with metastatic solid tumour who were randomized to receive chemotherapy with or without Aloe. According to tumour histotype and clinical status, lung cancer patients were treated with cisplatin and etoposide or weekly vinorelbine, colorectal cancer patients received oxaliplatin plus 5-fluorouracil (5-FU), gastric cancer patients were treated with weekly 5-FU and pancreatic cancer patients received weekly gemcitabine. *Aloe arborescens* was given orally at a dose of 10 ml thrice daily of a mixture consisting of 300 g of Aloe fresh leaves in 500 g of honey plus 40 ml of 40% alcohol (m/m or V/V unknown), every day without interruption, either during or after chemotherapy, until the progression of disease, starting 6 days prior to the onset of chemotherapy. The clinical response and toxicity were assessed according to WHO criteria. The clinical responses were radiologically evaluated after at least three cycles of chemotherapy by repeating the same radiological investigation used prior to the onset of chemotherapy, including CT scan, NMR and PET. The percentage of both objective tumour regressions and disease control was significantly higher in patients concomitantly treated with Aloe than with chemotherapy alone, as well as the percent of 3 years survival patients. The results were statistically analysed by the chi-square test, Student's *t*-test and analysis of variance, as appropriate (Lissoni *et al.*, 2009):

Tumour	Poly-chemotherapy	Time frame
Small cell lung cancer (SCLC)	Cisplatin 20 mg/m ² i.v.	3 days / 21days
	Etoposide 100mg/m ² i.v.	
Non-small cell lung cancer (NSCLC)	Vinorelbine (VNR) 25 mg/m ² i.v.	weekly
Colorectal cancer	Oxaliplatin 85 mg/m ²	Day 1 and 8/28 days
	5-FU 500 mg/m ²	Day 1,8,15/28 days
	Folates 10 mg/m ²	Day 1,8,15/28 days
Gastric cancer	5-FU 375 mg/m ²	weekly
	Folates 10 mg/m ²	
Pancreatic Adenocarcinoma	Gemcitabine 1,000 mg/m ²	weekly





Assessor 's comment:

In spite of the summarized patients suffering from 5 different metastatic tumours entities and the not transferable extract specification the 3 year survival data study at least gives a hint that aloe is not cancerogenic especially in colorectal cancers.

The dried latex of the aloe plant (aloes) is one of several traditional remedies used for diabetes in the Arabian Peninsula. Its ability to lower the blood glucose was studied in 5 patients with non-insulindependent diabetes and in Swiss albino mice made diabetic using alloxan. During the ingestion of aloes (dried resin, HAD content unknown), half a teaspoonful daily for 4-14 weeks, the fasting serum glucose level fell in every patient from a mean of 273 +/- 25 (SE) to 151 +/- 23 mg/dl (p less than 0.05) with no change in body weight. In normal mice, both glibenclamide (10 mg/kg twice daily) and aloes (500 mg/kg twice daily) induced hypoglycaemia after 5 days, 71 +/- 6.2 and 91 +/- 7.6 mg/dl, respectively, versus 130 +/- 7 mg/dl in control animals (p less than 0.01); only glibenclamide was effective after 3 days. In the diabetic mice, fasting plasma glucose was significantly reduced by glibenclamide and aloes after 3 days. Thereafter only aloes was effective and by day 7 the plasma glucose was 394 +/- 22.0 versus 646 +/- 35.9 mg/dl, in the controls and 726 +/- 30.9 mg/dl in the glibenclamide treated group (p less than 0.01) (Ghannam *et al*, 1986).

Туре	Study	Test Product(s)	Number of	Type of	Outcomes	Statistical	Clinical
			subjects	subjects		analysis	relevance
Odes et al.,	Double blind	Capsules of 500 mg	35 m/f	Chronic constipation	Comparison 2 weeks pre-	Information	As a
1991	Randomized	were made up to	19 verum	>2 years	to last 2 weeks of	not	combinatio
	14d pre	contain the active	13 placebo		treatment	available	n product is
	28 days	ingredients celandine,	3 drop outs		Patients on celandine-		used the
	treatment	Aloe vera and psyllium			Aloe vera-psyllium took		results are
		in the ratio 6:3:1.			10.1 +/- 4.1 capsules per		only a
		Increasing 1-3 caps.			week and patients on		weak
					placebo 15.8 +/- 6.9		support to
					capsules (p=0.02). The		the laxative
					mean number of bowel		use
					actions per week in		
					verum patients increased		
					from 4.6 +/- 2.4 to 7.9		
					+/- 3.9 (p<0.002) and in		
					the placebo group from		
					3.9 +/- 1.6 to 4.3 +/- 2.1		
					(p-value not mentioned		
Siegers et al.,	Retrospective	Information not	3049 patients	Diagnostic colorectal	Incidence of	Information	Not
1993		available		endoscopy	pseudomelanosis coli was	not	relevant
					3.13% in healthy	available	regarding
					probands		efficacy
					with colorectal		
					adenomas, the incidence		
					increased to 8.64%		
					(p<0.01),		
					with colorectal		
					carcinomas it was 3.29%.		

Туре	Study	Test Product(s)	Number of	Type of	Outcomes	Statistical	Clinical
			subjects	subjects		analysis	relevance
Siegers et al.,	Prospective	Information not	1095	In healthy	9.8% (p=0.068) for	Relative risk	Not
1993		available		Patients the incidence	patients with adenomas	of 3.04	relevant
				of pseudomelanosis	18.6% for patients with	(1.18, 4.9;	regarding
				coli was 6.9%	colorectal carcinomas	95%	efficacy
						confidence	
						interval) for	
						colorectal	
						cancer as a	
						result of	
						chronic	
						anthranoid	
						laxative	
						abuse	
Kune <i>et al.</i> ,	Information	Information not	Information	Information not	Commercial laxative use	Information	No relevant
1988/1993	not available	available	not available	available	was similar in 685	not	cancerogen
					colorectal cancer patients	available	i-city
					and723 age/sex matched		
					European Union based		
					controls. Also, when		
					laxatives were subdivided		
					into various groups		
					containing		
					anthraquinones,		
					phenolphthalein mineral		
					salts and others, previous		
					laxative intake was		
					similar between cases		
					and controls. Previous		
					use of anthraquinone		

Туре	Study	Test Product(s)	Number of	Type of	Outcomes	Statistical	Clinical
			subjects	subjects		analysis	relevance
					laxatives and of phenolphthalein containing laxatives was not associated with the risk of colorectal cancer.		
Nusko <i>et al.</i> , 1993	Retrospective	Information not available	2277	Colonoscopy	Occurrence of colorectal adenomas and laxative use (relative risk of all patients exposed to laxatives = 1.72; of patients exposed to laxatives without melanosis coli = 1.47). The relative risk of adenoma development in patients with melanosis coli was 2.19.	Information not available	The Relative risk of adenoma developme nt in patients with melanosis coli was 2.19.
Loew <i>et al.,</i> 1994	Comparative	Information not available	423 patients with colorectal neoplasms 522 patients with benign proctologic disorders regular laxative users	Information not available	No significant difference of the PMC rates between carcinoma (6.1%) and the control groups (4.2%) (p≤0.197).	Information not available	Not relevant for efficacy
Jacobs and White 1998	Case-control study	Information not available	(424 incident cases and	Information not available	The adjusted relative risk (RR) was 2.0 [95%	Information not	Not relevant for

Туре	Study	Test Product(s)	Number of	Type of	Outcomes	Statistical	Clinical
			subjects	subjects		analysis	relevance
			414 random-		confidence interval (CI) =	available	efficacy
			digital-dial		1.2-3.6] for constipation		
			controls		12-51 times per year,		
					and 4.4 (95%CI = 2.1-		
					8.9) for constipation 52		
					or more times a year.		
					When adjusted for		
					constipation, commercial		
					laxative use was no		
					longer associated with		
					increased risk (RR = 0.3,		
					95%CI = 0.1-0.9 for less		
					than 350 uses; RR = 0.9,		
					95% CI = 0.4-2.3 for 350		
					or more uses).		
Nusko <i>et al.,</i>	Prospective	Information not	202 patients	Information not	No statistically significant	Information	Not
2000	case control	available	with newly	available	risk of anthranoid use for	not	relevant for
	study		diagnosed		the development of	available	efficacy
			colorectal		colorectal adenomas		
			carcinomas		(unadjusted odds ratio		
			238 patients		1.0; 95% CI 0.5-1.9) or		
			controls		carcinomas (unadjusted		
					odds ratio 1.0; 95% CI		
					0.6-1.8).		
Roberts et al.,	Population-	Information not	Information	Information not	Constipation was	Information	Constipatio
2003	based, case-	available	available	available	associated with a greater	not	n was
	control study				than two-fold risk of	available	associated
					colon cancer (OR 2.36;		with a

Туре	Study	Test Product(s)	Number of	Type of	Outcomes	Statistical	Clinical
			Subjects	Subjects	95% CI = 1.41-3.93) OR for constipation was slightly higher for distal than for proximal colon cancers No association with laxative use (OR 0.88; 95% CI = 0.69-1.11)		greater than two- fold risk of colon cancer
Joo <i>et al.,</i> 2004	Information is not available	Information not available	Information not available	Retrospective review of consecutive barium enemas performed on two groups of patients with chronic constipation (group 1, stimulant laxative use (n=29); group 2, no stimulant laxative use (n=26))	Loss of haustral markings occurred in 15 (40.5%) of the total stimulant laxative users Loss of haustra was seen in patients chronically ingesting bisacodyl, phenolphthalein, senna, and casanthranol	Information not available	Not relevant for efficacy
Lissoni <i>et al.,</i> 2009	Prospective controlled randomized CT	Chemotherapy with or without Aloe 10 ml thrice daily of a mixture consisting of 300 g of Aloe fresh leaves in 500 g of honey plus 40 ml of 40% alcohol (m/m or (V/V) unknown	240	Patients with metastatic solid tumor	The percentage of both objective tumor regressions and disease control was significantly higher in patients concomitantly treated with Aloe than with chemotherapy alone	Chi-square test, Student's <i>t</i> - test and analysis of variance	3 year oncology data: no cancerogen i-city in colorectal tumor patients

4.3. Clinical studies in special populations (e.g. elderly and children)

Generally, a change of nutrition is recommended in constipated children via an increase in daily fibre intake. According to the recommendations from a conference on dietary fibre in childhood, children older than 2 years of age should increase their intake of dietary fibre (increased consumption of a variety of fruits, vegetables, cereal and other grain product) to an amount equal or greater than their age plus 5 g (e.g. 8 g/day at age 3) (Williams *et al.*, 1994). Change in nutrition should be accompanied with behaviour modification, e.g. increased physical exercise. There are no systematic clinical data available, which evaluate the use of aloe dried juice as a laxative in children.

According to the ESCOP and WHO monographs, the use in children under 10 years of age cannot be recommended.

According to the "Note for guidance on clinical investigation of medicinal products in the paediatric population" (CPMP/ICH/2711/99), the age limit between "children" and "adolescents" is set to "12 years of age".

4.4. Overall conclusions on clinical pharmacology and efficacy

In the absence of clinical studies, the laxative effect of barbados aloes and cape aloes is mainly based on pharmacological data, clinical experiences and class-effects comparable with other hydroxyanthracene derivatives containing herbal substances. Clinical and pharmacological data obtained on other anthranoid-containing laxatives (please refer to the assessment report on "Cassia senna L. and Cassia angustifolia Vahl, folium") support the efficacy of these anthranoid-containing herbal substances for short-term use in cases of occasional constipation.

Other effects have been predominantly investigated in experimental studies. Adequate clinical trials are not available.

Because of the possible genotoxic or tumorigenic risk in experimental investigations and the results of Siegers (1993), pharmacovigilance actions for anthranoid-containing laxatives were initiated in Germany in 1996: the daily dose and the duration of administration were limited and children, pregnant women and nursing mothers were excluded from the application of aloe containing laxatives.

The results of the most recent studies are inconsistent and the question of a possible carcinogenic risk of long-term use of anthranoid-containing laxatives is still open. Some studies revealed a risk for colorectal cancer associated with the use of anthraquinone-containing laxatives, some studies did not. However, a risk was also revealed for constipation itself and underlying dietary habits. Further investigations are needed to determine the carcinogenic risk definitely.

Long-term administration of anthranoid-containing medicinal products leads to the development over a period of 4 - 13 months of pseudomelanosis coli – pigmentation of the gut wall in the caecum and colon. This condition is produced by the accumulation of macrophages that have stored a brown pigment from the breakdown products of anthranoid (probably lipofuscin) and consequently cause the mucosa to appear brown to blackish-brown in colour. Prevalence among patients with chronic constipation is reported to be 12 - 31%, and 62% following chronic ingestion of anthranoid-containing laxatives. This finding disappears 6 - 12 months after stopping chronic laxative administration.

Long-term stimulant laxative use may result in anatomic changes in the colon characterised by loss of haustral folds. These considerations also contribute to the limitation of the maximum duration of use.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

The relationship between sigmoid cancer (SC) and constipation, anthranoid laxative use, and melanosis coli using ACF analysis (aberrant crypt foci analysis) was investigated. Fifty-five surgical patients with SC, 41 surgical patients with diverticular disease (DD), and 96 age- and sex-matched subjects without intestinal disease (controls) were interviewed on their history of constipation and anthranoid laxative use. Melanosis coli and ACF characteristics were investigated on sigmoid mucosa in patients with SC or DD. Constipation and anthranoid laxative use were similar between patients with SC (30.9% and 32.7%, respectively) and those with DD (39% and 26.8%) but higher than among controls (18.8% and 8.3%). Melanosis coli was found in 38.2% of patients with SC and in 39% of those with DD. Mean ACF frequency was higher in patients with SC (0.24/cm(2)) than in those with DD (0.10/cm(2); P < 0.0001), and it did not vary according to constipation, laxative use, or melanosis coli in either group. This study confirms the association of ACF frequency with colon cancer and does not support the hypothesis of a cause-effect relationship of CRC with constipation, anthranoid laxative, use or melanosis coli (Nascimbeni *et al.*, 2002).

 Table 6: Clinical safety data from clinical trials

Туре	Study	Test Product(s)	Number of	Type of subjects	Adverse reactions	Comments
			subjects			
Nascimbeni et	prospective	Not specified	55 SC	Fifty-five surgical	Melanosis coli and ACF	Melanosis coli had
<i>al.,</i> 2002			41 DD	patients with	characteristics were	no prevalence for
			96 controls	sigmoid cancer	investigated on sigmoid	SC.
				(SC), 41 surgical	mucosa in patients with	
				patients with	sigmoid cancer (SC) or DD.	
				diverticular	Constipation and	
				disease (DD), and	anthranoid laxative use were	
				96 age- and sex-	similar between patients with	
				matched subjects	SC (30.9% and 32.7%,	
				without intestinal	respectively) and those with	
				disease (controls)	DD (39% and 26.8%) but	
				were interviewed	higher than among controls	
				on their history of	(18.8% and 8.3%).	
				constipation and	Melanosis coli was found in	
				anthranoid laxativ	38.2% of patients with SC	
				e use.	and in 39% of those with	
					DD.	

5.2. Patient exposure

No data available. The above cited studies do not include specific data for consumption of Aloe extracts standardized for their HAD content. The frequency of adverse reactions can therefore not be specified.

5.3. Adverse events, serious adverse events and deaths

Chronic use or abuse of aloe dried juice preparations may lead to hypokalaemia. This hypokalaemia and the increased loss of potassium may increase the activity of cardiac glycosides and interfere with the action of anti-arrhythmic agents (interaction with antiarrhythmic medicinal products, which induce reversion to sinus rhythm, e.g. quinidine) and medicinal products inducing QT-prolongation (Haverkamp *et al.*, 2002). Concomitant use with medicinal products inducing hypokalaemia (e.g. diuretics, adrenocorticosteroids and liquorice root) may aggravate electrolyte imbalance.

Prolonged use of aloe containing preparations is associated with watery diarrhoea leading to electrolyte imbalance and the increased loss of potassium can lead to hypokalaemia (Cooke, 1981).

A case of a 47 years old man from Soweto, South Africa is reported, who developed acute oliguric renal failure and liver dysfunction after ingestion of an herbal remedy. The patient's renal function recovered slowly, and dialysis was discontinued after several weeks, although serum creatinine did not return to the normal range. Mass spectrometric and chromatographic analysis of the herbal remedy used by the patient revealed the presence of Cape aloes (Luyckx *et al.*, 2002).

Lee *et al.* (2004) described a patient with massive intraoperative bleeding after oral consumption of *Aloe vera* tablets. A 35 years old woman lost 5I of blood during surgery as a result of a possible interaction between *Aloe vera* and sevoflurane. The authors stated that compounds contained in *Aloe vera* can cause a reduction in prostaglandin synthesis, which may inhibit secondary aggregation of platelets. Sevoflurane inhibits thromboxane A2 formation by suppression of cyclooxygenase activity, impairs platelet aggregation, and prolongs bleeding. Although the vascularity and size of the haemangioma were the most important factors for the massive intraoperative blood loss, the authors concluded that concomitant use of sevoflurane and *Aloe vera* played a contributory role and that this adverse event was possible as a result of the sevoflurane and *Aloe vera* interaction.

Assessor's Comment:

The information given in the publication is insufficient. The Aloe vera preparation may not be comparable to the aloe preparations assessed in this report.

Increased loss of potassium may potentiate the actions of conventional drugs such as cardiac glycosides and corticosteroids. Such interactions may result in cardiac arrhythmias and hypertension.

5.4. Laboratory findings

Not applicable

5.5. Safety in special populations and situations

The importance of hydration and electrolyte replacement has been recognized in the management of patients with hyperosmolar hyperglycemic syndrome (HHS) (Kitabchi *et al.* 2001, Waldhäusl *et al.* 1979). Isotonic saline (0.9% NaCl) is recommended at 15–20 ml/kg during the first 1–2 h, followed by 250–500 ml/h until resolution of the hyperglycemic crisis. Fluid replacement alone has been shown to reduce glucose concentration by 75–100 mg/h, due to a reduction in counter regulatory hormones and

improvement of renal perfusion (Matz, 1999). In addition, many patients with HHS have high serum potassium despite total body potassium deficit due to insulin deficiency and hyperosmolality, which cause a shift of potassium from the intracellular compartment into plasma (Cruz-Caudillo and Sabatini, 1995, Ennis *et al.*, 1994). During insulin treatment and hydration, serum potassium levels rapidly fall; therefore, it is recommended that potassium replacement should be initiated when serum levels fall <5.5 mEq/l, with the goal to maintain a serum potassium concentration in the range of 4–5 mEq/l (Pasquel and Umpierrez, 2014).

The basics of treatment of a diabetic ketoacidosis (DKA) are intravenous fluid replacement with large volumina and the intravenous substitution with insulin as well. An additional replacement of high amounts of potassium is critical, because the efficacy of the insulin shifts the potassium from the extracellular volume into the cells. From the hyperkalemia a hypokalemia is developing. With underlying potassium depletion from chronic laxative abuse a higher intravenous potassium supplement is essential (Gosmanov *et al.*, 2014).

5.5.1. Use in children and adolescents

Children should not use hydroxyanthracene containing products to treat constipation. Diet changes and physical exercise should be used to change bowel habits.

5.5.2. Contraindications

Aloe preparations should not be used by patients with known hypersensitivity to aloe.

There are several publications available dealing with allergic reactions. Most of all these reactions were caused by local application and the aloe preparations used are not exactly specified. Ernst (2000) reported that remedies which can cause dermatological side-effects include *"Aloe vera"* besides others. Anliker *et al.* (2002) described a case of an anaphylactic shock due to local application of *"Aloe vera"* leaves". Schempp *et al.* (2002) categorised *"aloe"* as sensitising plant in cosmetics.

Furthermore, like all anthranoid-containing laxatives, aloe-containing medicinal products should not be used in cases of intestinal obstructions and stenosis, atony, appendicitis, inflammatory colon diseases (e.g. Crohn's disease, ulcerative colitis), abdominal pain of unknown origin, severe dehydration states with water and electrolyte depletion.

5.5.3. Special warnings and precautions for use

The following warnings and precautions for use are recommended:

- Long-term use of stimulant laxatives should be avoided, as use for more than a brief period of treatment may lead to impaired function of the intestine and dependence on laxatives. If laxatives are needed every day the cause of the constipation should be investigated. Senna pod preparations should only be used if a therapeutic effect cannot be achieved by a change of diet or the administration of bulk forming agents.
- Patients taking cardiac glycosides, antiarrhythmic medicinal products, medicinal products inducing QT-prolongation, diuretics, adrenocorticosteroids or liquorice root, have to consult a doctor before taking aloes concomitantly.
- Like all laxatives, aloes should not be taken by patients suffering from faecal impaction and undiagnosed, acute or persistent gastro-intestinal complaints, e.g. abdominal pain, nausea and

vomiting unless advised by a doctor because these symptoms can be signs of potential or existing intestinal blockage (ileus).

- Patients with kidney disorders should be aware of possible electrolyte imbalance.
- If the symptoms worsen during the use of the medicinal product, a doctor or a pharmacist should be consulted.
- For liquid dosage forms containing ethanol the appropriate labelling for ethanol, taken from the 'Guideline on excipients in the label and package leaflet of medicinal products for human use', must be included.

It cannot be definitely assessed if a longer than a brief period of treatment with stimulant laxatives leads to dependence requiring increasing quantities of the medicinal product, to an atonic colon with impaired function and to aggravation of the constipation.

Müller-Lissner (2005) concluded in his review that the arguments in favour of laxative-induced damage to the autonomous nervous system of the colon are based on poorly documented experiments and that, in contrast, the investigations that do not support such damage are well done. The studies in the cited references (Smith 1968; Riemann *et al.* 1980 and 1982; Berkelhammer *et al.*, 2002; Meisel *et al.*, 1977; Pockros and Foroozan, 1985) showed abnormalities observed in humans (damage to enteric nerves, smooth muscle atrophy; distension or ballooning of axons, reduction of nerve-specific cell structures and increase in lysosomes, and sometimes a total degeneration of whole nerve fibres; short-lived superficial damage to the mucosa). They were uncontrolled observations and the author therefore concluded that the cause of these damages can also be the constipation itself or pre-existing changes of unknown aetiology.

The only study comparing the morphology of the autonomous nervous system of constipated patients taking anthraquinones (Aloe) to that of an appropriate control group of constipated patients without laxative intake (Riecken *et al.*, 1990) did not support the hypothesis that anthraquinone-containing laxatives are able to provoke relevant degenerative changes in the colonic nerve tissue. But this investigation was conducted in 11 matched pairs only.

5.5.4. Drug interactions and other forms of interaction

The available data on pharmacokinetic interactions do not reveal any specific pharmacokinetic interaction. Interactions associated with potassium metabolism are addressed in the monograph in the section "Interactions".

Hypokalaemia (resulting from long-term laxative abuse) potentiates the action of cardiac glycosides and interacts with antiarrhythmic medicinal products. Concomitant use with diuretics, adrenal corticosteroids and liquorice root may enhance loss of potassium.

5.5.5. Fertility, pregnancy and lactation

Safety during pregnancy and lactation has not been established. In the absence of sufficient data and because of concerns on genotoxic and cancerogenic potential, the use during pregnancy and lactation is contraindicated.

5.5.6. Overdose

Like for all anthranoid-containing laxatives, the major symptoms of overdose/abuse are griping pain and severe diarrhoea with consequent losses of fluid and electrolyte, which should be replaced. Diarrhoea may cause potassium depletion, in particular. Potassium depletion may lead to cardiac disorders and muscular asthenia, particularly where cardiac glycosides, diuretics or adrenocorticosteroids are being taken at the same time.

Treatment should be supportive with generous amounts of fluid. Electrolytes, especially potassium, should be monitored. This is especially important in the elderly.

Furthermore chronic ingestion of overdoses of anthranoid-containing medicinal products may lead to toxic hepatitis (see below).

Hepatitis

Beuers *et al.* (1991) reported a case of toxic hepatitis related to abuse of senna glycosides in a 26 years old female, who had taken an extract of senna fruits corresponding to 100 mg of sennoside B daily in addition to the usual dose of 10 g senna leaves twice a week in a laxative tea. When the patient stopped taking senna, aminotransferases fell by 70% within a week and ranged from 20 - 40 U/I subsequently. When the patient took senna alkaloids again, 2 months later, liver function rapidly deteriorated and improved once more when the product was stopped.

Vanderperren *et al.* (2005) reported a case of a 52 years old woman, who had ingested, for more than 3 years, one litre of an herbal tea each day made from a bag containing 70 g of dry senna fruits. She developed renal impairment and acute hepatic failure with increase in prothrombin time (international normalised ratio > 7) and development of encephalopathy. The patient recovered with supportive therapy. Surprisingly, large amounts of cadmium were transiently recovered in the urine.

According to the Rucam score (Roussel UCLAF causality assessment method - for detailed information, please see the assessment report on "Cassia senna L. and Cassia angustifolia Vahl, folium"), these hepatoxic cases are related to the chronic ingestion of overdoses. Concentrated and dried juice of the leaves of *Aloe barbadensis* Mill. and *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids) being anthranoid-containing herbal substances, the possibility of toxic hepatic reactions is referred to in the section 'Overdose' of the European Union herbal monograph on aloe.

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

Not applicable

5.5.8. Safety in other special situations

Like all anthranoid-containing laxatives, aloe preparations may produce abdominal pain and colicky gastrointestinal symptoms and passage of liquid stools, in particular in patients with irritable colon. However, these symptoms may also occur generally as a consequence of individual over dosage. In such cases dose reduction is necessary. The correct individual dose is the smallest required to produce a comfortable soft-formed motion.

As mentioned above hypersensitive reactions may occur.

Chronic use may lead to disorders in water equilibrium and electrolyte metabolism, and may result in albuminuria and haematuria.

Furthermore, use over a long period may lead to pigmentation of the intestinal mucosa (pseudomelanosis coli), which usually recedes when the patient stops taking the preparation (see section 4.4).

Yellow or red-brown (pH dependent) discolouration of urine by metabolites, which is not clinically significant, may occur during the treatment (see section 4.1.2).

Luyckx *et al.* reported 2002 a case of a 47 years old man from Soweto, South Africa, who developed acute oliguric renal failure and liver dysfunction after ingestion of an herbal remedy. The patient's renal function recovered slowly, and dialysis was discontinued after several weeks, although serum creatinine did not return to the normal range. Mass spectrometric and chromatographic analysis of the herbal remedy used by the patient revealed the presence of "Cape aloes".

The causality cannot be assessed definitely.

The German Health Authority has received one report of an adverse event concerning a monopreparation (a Mexican aloe extract). A 45 years old patient, who regularly takes levothyroxine, developed glycosuria, albuminuria, haematuria, and leucocyturia after taking the aloe extract. This patient was suspected to have a toxic-interstitial nephritis and nutritive-toxic tubular injury. One week after dechallenge the urinary findings improved. The aloe extract was suspected to cause these adverse events. The extract was not specified. Therefore an assessment whether the extract is comparable with the preparations described in this assessment report or not is not possible. Nevertheless albuminuria and haematuria are known adverse reactions of chronic misuse of aloe preparations.

5.6. Overall conclusions on clinical safety

The above mentioned safety concerns are balanced by an adequate benefit in treating short term constipation.

6. Overall conclusions (benefit-risk assessment)

Well-established use

Short term use of occasional constipation.

There are no clinical studies available, which evaluate the clinical efficacy of barbados aloes and cape aloes in patients with occasional constipation. Well-designed studies with mono-preparations of *Aloe vera* containing laxatives are missing. There are herbal medicinal products with herbal preparations of Aloe, folii succus siccatus on the market, which fulfil the criteria of a marketing authorization for the period of ten years.

The postulated laxative effect is mainly based on pharmacological data, clinical experiences and analogy to class-effects of other hydroxyanthracene derivatives. Clinical and pharmacological data obtained on other anthranoid-containing laxatives (primarily senna leaf preparations) support the efficacy of these anthranoid-containing herbal substances for short-term use in cases of occasional constipation.

The use in children under 12 years of age, during pregnancy and lactation is contraindicated.

Due to the particulars of hydroxyanthracene derivatives adequate precautions and special warnings are necessary. With respect to misuse for longer periods and especially because of some remaining concerns on carcinogenicity the duration of use is limited to a maximum use of one week.

The hydroxyanthracene containing plant extracts are standardised extracts according to their hydroxynthracene content.

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Traditional use

Besides the use as a laxative, the use as an emmenagogue and the external use for wounds and abscess are described in most references mentioned above. But as already mentioned in the Dispensatory of the United States of America 1918, it is extremely doubtful whether aloe exercises any action upon the pelvic organs which is not attributable to its cathartic effects. There are no plausible pharmacological data for this indication, nor for haemoptysis, jaundice or gout etc. Furthermore, the preparations used are not described sufficiently. For external use there are no defined preparations.

A monograph on traditional use is not established as the above mentioned references are not sufficient to prove that the respective products are not harmful in the specified conditions of use and that the pharmacological effects or efficacy of the medicinal product are plausible.

Annex

List of references