European Medicines Agency
Post-authorisation Evaluation of Medicines for Human Use

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ASSESSMENT REPORT FOR

Rhubarb (Rhei radix)

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I.

REGULATORY STATUS OVERVIEW[1]

MA: Marketing Authorisation;
TRAD: Traditional Use Registration;
Other TRAD: Other national Traditional systems of registration;
Other: If known, it should be specified or otherwise add 'Not Known'

Member State	**Regulatory Status**				**Comments**[2]
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	

[1] This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.
[2] Not mandatory field

II. ASSESSMENT REPORT FOR HERBAL SUBSTANCE(S), HERBAL PREPARATION(S) OR COMBINATIONS THEREOF WITH WELL-ESTABLISHED USE AND/OR TRADITIONAL USE

RHUBARB (RHEI RADIX) AND HERBAL PREPARATION(S) THEREOF WITH WELL-ESTABLISHED USE AND/OR TRADITIONAL USE

BASED ON ARTICLE 10A OF DIRECTIVE 2001/83/EC AS AMENDED

(WELL-ESTABLISHED USE)

BASED ON ARTICLE 16 D(1) AND ARTICLE 16F AND 16H OF DIRECTIVE 2001/83/EC AS AMENDED

(TRADITIONAL USE)

Herbal substance(s) (binomial scientific name of the plant, including plant part)	The whole or cut, dried underground parts of *Rheum palmatum* L. or of *Rheum officinale* Baillon or of hybrids of these two species or of a mixture
Herbal preparation(s)	Standardised herbal preparations thereof
Pharmaceutical forms	Herbal substance or preparation for oral use
Rapporteur	Dr Werner Knöss

II.1 INTRODUCTION

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

Rhubarb belongs to the stimulating laxatives containing hydroxyanthracene derivatives and is intended "for short-term use in cases of occasional constipation". Therefore we refer to the assessment reports for senna, aloe, frangulae cortex and cascara.

Constipation is a common complaint in 1-6 % middle-aged and 20-80 % of the elderly people, which is treated by laxatives. Constipation also tends to be more prevalent among women. The functional constipation is the most common type without any specific etiology (59). Among the most commonly used laxatives are either preparations containing stimulating hydroxyanthracene derivatives or lubricating laxatives (vegetable or mineral oils) or bulk forming agents.

Rhubarb consists of the whole or cut, dried underground parts of *Rheum palmatum* L. or of *Rheum officinale* Baillon or of hybrids of these two species or of a mixture. The underground parts are often divided; the stem and most the bark with the rootlets are removed. It contains not less than 2.2 per cent of hydroxyanthracene derivatives, expressed as rhein ($C_{15}H_8O_6$, M_r 284.2), calculated with reference to the dried herbal substance. This complies with the European Pharmacopoeia (1, 2).

Herbal preparations thereof have to be standardised to their amount of hydroxyanthracene derivatives, calculated as rhein.

Rhubarb contains a complex mixture of different hydroxanthracene derivatives. The amount is 3 to 12 % depending on the method of determination. These hydroxanthracene derivatives mainly (60 – 80 %) consist of mono- and diglycosides of 1,8-dihydroxyanthrachinons aloeemodin, chrysophanol, emodin, physcion and rhein (6), and only small amounts of the respective aglycones. Dianthrone glycosides (sennosides) are also present (10 - 25 %). Oshio H et al. 1974 (15) isolated sennosides E and F besides the sennosides A, B and C from the rhizome of *Rheum palmatum* L. Small amounts of anthrone glycosides depending on the time of harvesting and the conditions of drying are also found. The level of the oxidized forms is maximal in the summer and almost nil in the winter (3, 4, 5).

Rhubarb also contains tannin agents like gallotannins (ca. 5 %) (3, 4).

Chemotaxonomical investigations have shown that the underground parts of *Rheum palmatum* L. or of *Rheum officinale* Baillon do not contain stilbenes like rhaponticin (74). Other analytical investigations discovered several different stilbenes. Kubo I et al. 1991 (20) isolated two stilbene glycosides, 4'-O-methylpiceid and rhapontin, from a methanolic extract of the root of *Rheum palmatum*, which was purchased at a marketplace in Indonesia. Further investigations are necessary to clarify the analytic and the kind of herbal substances investigated whether these were not any falsifications (3). According to the European Pharmacopoeia it has to be shown that the herbal substance does not contain rhaponticin.

II.1.2 Information on period of medicinal use in the Community regarding the specified indication

II.2 NON-CLINICAL DATA

II.2.1 Pharmacology

II.2.1.1 Overview of available data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Primary pharmacodynamics

Laxative effect

Rhubarb belongs to the anthranoid-containing laxatives. 1,8-dihydroxanthracene derivatives possess a laxative effect (28). The mode of action is based on two mechanisms. Firstly, colonic motility is increased leading to a reduced transit time and reduced fluid absorption, and secondly, an influence on secretion processes by two concomitant mechanisms viz. inhibition of absorption of water and electrolytes (Na+,Cl-) 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 (secretagogue effect) resulting in enhanced concentrations of fluid and electrolytes in the lumen of the colon. These findings are based on investigations with different anthrones deriving also from other anthranoid-containing herbal substances, but the results of these investigations are not always consistent.

Chirikdjian JJ et al. 1983 isolated physcion diglycoside from rhubarb roots and showed that this compound had a laxative activity similar to physcion monoglycoside in mice, orally administered, although with physcion diglycoside the onset of the purgative effect was observed 2 hours later than with physcion monoglycoside. Physcion itself showed no remarkable effect. Only higher doses (300 and 450 mg/kg b.w.) slightly increased the number of defaecations. Stool consistency did not change (7).

Leng-Peschlow E 1986 measured quantity and consistency of the faecal output, large intestine transit time, and colonic net absorption in rats after oral administration of sennoside A + B (12.5 – 200 mg/kg). The release of normal faecal pellets was accelerated 3-4 h after drug administration. Excretion of soft faeces reached its maximum 5-7 h after administration. Large intestine transit time was dose- and time-dependently influenced. Net fluid absorption in the colon was inhibited. The increase in fluid volume due to net fluid secretion is delayed compared with the acceleration of large intestine transit. The author concluded that the laxative effect of the sennosides consists of changes in colon motility as well as in colonic fluid absorption, but motility may be an earlier and more sensitive parameter than net absorption (29).

Leng-Peschlow E 1989 administered bisacodyl or sennosides A + B (10-100 mg/kg each) orally and intracaecally to rats. Similar quantity of soft faeces within 24 h and a similar acceleration of large intestine transit time were induced, but in each case bisacodyl had a prolonged action. Net fluid absorption in the perfused rat colon was reduced by rhein (10^{-3} mol/l), an active metabolite of sennosides, comparable to the effect of bisacodyl, however recovery was delayed after bisacodyl (30).

Harima S et al. 1994 measured sennoside A content in hot-water extracts from 17 varieties of rhubarb obtained from the Japanese market. The contents are between 0.4 and 18.8 mg/extract g. The authors also examined the respective cathartic effects of the different varieties in mice. A positive correlation was confirmed between the sennoside A content and the cathartic effects (8).

Yagi T et al. 1997 tried to explore the mechanism involved in the synergistic purgative action of aloe-emodin anthrone and rhein anthrone, the active metabolite of sennoside C, a purgative constituent of rhubarb and senna. 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 µmol/kg in mice (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

achieve net secretion, at ½ dose. The mixture significantly decreased net water absorption and reversed it into net secretion at this dose. These anthrones did not stimulate mucus secretion in the colon lower than ½ dose. The authors conclude 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 (9).

Yagi T et al. 1999 investigated the purgative effects of intracaecally administered rhein anthrone and anthraquinones such as aloe-emodin and chrysophanol, isolated from rhubarb, emodin and rhein, and the possible synergistic effects of the anthraquinones with rhein anthrone in mice. The anthraquinones were less potent purgatives than rhein anthrone, but the equimolar mixture of aloe-emodin and rhein anthrone had synergistic potentiating effects. An equimolar mixture of other anthraquinones and rhein anthrone tended to potentiate the purgative action (10).

Rhubarb acts within 8 to 12 hours due to the time taken for transport to the colon and metabolisation into the active compounds (4). The content of tannin agents may possibly counteract the laxative effect of the anthraquinones , and rhubarb is therefore considered to be a milder laxative than other anthraquinones.

Secondary pharmacodynamics

Due to the content of tannin agents rhubarb preparations were used for diarrhoea, for gastritis and enteritis, and as a styptic (3, 4).

Bae EA et al. 1998 tested different herbal substances for their inhibitory effect on *Helicobacter pylori* (HP) and on the HP urease in vitro. HP was isolated from the gastric antrum of chronic gastric patients. HP also produces a vacuolating toxin and its toxicity may be potentiated by urease-mediated ammonia production. HP urease is considered to play critical roles in the pathogenesis of gastric and peptic ulcer. Therefore, eradication of this bacteria and inhibition of the HP urease seems to be important for the treatment. The water extract of *Rheum palmatum* very strongly inhibited the growth of HP at 1 mg/ml, but it has no inhibitory effect on the urease activity (11).

Sydikes RJ et al. 1991 tested the virucidal effects of hot glycerine extracts from *Rheum officinale, Aloe barbadensis, Rhamnus frangula, Rhamnus purshianus*, and *Cassia angustifolia* against herpes simplex virus type 1. All the plant extract inactivated the virus. The active components in these plants were separated by thin-layer chromatography and identified as anthraquinones. Anthraquinone-glycosides should be ineffective. A purified sample of aloe-emodin was prepared from aloin, and its effects on the infectivity of herpes simplex virus type 1 and type 2, varicella-zoster virus, pseudorabies virus, influenza virus, adenovirus, and rhinovirus were tested by mixing virus with dilutions of aloe-emodin for 15 min at 37 degrees C, immediately diluting the sample, and assaying the amount of infectious virus remaining in the sample. The results showed that aloe-emodin inactivated all of the viruses tested except adenovirus and rhinovirus. Electron examination of anthraquinone-treated herpes simplex virus demonstrated that the envelopes were partially disrupted. These results show that anthraquinones extract from a variety of plants are directly virucidal to enveloped viruses (67).

Hsinag CY et al. 2001 screened 31 herbs in 5 different preparations (cold aqueous, hot aqueous, ethanolic, acidic ethanolic, and methanolic) for their antiviral activities. 7 extracts, which showed significant antiviral activities, were further investigated for their antiviral mechanisms in vitro. Ethanolic extract of *Rheum officinale* prevented the process of *Herpes simplex* attachment and penetration (12).

Blaszczyk T et al. 2000 screened 56 dried Chinese plants for their antimycotic properties in vitro. They used 10 % aqueous extracts. The extract of *Rheum palmatum* L. (radix et rhizoma) showed antimycotic activity against *Aspergillus fumigatus* and *Candida albicans* comparable to that of nystatin. The growth of *Geotrichum candidum* and *Rhodotorula rubra* was also inhibited, but to a lesser extent (13).

Cyong JC et al. 1987 screened in vitro 178 Chinese herbs for their antibacterial activity against *Bacteroides fragilis*, a major anaerobic microorganism in the intestinal flora in humans. Only rhubarb root (*Rheum officinale*) was found to have significant activity and the purified active substance was identified as rhein. Rhein also has potent activity against *Candida albicans* and weak activity against *Escherichia coli* and *Bacillus subtilis*. Negligible or no activity was shown against e.g. *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Staphylococcus aureus* (14).

Several in vivo animal investigations deal with renal failure.

Yokozawa T et al. 1984 (16), 1985 (21), 1986 (17) and 1987 (22) examined the effect of orally administered aqueous extract of roots of *Rheum officinale* Baillon in rats with chronic renal failure induced by an adenine diet. The herbal preparation caused a marked decrease of blood urea nitrogen and creatinine, and a decrease of methylguanidine and guanidinsuccinic acid levels. Hypocalcaemia, hyperphosphataemia, and free amino acid patterns were also improved.

Yokozawa T et al. 1997 administered an aqueous dry extract of rhubarb (*Rheum officinale* Baillon) 125 mg/kg b.w./day orally to rats with diabetic nephropathy induced by subtotal nephrectomy and injection of streptozotocin for 80 consecutive days. High blood and urinary glucose levels were ameliorated. Furthermore, improvement of hyperlipidaemia, and accelerated excretion of urinary urea nitrogen and creatinine were observed. The changes were significant compared to untreated controls (18).

Zhang G et al. 1996 studied the effect of orally administered aqueous extract of dry roots of *Rheum palmatum* (150 mg/day from day 30 to day 120 in drinking water) on the course of chronic renal failure in rats submitted to subtotal nephrectomy. Rhubarb treatment had no effect on the systemic hypertension. Rhubarb-treated rats had significantly less proteinuria when compared to the untreated group. Renal function was comparable in both groups, but the severity of glomerulosclerosis was significantly reduced in the treated group (19).

Kosuge T et al. 1985 tried to identify the anticoagulative principle of *Rheum palmatum* L. by a combination of partition, fractional precipitation and column chromatography on silica gel and plasma recalcification time in mice, because the herb is used as an anticoagulant and haemostatic agent in Chinese medicine. D-catechin was identified as the anticoagulative principle. D-catechin has been also isolated from *Sanguisorba* and *Hypericum* species, although these herbs are commonly used as haemostatics in Chinese medicine (23). The clinical relevance concerning efficacy and safety has to be proven. Up to now no adverse event dealing with a bleeding event has been reported during short-term use in cases of occasional constipation.

Several investigations deal with the possible anticancer effect of *Rheum palmatum*, especially with emodin isolated from the root.

An extract of *Rheum palmatum* L. increased the sensitivity to paclitaxel at a concentration of 10 µg/ml and 50 µg/ml significantly, not to 5-fluorouracil in HeLa cells (human cervical carcinoma cell line). Paclitaxel is a MDR1 substrate whereas 5-fluorouracil is not such a substrate. Rhodamine123 was used to evaluate the MDR1-mediated transport. Rhodamine123 uptake by HeLa cells was significantly increased by the presence of rhubarb at 100 µg/ml. The authors concluded that the effect is mediated by an inhibition of MDR1 function in tumour cells and that the combination of anticancer drugs with some herbal extracts contributes to the enhancement of clinical outcomes in cancer chemotherapy (36). (see also pharmacokinetic interactions)

A cytotoxic anthraquinone glycoside, pulmatin, 1,8-dihydroxy-3-methyl-anthraquinone-1-O-ß-d-glycoside, and its congeners, chrysophanein and physcionin, have been isolated as minor components from a methanolic extract of the root of *Rheum palmatum*. These anthranquinone glycosides exhibited moderate cytotoxic activity against several types of carcinoma cells (HeLa epithelioid carcinoma cells, BT-20 human breast carcinoma cells). The authors also

isolated two stilbene glycosides, 4'-O-methylpiceid and rhapontin. Therefore falsifications of the herbal substance cannot be ruled out (40).

An in vitro investigation shows that emodin, selectively inhibits casein kinase II (CKII), a Ser/Thr kinase, as a competitive inhibitor (24).In human lung adenocarcinoma cells emodin induces apoptosis through a reactive oxygen species-dependent mitochondrial signaling pathway (25).In various human cancer cell lines (breast cancer, hepatocarcinoma, skin squamous carcinoma) emodin significantly inhibits epidermal growth factor (EGF)-induced migration which is one of the underlying mechanisms in cancer invasion and metastasis (26). Furthermore emodin inhibits vascular endothelial growth factor-A-induced angiogenesis by blocking receptor-2 (KDR/Flk-1) phosphorylation (27).

Kazuhiro S et al. 2000 reported about phytoestrogen properties of emodin isolated of an aqueous extract of the rhizome of *Rheum palmatum* for the first time. Emodin binds to the estrogen receptor and activates transcription through estrogen responsive elements (ERE). However, the exact mechanism is still unclear. The inhibition of casein kinase II (CKII) by emodin may play a role because this kinase phosphorylates serine-167 on the human estrogen receptor, which results in increased estrogen response element binding and transcriptional activation (37).

Several Chinese plant extracts were tested to screen pharmacological activities that could be relevant to the treatment of cognitive disorders. A simple and rapid enzyme essay on thin layer chromatography (TLC) plates has been developed for the screening of acetylcholinesterase and butyrylcholinesterase inhibition in plant extracts. The hexane extract of *Rheum officinale* Baillon showed a clear activity whereas the methanolic and the chloroform extract showed no activity (38).

Safety pharmacology

There are no special investigations available.

Pharmacodynamic interactions

Chronic use or abuse of rhubarb preparations may lead to hypokalaemia like the abuse of all anthranoid-containing laxatives. This hypokalaemia and the increased loss of potassium may increase the activity of cardiac glycosides and interfere with the action of antiarrythmic agents (interaction with antiarrhythmic medicinal products, which induce reversion to sinus rhythm, e.g. quinidine) and medicinal products inducing QT-prolongation. Concomitant use with medicinal products inducing hypokalaemia (e.g. diuretics, corticosteroids and liquorice root) may aggravate electrolyte imbalance.

II.2.1.2 Assessor's overall conclusions on pharmacology

The postulated laxative effect is supported by the pharmacological data, even if the most data derive from investigations with isolated constituents of rhubarb and not with the preparation or the herbal substance itself. Pharmacological data obtained from other anthranoid-containing laxatives complete these scientific findings.

In Asia (China, Japan) rhubarb is used as an anticancer agent among others. Therefore several investigations try to clarify the underlying pharmacodynamic actions. Up to now the clinical relevance of the findings is doubtful.

The phytoestrogen properties of emodin need to be confirmed and to elucidated by further investigations.

II.2.2 Pharmacokinetics

II.2.2.1 Overview of available data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Absorption, distribution, metabolism, elimination

No pharmacokinetic data obtained with rhubarb or its preparations are available.

According to Witte P and Lemli L 1990 (31) anthrone-glycosides are carried, unabsorbed, to the large intestine, where the active aglykon is released by bacterial hydrolysis of the sugar. The intestinal bacterial flora also accounts for the reduction of anthraquinone aglyka to the corresponding anthrones. After absorption, the anthranoids are transformed mainly to their corresponding glucuronide and sulfate derivatives, which appear in urine and bile.

In the case of senna, animal experiments with radio-labeled rhein-anthrone administered directly into the caecum demonstrated absorption < 10 % (32).

Experiments with radiochemical anthranoids showed a significant clearance of tissue-bound activity of all organs, except the kidneys, which exhibited a pronounced retention of anthranoid equivalents.

After oral administration of 4.5 mg/kg 14C-aloe-emodin (AE) to rats 20 – 30 % of the dose was excreted in urine and the rest in faeces. Aloe-emodin was quickly meatobolised 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 (33).

Emodin was admininstered to rabbits by i.v. bolus. The AUC of emodin was 518 µg.min/ml, clearance was 72.3 ml/min, and elimination half life was 227 min. Oral administration at 10 mg/kg b.w. resulted in a very low serum concentration (approximately 2.5 µg/ml). Emodin was found to be highly bound (99.6%) to serum protein, investigated by the equilibrium dialysis method (34).

Müller SO et al. 1998 (35) presented studies which were designed to elucidate the enzymes involved in the biotransformation of naturally occurring 1,8-dihydroxyanthraquinones and to investigate whether biotransformation of 1,8 dihydroxyanthraquinones may represent a bioactivation pathway. First the metabolism of emodin was studied. With rat liver microsomes, the formation of two emodin metabolites, omega-hydroxemodin 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 omega-hydroxyemodin seems to be catalysed by several cytochrome P450 enzymes at low rates. The formation of 2-hydroxemodin was increased in liver microsomes from 3-methylcholanthracene-pretreated rats and was inhibited by alpha-naphthoflavone, by an anti-rat cytochrome P450 1A1/2 antibody, and to a lesser degree, by anti-rat cytochrome P450 1A1 antibody. These data suggest the involvement of cytochrome P450 1A2 in the formation of this metabolite. However, other cytochrome P450 enzymes also seem to catalyse this reaction. The anthraquinone chrysophanol is transformed, in a cytochrome P450-dependent oxidation, to

aloe-emodin as the major product formed. Further on Müller SO et al. compared the mutagenicity of the parent dihydroxyanthraquinones and their metabolites 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. These data indicate that the cytochrome P450-dependent biotransformation of emodin and chrysophanol may represent bioactivation pathways for these compounds.

Pharmacokinetic interactions

Takara K et al. 2005 (36) concluded an inhibition of MDR1 function in tumour cells by a rhubarb extract from their in-vitro investigations (see secondary pharmacodynamics). However, rhodamine123, the substrate used is also transported by transporters for organic cations, and therefore it is not a selective substrate. It is not known whether the HeLa cell line expressed MDR1-gene in a constant way.

This might be a hint that rhubarb influences this transport system in some way but up to now this hint is too weak to draw some conclusion concerning possible interactions.

A recherche in the database XMEDALL with the keywords “rhubarb (Rheum)” and “MDR” or “PGP (glycoprotein)” did not show any further publications. There are no reports of clinical relevant pharmacokinetic interactions available. We also take into account that rhubarb is only used for short term.

II.2.2.2 Assessor’s overall conclusions on pharmacokinetics

Anthrone-glycosides are carried, unabsorbed, to the large intestine. They are converted by the bacteria of the large intestine into the aglycones and subsequently to the active compounds, the anthrones. Absorption seems to be low.

The data available concerning some pharmacokinetics interactions are too weak to draw some conclusions.

II.2.3 Toxicology

II.2.3.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

No specific data are available for single dose toxicity and reproductive toxicity for rhubarb or the preparations thereof.

The study of Yan M et al. 2006 focuses on the toxicity of total rhubarb (rhizomes of *Rheum palmatum* L.) anthraquinones (TRAs) on Sprague Dawley rats. TRAs were orally administered for 13 weeks. 20 animals were randomly allocated into four groups. TRAs were dissolved in 0.5% sodium carboxymethylcellulose solution and each group was administered per os once daily for 13 weeks a dose of 0, 140, 794, 4500 mg/kg b.w. After that, rats were sacrificed under halothane anesthesia and all main organs and glands were taken for pathohistology studies. In the highest dose group, nephrotoxicity were discernible at 13 weeks. There was no clear morphologic change in the kidney of the control group, while in TRAs tested group, swelled and denatured renal tubule epithelial cells were observed. The results of gene differential expression study indicated the TRAs affect mostly at oxidative stress pathway, cell cycles, nutrients metabolism, thus caused renal tubule epithelial cells swelled and denatured in histopathology study. CYP1A1, which is regarded as a carcinogen-metabolising enzyme, was dramatically up-regulated and may account to genotoxicity. Mitogen-activated protein kinase (MAPK) kinase 6 was identified to be the target gene which cause cell cycle arrest and proliferation inhibition and contributes to the nephrotoxicity on rats. However, the discernible nephrotoxicity was only observed at the high dose group.

According to the authors therapeutic dosage of TRAs is 420 mg/day, 7 mg/kg b.w. respectively, if one person weighs 60 kg. The dosage used in the experiment is 642 times ordinary clinical dose (39).

Paneitz et al. 1999 compared ethanolic extracts of different *Rheum* species with respect to their mutagenicity in the *Salmonella*/microsome assay with strains TA 98, TA 100 and TA 1537. The root extract of *Rheum officinale* Baillon was weakly mutagenic in strain TA 1537 even without metabolic activation at a dosage of 300 µg/plate and the effect is enhanced by addition of S9-mix, already at dosage of 30 µg/plate. The authors concluded that the positive effect without metabolic activation might be due to the content of aloe-emodin, which is the only direct-acting mutagenic anthraquinone present in *Rheum* species, whereas the enhancement of the mutagenic potency after addition of S9-mix is probably related to emodin (41).

In a dose 5 mg/plate, an aqueous extract (1:6) of *Rhei radix* (no further specification is given) had a mutagenic effect on *Salmonella typhimurium* TA 98 following metabolic activation with S9-mixture. However, this effect was not obtained with *Salmonella typhimurium* TA 100. In a rec-assay with *Bacillus subtilis* for DNA-damage the same extract (6 mg/plate) gave a positive result. A methanolic (1:6) extract gave no response in the same tests. Sennoside B and rhein did not induce significant numbers of chromosomal aberrations or aberrant cells in bone marrow cells of Swiss mice (53).

Further data exist for different hydroxanthraquinones and other anthranoid-containing herbal substances like senna.

Older toxicological data indicate that the two hydroxyanthraquinones, emodin and aloe-emodin might represent a genotoxic or carcinogenic risk (Mori 1990 (42), Siegers 1992 (43), Brusick 1997 (44)). While most studies gave negative responses, results from some of the studies suggest a genotoxic activity by both (Wölfle 1990 (45), Westendorf 1990 (46), Westendorf 1993 (47)). These were Ames tests showing an interaction with Salmonella DNA resulting in the production of frameshift mutations (Westendorf 1990 (46), Sandnes 1992 (48), Heidemann 1993 (49)). Other sennosides and rhein were mostly negative in the respective tests. In three in vivo studies the crude senna herbal substance at a concentration of 1 or 1.5 g/kg body weight showed no evidence of any genetic effects (Heidemann 1993 (49)). In vitro assays overestimate the potential hazard from exposure and must be reevaluated by in vivo experiments.

Westendorf et al. 1990 (46) reported that in the Ames test aloe-emodin was mutagenic in S. *typhimurium* strain TA1537 and furthermore active against TA98, TA1538 and TA97 (all frameshift mutant sites). The activity was independent of metabolic activations; in fact, the addition of S9 mix tends to suppress the mutagenicity. In the Mammalian Cell Mutation Test Westendorf et al. reported that aloe-emodin was mutagenic to V79 cells. However, other scientists question this conclusion. The highest concentration employed was 30 µg/ml and did not show much, if any toxicity (see publication). This indicates the possibility of a problem, since mutagenic effects in this assay are typically associated with toxicity. The apparent positive response was based on a very low spontaneous mutant frequency. Numerous laboratories have recognised that the spontaneous background for HGPRT-mutants (hypoxanthine-guanine phosphoribosyl transferase) is quite variable and increase of at least 3-5 fold are required in duplicate tests to confirm an effect. In the in vitro UDS assay, also conducted by Westendorf et al. 1990, aloe-emodin was associated with a significant increase in net grains/nucleus. 2 trials were reported. The concentrations range in both covered 6.3 µg/ml to 100 µg/ml. At a concentration of 25 µg/ml the net grains/nucleus reached the criteria to call the response positive. In the *Salmonella*/ microsome assay emodin, chrysophanol and physcion were weakly mutagenic in strain TA1537 in the presence of S9 mix only. Chrysophanol was also weakly mutagenic in strain T102 without and with exogenous metabolic activation for induction of mutagenicity. No mutagenic effects were observed in the V79-HGPRT mutation assay and in the unscheduled DNA synthesis (UDS)

assay for chrysophanol and physcion. Emodin was highly mutagenic in the V79-HGPRT mutation assay. In the UDS assay emodin was a string inducer of UDS in primary hepatocytes. Emodin was also tested with respect to its transforming activity in C3H/M2 mouse fibroblasts in vitro. Emodin was clearly active in this in this assay.

Sandnes D et al. 1992 (48) investigated the mutagenicity of senna glycosides and extracts of senna folium and senna fructus in the Salmonella typhimurium reversion assay. Senna glycosides were inactive in all strains, except for a slight, but significant increase in mutant frequency in TA102 in the absence and presence of liver microsomes. Extracts of senna fructus and senna folium demonstrated weak activity in TA97a, TA100 and TA102 in the presence of liver microsomes, and in TA97a and TA102 in the absence of liver microsomes. A strong increase in mutant frequency (3- to 5-fold above background frequency) was observed with all extracts in TA98 in the presence of liver microsomes. This activity increased further following enzymatic hydrolysis with hesperidinase of extracts of senna fructus from one source, and could be correlated to the release of the flavonol aglyka kaempferol and quercetin.

Helmholz H et al. 1993 (114) investigated the mutagenic and genotoxic activities of the glycosides emodin and frangulin, and of an alcoholic extract of frangula bark, and of a commercial frangula bark preparation Sanurtin N® with the aid of the in vitro *Ssalmonella*/microsome mutagen test and the deoxyribonucleic acid (DNA) repair test of primary rat hepatocytes. 1 g of the alcoholic extract contained 50.76 mg glucofrangulin, 86.84 mg frangulin, 30.88 mg emodin, 10.3 mg pyscion, and 14.32 mg chrysophanol. One coated tablet of Sanurtin N® contained 8.28 mg glucofrangulin, 0.21 mg frangulin, <0.1 mg emodin, and physcion and chrysophanol only in traces. The tests provided evidence of a dose-dependent increase in the mutation rate or the induction of DNA repair, for the glycosides, the extract of the crude herbal substance and the commercial preparation. The mutagenic potency was larger for emodin than for the alcoholic extract than for frangulin than for Sanurtin N®. The authors concluded that phytotherapeuticals based on frangula bark can cause genotoxic effects and are potential tumour promoters.

The three in vivo studies by Heidemann 1993 (49) which showed no evidence of any genetic effects, were the Chromosome Aberration Test, the Mouse Spot Test, and the in vivo/in vitro UDS (unscheduled DNA synthesis) in rat hepatocytes.

Mengs U et al. 1997 (54) investigated the potential of emodin to induce micronuclei in polychromatic erythrocytes (PCEs). Mice of both genders received a single oral dose of 2000 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 examination confirmed the systemic availability of emodin. Plasma levels of up to 190 µg emodin/ml represented concentrations being in the concentration range that induced positive responses in several genotoxicity cell culture assays.

Jahnke GD et al.2004 (55) evaluated emodin for potential effects on pregnancy outcome. 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 ~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 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 unaffecte, 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 +/- 1700 ppm. A LOAEL was not established.

2001 the National Toxicology Programm (NTP) of the U.S. Department of Health and Human Services published a technical report on toxicology and carcinogenesis studies of emodin (50).

16-day study in F344/N rats

Groups of 5 male and 5 female rats were fed diets containing 0, 600, 2000, 5,500, 17,000, or 50,000ppm emodin. This corresponds in males to average daily doses of approximately 50, 170, 480, 1,400, or 3,700 mg emodin/kg bw and in females to 50, 160, 460, 1,250, or 2,000 mg/kg bw. 3 female rats died before the end of the study. Mean body weights of males and females exposed to 5,500 ppm and 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 $B6C3F_1$ mice

The size of the groups and the administered concentrations were the same as described above. The concentrations correspond in males to average daily doses of approximately 120, 400, 1,200 or 3,800 mg/kg bw and in females to 140, 530, 1,600 or 5,000 mg/kg bw. 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 was greater than by the controls. Macroscopic lesions were present in the gallbladder and kidney exposed to 17,000 ppm.

14-week study in rats

Groups of 10 male and 10 female rats were fed diets with 0, 312.5, 625, 1,250, 2,500 or 5,000 ppm emodin. This corresponds to average daily doses of approximately 20, 40, 80, 170, or 300 mg/kg bw in males and females. Among others 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

The size of the groups and the administered concentrations were the same as described above. This corresponds 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. 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 1,250 ppm or greater.

2-year (105 weeks) 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).

3 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 12-month 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 of 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-year study in mice

Groups 0f 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) and 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). 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 concentration of exposure. At 2-years, the incidences of renal tubule pigmentation were significantly increased in all exposed groups; severities also gained with increasing concentration of exposure.

Genetic toxicology

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. Three separate in vivo micronucleus tests were performed with emodin. A male rate bone marrow micronucleus test, with emodin administerd by 3 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 the 14-week study, negative results were seen in male mice, but a weekly positive response was observed in similarly exposed females.

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

- The studies give no evidence of carcinogenic activity for male rats and female mice, and equivocal evidence for 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 2-hydroxyemodin acts as the genotoxin (51).

2002 the American Herbal Products Association submitted a review of the data from the National Toxicology Program and relevant to the status of Cascara sagrada ingredients (52). All of the NTP oral feeding studies were of durations which exceed the duration of human exposure. The studies that are nearest the human exposure are the 16 day studies in mice and rats. The most conservative no adverse level (NOAEL) reported for these studies, is 160 mg/kg in female rats and 400 mg/kg in male mice. Assuming that an adult of 70 kg body weight consumes 21 – 100 mg hydroxanthracenes daily (0.3 – 1.43 mg per kg body weight), the association calculated a standard conservative 100-fold margin of safety.

Based on the maximal daily dose of 30 mg hydroxyanthracene recommended in the HMPC monograph of rhubarb (0.43 mg per kg body weight) a standard conservative 370-fold margin of safety can be calculated.

Concerning the evaluation of carcinogenicity the association concluded that the equivocal evidence of carcinogenicity in female rats in the 2-year feed study is clearly not relevant to humans because humans do not have a Zymbal's gland and there was no additional data on which the finding of equivocal evidence was made. The equivocal evidence of carcinogenic activity of emodin in male mice based on a single occurrence of uncommon renal tubule neoplasm in each of the highest doses (i.e., at 35 and at 70 mg/kg body weight) but not in the low dose (15 mg/kg body weight). Because of the short-term duration of exposure to cascara this finding would be of questionable relevance to human exposure, just as the sex-specific differences.

Heidemann A et al. 1996 (56) undertook in vitro and in vivo 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 in vivo studies (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]) 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 unscheduled DNA synthesis (UDS) assay. Hepatocytes of aloe-emodin treated male Wistar rats did not show DNA damage via repair synthesis. These 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.

The aim of the study of Schörkhuber M et al. 1998 (57) was to demonstrate the effect of the 1,8-dihydroxyanthraquinone (DHA)-laxatives, danthrone, rhein, aloe-emodin and sennidine, on colorectal tumour cells because the available information is 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 or the biphenylic laxative bisacodyl. DNA synthesis was not similarly reduced: 0.4-4 microM 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/10(6) cells, 18 microM rhein and 0.7 microM 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 preceeded by specific phosphorylation of cellular proteins with molecular weights of 110, 78, 63, 57 kCa, indicating the specific induction of a cellular signalling cascade by the laxatives.

Mitchell JM, Mengs U et al. 2006 (115) conducted an oral carcinogenicity and toxicity study of senna in rats. The administered senna preparation were powdered Tinnevelly senna pods containing 1.829% of sennosides A-D, 1.596 % of potential rhein, 0.111% of potential aloe-emodin, 0.014% of total emodin, and 0.004% of total chrysophanol (sum of potential hydroxyanthraquinones 1.725%). Senna was administered by gavage to Sprague Dawley rats once daily at dose levels of 0, 25, 100 and 300 mg/kg/day for up to 104 consecutive weeks. Based upon clinical signs related to laxation effect of senna, the highest dose (300 mg/kg/day) was considered to be a maximum tolerated dose. The primary treatment-related clinical observation was mucoid faeces seen at 300 mg/kg/day. In the highest dose group animals had slightly reduced body weights, increased water consumption and notable electrolyte changes in serum and urine. At necropsy, dark discolouration of the kidneys was observed in all treated groups. Histological changes were seen in the kidneys of animals of all treated groups and included slight to moderate tubular basophilia and tubular pigment deposits like already described above. For all treated groups, minimal to slight hyperplasia was evident in the colon and caecum. Under the conditions of the study there were no alteration seen in the colonic nervous plexus. Even in the highest dose group, there was no indication of any pigment deposits in the mucous membranes of the large intestine. No treatment-related neoplastic changes were observed in any of the examined organs. The authors concluded that senna did not reveal any evidence of carcinogenicity in this study.

II.2.3.2 Assessor's overall conclusions on toxicology

Experimental data, mainly in vitro tests showed a genotoxic risk of several anthranoids. Most of the in-vivo studies showed no effect or only equivocal effects.

Mueller SO et al. 1999 concluded in their publication "Occurrence of emodin, chrysophanol and physcion in vegetables, herbs and liquors. Genotoxicity and anti-genotoxcity of the anthraquinones and of whole plants" (58), although three common vegetables have been shown to contain anthraquinones, data on their genotoxicity alone should not be used to derive a human risk. The authors analysed vegetables, herbs and herbal-flavoured liquids for the quantitative determination of the content of the aglycosidic anthraquinones emodin, chrysophanol and physicion. For example emodin, chrysophanol and physcion were found in lettuce and beans, and emodin and physcion in peas. For emodin the authors had shown unequivocal genotoxic potency in mammalian cells. On the contrary, chrysophanol and physcion showed borderline effects, and physcion was cytotoxic at higher concentrations. The lyophilisates of these vegetables were tested in the micronucleus test in lymphoma cells, alone and in combination with the known genotoxic and carcinogenic anthraquinone danthrone. None of the lyophilisates increased the frequency of micronuclei. Surprisingly, the genotoxicity of danthron was reduced significantly by addition of the vegetables lyophilisates. The authors concluded that the vegetables as a whole contain numerous other constituents some of which might also have protective effects and therefore for evaluation of a putative human health risk from dietary mutagens, the assessment should not be based on measured concentrations of mutagens.

This is also to be considered in anthranoid-containing herbal substances like rhubarb which also are combinations of numerous constituents.

Nephrotoxicity observed in mice occurred after administration during 13 weeks and with a 642 times higher dose than the ordinary clinical dose according to the authors.

Pharmacovigilance actions were initiated in Germany in 1996 (60) to minimise a possible risk. The maximal dose and the duration of use were limited.

II.3 CLINICAL DATA

II.3.1 Clinical Pharmacology

II.3.1.1 Pharmacodynamics

II.3.1.1.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) including data on constituents with known therapeutic activity.

There exist several investigations with different Chinese and Japanese herbal preparations which are not specified exactly. We mainly mention the investigations with *Rheum palmatum* L. or *Rheum officinale* Baillon

Mitsuma T et al. 1998 investigated the differences in cathartic actions of three different types of rhubarb: rhubarb A (produced in the Province of Sichuan, China), rhubarb B (Rheum coreanum, cultivated and processed in Japan), rhubarb C (tablets manufactured with the processed rhizome of *Rheum palmatum* from the Province of Qing-Hai, China). These three types were administered to 12 healthy volunteers for 3 days. Pharmacological effects were evaluated in terms of the number of bowel movements, bowel sounds, urinary volume and various blood chemical parameters. From the results, the authors concluded that the processed rhubarb (C), with a weaker cathartic action, was suitable for therapeutic use in patients with chronic renal failure (63).

II.3.1.2 Pharmacokinetics

II.3.1.2.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) including data on constituents with known therapeutic activity.

Vyth A et al. 1979 (61) isolated rhein, aloe-emodin, emodin, physcion and chrysophanol from powdered root of rhubarb (no further specification is given) after oxidative hydrolysis. After oral administration of 65 mg or 200 mg of powdered root of rhubarb in 2 volunteers, rhein was found in human urine. The sample collected in the morning contained more anthraquinones than those collected noon.

Zhu W et al. (62) compared the pharmacokinetic parameters of rhein administered by retention enemas with those conventional oral dosing of rhubarb extract (aqueous extract of *Rheum officinalis* Baillon). The amounts of rhein, emodin, aloe-emodin, chrysophanol, physcion on the extract were 12.308, 1.337, 1.594, 1.204, 1.615 mg/g, respectively. Eight healthy male volunteers were enrolled in a prospective crossover study. All subjects received a single dose of rhubarb extract (50 mg/kg corresponding to about 54 mg hydroxyanthracene derivatives, body weight 60 kg) on two separate occasions, once orally, once by a retention enema. Rhein plasma concentration was measured by HPLC. The plasma rhein levels rose rapidly after oral administration and then declined slowly. The curve fitted a two-compartment model well. Concerning the retention enema administration, the distribution of rhein reached its C_{max} at 53.46 +/- 33.10 minutes after a rapid ascent, characteristic of fast absorption. The distribution and elimination of the plasma rhein followed a one-compartement model. The C_{max}, $AUC_{0-\infty}$, AUMC (area under the first moment of plasma concentration-time curve) were significantly higher in oral administration, while V_d (volume of distribution) of rhein after oral administration was significantly lower. However, no statistically significant differences between the two treatments were observed for any other pharmacokinetic parameters like T_{max}, $t_{1/2}$, $MRT_{0-\infty}$ (mean residue time), CL (body clearance).Two subjects reported mild to moderate diarrhoea, which resolved without treatment. No clinically meaningful changes in examinations findings or vital signs were observed.

II.3.1.3 Assessor's overall conclusions on pharmacodynamics and pharmacokinetics

There are only limited human pharmacological data available. Based on animals experiments and experiences with other anthranoid-containing herbal substances it is assumed that the laxative effect in humans is also caused by the two mechanisms described in chapter II.2.1.1. Rhubarb acts within 8 to 12 hours due to the time taken for transport to the colon and metabolisation into the active compounds.

II.3.2 Clinical Efficacy[3]

II.3.2.1 Dose response studies

There are no dose response studies available.

The German monograph of Rhei radix (2) indicates a daily dose of 20 – 30 mg hydroxyanthracene derivatives calculated as rhein, but recommends that the pharmaceutical form must allow lower dosages than the usual daily dose.

The ESCOP monograph for "RHEI RADIX", 2nd edition, (4) recommends 15 – 50 mg hydroxyanthracene derivatives.

The WHO monograph on Rhizoma Rhei (5) recommends 10 – 30 mg hydroxyanthracene derivatives.

The recommendation in the German pharmacovigilance actions for anthranoid-containing laxatives of 21 June 1996 (2) only determines a daily maximum limit of 30 mg hydroxyanthracene derivatives in consideration of the toxicological data. The patient has to be informed that the correct individual dose is the smallest required to produce a comfortable soft-formed motion.

Taken into account that rhubarb also contains tannin agents which may possibly counteract the laxative effect of the anthraquinones and according to the German pharmacovigilance actions we recommend a dose range from 20 – 30 mg hydroxyanthracene derivatives calculated as rhein like the German monograph.

Normally it is sufficient to take an anthranoid-containing laxative up to two to three times a week (64).

II.3.2.2 Clinical studies (case studies and clinical trials)

Laxative effect

Fotiades P et al. 1976 (68) investigated the efficacy of Laxariston® in the treatment of constipation. 3 g of this preparation contain 0.9 g methyl cellulose, 0.3 g frangula bark (13.5 mg hydroxyanthracene derivatives), 0.3 g senna leaves (7.5 mg hydroxyanthracene derivatives), 0,15 g rhubarb root (6.75 mg hydroxyanthracene derivatives) and 0.015 g achillea extract. Laxariston® was given to 61 inpatients with mainly arthritic illness (3 g daily for 26.1 days on average) and to 33 outpatients mainly after abdominal surgery (7.6 g daily for 88.9 days). 31 patients of the whole study population had acute complaints, 20 patients suffered from chronic constipation and 41 patients from "functional" constipation. Special complaints are not mentioned in the publication. The time until disappearance of complaints was evaluated as follows: 0-2 days: very good efficacy; 3 – 14 days: good efficacy; 15 – 28 days: sufficient efficacy; more than 28 days: insufficient efficacy. Laxariston® had a very good efficacy in 71 patients (77.2%), a good efficacy in 19 patients (20.7%) and a satisfactory efficacy in 2 patients (2.1%). In the group with acute complaints the efficacy was very good in 77.4% and good in 22.6%. In the group with chronic complaints the efficacy was very good in 35%, good in 55% and satisfactory in 10 %. In the group with functional complaints the efficacy was very good in 97.6% and good in 2.4%. The

[3] In case of traditional use the long-standing use and experience should be assessed.

tolerance of the preparation was good in all these patients. The efficacy in 2 patients was not evaluated because these patients developed abdominal pain.

Bauer H 1977 (69) administered Laxariston® (specification see above) to 73 patients with gynaecological diseases and to 95 pregnant women suffering from constipation. Special complaints are not mentioned in the publication.

The first group consisted of 30 patients with laparotomy in the past, of whom 15 patients additionally took oestrogens, 6 patients with oestrogens and conservative gynaecological diseases, 7 patients with oestrogens and other medicinal products which influence the intestine motility, 13 patients with pathological-anatomic alteration in the pelvis minor, and 7 patients with constipation not caused by the gynaecological diseases. On average the women took this medicinal product for 47.2 days and the complaints disappeared in 5.3 days with a daily dose of 5.3 g. The time until disappearance of constipation complaints was assessed as follows: 0-3 days: very good efficacy; 4-5 days: good efficacy; 6-7 days: satisfactory efficacy; >7 days: insufficient efficacy. Efficacy was very good in 41 patients, good in 20 patients and satisfactory in 11 patients. One patient dropped out (reason not given). 6 patients (8.2%) complained about adverse reactions (spasms, tenesms, and nausea). 21 patients (28.8%) reported about positive reactions like weight reduction, decrease of haemorrhoidal complaints, and decrease of flatulence.

In the second group 14 pregnant women were in the first trimenon, 15 in the second, and 66 women in the third trimenon. On average Laxariston® was administered for 61.4 days and the complaints disappeared in 3.9 days with a daily dose of 3.9 g. Efficacy was very good in 55 patients, good in 31 patients, satisfactory in 7 patients and insufficient in 2 patients. This result was not analysed with regard to the different trimenons. 4 patients (4.2%) complained about adverse reactions. 29 patients (30.5%) reported about positive reactions. 12 women in the second group were gynaecologically treated because of a threatening abortion. One of these women only miscarried.There is no information about the state of the new-borns.

3 g of Laxariston® already contains 27.75 mg hydroxyanthracene derivatives, nearly 25% derive from rhubarb.

Borgia M et al. 1985 (70) carried out a double-blind controlled trial in 4 centers. 359 patients with slight or moderate functional disorders of the gastrointestinal tract were included. The effect of a combination of herbs with rhubarb, gentian, boldus and cascara (91 patients) was compared with the effect of placebo (90 patients), the effect of a combination with rhubarb and gentian (90 patients), and the effect of a combination with boldus and cascara (88 patients). The amount of anthranoides is not specified. The test preparation with rhubarb, gentian, boldus and cascara was more effective than the inert control excluding the disturbances not related to the pharmacological activities of the components. Combinations of the components “rhubarb + gentian” and “boldus + cascara” tested as active controls, showed significant favourable effects on disturbances of appetite and digestion and on constipation respectively.

Moser EH and Hübner WD 2002/2003 (65, 66) enrolled 284 patients between 19 and 70 years suffering from irritable bowel syndrome (IBS) in a 12-week double blind, controlled, randomised, multi-centre and prospective clinical trial to compare the efficacy as well as the tolerance of Eucarbon® tablets (containing as active ingredients “180 mg Carbo ligni”, i.e. vegetable, non-activated charcoal, “105 mg Fol. Sennae, 25 mg Extr. Rhei”) to Carbo ligni (CL) containing tablets. Men and women who met the Rome criteria for IBS (all forms) for at least 3 months were eligible. 145 patients received Eucarbon® and 139 patients Carbo ligni. During the first 4 weeks, the physician was allowed to adapt the dosage to a patient’s individual needs, from one to eight tablets per day. No dosage changes were allowed after the fourth week. The number of tablets prescribed daily (1-3, 4-6, or >6) was similar between groups, although a tendency to use fewer tablets was evident in the

Eucarbon® group. After the 12-week treatment period, 262 patients were available for ITT analysis and 144 for PP-analysis whereby changes of the disease were evaluated with scores based on the Francis IBS system (38) modified with an open upper boundary (a patient-administered questionnaire that uses a VAS (0%-100%) to score the severity of pain, distension, bowel dysfunction, and quality of life/global well-being) as the primary efficacy parameter. Scores on the VAS for overall well-being decreased in the PP population from 48 with Eucarbon® and 46 with CL before treatment (ITT, 47 and 47) to 18 and 20 after 12-week treatment (ITT, 19 and 22). This translates to an amelioration of symptoms in the PP population by 62.5% with Eucarbon® and 56.5% with CL; respective values in the ITT population were 59.6% and 53.2%. The relative gain in efficacy with Eucarbon® compared with its basic component (charcoal) was therefore only about 8% to 9% without statistical significance. Differences in the Francis score became more prominent in some subgroups selected for exploratory analysis. The patients who described "often normal stools" at baseline achieved significantly greater overall well-being after treatment with Eucarbon® (p=0.038, Wilcoxon test, PP population). Similar improvement in the subgroup admitting to "movements often hard" was more pronounced with Eucarbon® than with CL (not statistically significant). Both treatments were well tolerated, adverse events occurred with similar frequency in both groups (22% of patients treated with Eucarbon® vs. 17% treated with CL). In most cases, it was not possible to distinguish the event from symptoms of IBS.

The ingested dose of hydroxyanthracene derivatives is not mentioned in the publication. The leaflet of the chemical-pharmaceutical factory F. Trenka, Vienna, Austria, indicates an amount of 2.65 – 3.95 mg anthraquinone per tablet.

Other effects

From 1989 to 1992, 151 chronic renal failure (CRF) patients with initial serum creatinine level of 328 +/- 92 mmol/l (3.7 mg/dL) were enrolled to compare the clinical effectiveness of rhubarb (*Rheum palmatum* L.), an ACE inhibitor as well as a combined regimen of rhubarb and ACE inhibitor, captopril, in a prospective open-label trial. All patients were also kept on a low-protein (0.6 g/kg/day) and low-phosphorus (10 mg/kg/day) diet. After follow up of an average of 32.5 months (range, 15 to 62), uraemic symptoms of nausea and anorexia improved in most of the treated patients. The frequency of reaching a serum creatinine greater than or equal to 8 mg/dL was 54.3 % for the captopril group, 25.9 % for the rhubarb-treated group, and 13.1 % for those receiving the combined regimen. The slope of the reciprocal serum creatinine versus time in months suggests that the progression rate of renal failure was slowed down in the groups of patients treated with rhubarb. Rhubarb also lowered the cholesterol and triglyceride levels of CRF patients. The authors concluded that this effect might be helpful in preventing the development of glomerulosclerosis (72, 73, 113).

462 patients with upper gastrointestinal bleeding took *Rheum palmatum* L. (Dahunag) up to a maximum of 15.4 g herbal substance. Blood was not found any more in the faeces after 1.5 days in 97 % of the patients. The data are limited because the original Chinese publication is not available (3).

Zhou H et al. 1990 have studied alcoholic extracted tablets of rhubarb for 10 years. 312 patients with gastric and duodenal ulcer bleeding were divided into three groups, namely, *Rheum officinale* Baillon, *Rheum palmatum* L, and *Rheum tanguticum* Maxim ex Balf. By using double-blind measurement of effect, the efficiencies of the groups appeared to be 90.7 %, 93.7 %, and 92.8 % respectively. The time taken for the stool occult blood changing from positive into negative was 57.1, 53.4 and 56 hours respectively. The differences were not significant ($p > 0.05$). The data are also limited because the language of the original publication is Chinese and we can only focus on the English abstract (75).

Saller R et al. investigated the efficacy of a combined topical preparation with 23mg/g rhubarb (standardised dried aqueous-ethanolic extract from the roots of *Rheum palmatum* L. and *Rheum officinale* Baillon with 4.0 – 6.0 % hydroxyanthracene derivatives, calculated as

rhein) and 23 mg/g sage (aqueous extract from the leaves of *Salvia officinales*), of a single-agent preparation with sage extract and of a reference treatment Zovirax® Creme (50 mg acyclovir/g) for topical treatment of Herpes labialis in a double-blind, comparative, randomised trial. Out of 149 patients 145 patients (111 female, 34 male) could be evaluated by intention-to-treat analysis. 64 patients received the combination, 40 the sage cream, and 41 Zovirax cream. The mean time to crust formation in all cured patients was 7.2 days, 7.8 days, and 6.6 days respectively. The mean time to healing was 6.7 days, 7.6 days and 6.5 days respectively. No statistically significant differences could be found. There were statistically significant differences in the course of the symptoms. For the parameter "swelling", at the first follow-up visit there was a significant advantage for Zovirax cream compared to the sage cream, and for the parameter "pain", at the 2nd follow-up visit there was a significant difference in favour of the combination compared to the sage cream. No significant differences were found between the Zovirax treated and the sage treated group. The authors concluded that the combination was as effective as Zovirax and tended to be more active than the sage cream (76).

II.3.2.3 Clinical studies in special populations (e.g. elderly and children)

Use in children

First of all change of nutrition is recommended in constipated children with an increase in daily fibre intake. It is recommended that children older than 2 years of age should increase their intake of dietary fibre to an amount equal or greater than their age plus 5 g (77). The behaviour has to modify additionally, e.g. increased physical exercise.

There are no systematic clinical data available which evaluate the use of rhubarb as a laxative in children.

In China herbal treatment of neonatal jaundice (NNL) has been practiced for a long time. Even to-date, a variety of herbals, including *Rheum officinale* (Da-huang), are still being prescribed to jaundiced infants, often in combination with modern treatment such as phototherapy and exchange transfusion. Their efficacy and their safety have, however, not been tested systematically. No data are available of the exact preparation, the dose administered and the positive and negative outcomes (78).

There are several reports of local intolerance of a high dose senna preparation on skin in children wearing napkins. These skin irritations were bullous and comparable with skin irritations caused by scalds (78).

According to the ESCOP and WHO monographs the use for children less than 10 years cannot be recommended. According to the "NOTE FOR GUIDANCE ON CLINICAL INVESTIGATION OF MEDICINAL PRODUCTS IN THE PAEDIATRIC POPULATION" (CPMP/ICH/2711//99) of 27 July 2000 and other monographs age limit for children should be determined to "12 years of age".

II.3.2.4 Assessor's overall conclusions on clinical efficacy

There are no recent clinical investigations available, which evaluate rhubarb alone and not in combination with other laxatives in a representative study population. 2 non-controlled investigations of the seventies assessed the efficacy of a combination preparation in patients with constipation. 3 g of this preparation contain 27.75 mg hydroxyanthracene derivatives, nearly 25 % derive from rhubarb, and 0.9 g of the bulk forming agent methyl cellulose. The daily dose was 3 to 7.6 g on average. A contribution of rhubarb to the efficacy of the investigated product is supposable.

Two double-blinded controlled trials were also conducted with combinations which showed positive effects if different functional disorders of the gastrointestinal tract. The ingested doses of hydroxyanthracene derivatives derived from rhubarb are not mentioned. The contribution of rhubarb to the efficacy cannot be assessed.

The postulated laxative effect is mainly based on the pharmacological data, experts' opinions (German monograph, ESCOP monograph, WHO monograph etc.) and clinical experiences. Clinical and pharmacological data obtained from other anthranoid-containing laxatives (we refer to the assessment report of *Cassia senna* L. et *Cassia angustiolia* Vahl, folium) and the 2 non-controlled investigations with Laxariston® support the efficacy of this also anthranoid-containing herbal substance for short term use in cases of occasional constipation. The current level of evidence for "the short term use of occasional constipation" can be identified as III.

The investigations concerning other indications than occasional constipation are insufficient to support further indications.

Use for topical treatment of *Herpes labialis* in a combination with sage needs further investigation whether the part of rhubarb is essential for the efficacy or not.

II.3.3 Clinical Safety/Pharmacovigilance

II.3.3.1 Contraindications

Rhubarb preparations should not be used by patients with hypersensitivity to rhubarb.

Furthermore rhubarb 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 like all anthranoid-containing laxatives.

II.3.3.2 Special warning/precautions for use

Patients taking cardiac glycosides, antiarrhythmic medicinal products, medicinal products inducing QT-prolongation, diuretics, corticosteroids or liquorice root, have to consult a doctor before taking rhubarb preparations concomitantly.

Like all laxatives, rhubarb containing medicinal products 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).

If laxatives are needed every day the cause of the constipation should be investigated. Long-term use of stimulating laxatives should be avoided.

Use for more than 1 - 2 weeks requires medical supervision. Rhubarb preparations should only be used if a therapeutic effect cannot be achieved by a change of diet or the administration of bulk forming agents.

See also point 3.3.5 drug abuse.

II.3.3.3 Adverse events

Like all anthranoid-containing laxatives rhubarb 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 overdosage. In such cases dose reduction is necessary. The correct individual dose is the smallest required to produce a comfortable soft-formed motion (60).

Like mentioned above hypersensitive reactions may occur.

Chronic use may lead to disorders in water equilibrium and electrolyte metabolism.

Chronic use 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.

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

1 case of development of generalised flush 2 h after drinking a 10% aqueous solution of radix e rhizome rhei for constipation is described (80).

II.3.3.4 Overdose

Like for all anthranoid-containing laxatives the major symptoms of overdose / abuse are griping 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 corticosteroids 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 ingested overdoses of anthranoid containing medicinal products may lead to toxic hepatitis (we refer to the assessment report of senna and cascara).

Yuen MF et al. 2006 studied the clinical outcome of traditional Chinese medicine (TCM)-induced hepatotoxicity in chronic hepatitis B patients. 45 chronic hepatitis B patients in 2004 with liver dysfunction requiring hospitalisation in the Queen Mary Hospital, The University of Hong Kong, Hong Kong, were screened prospectively for traditional Chinese medicine intake. The inclusion criteria were HBsAg positivity, intake of TCM within 6 months prior to admission, elevated bilirubin levels of more than two times upper limit of normal or elevated level of at least one of the liver enzymes. Patients with other possible cause for hepatotoxicity, e.g. any other virus hepatitis, alcohol intake, hepatotoxic medication were excluded. Seven patients had liver derangement attributable to the intake of TCM. Possibly hepatotoxic components were identified by extensive literature research. *Rheum palmatum* L. and *Cassia obtusifolia* L. were identified to be one of the hepatotoxic components, though these components were taken together with more than ten other Chinese herbal substances. From our point of view the causality cannot be assessed definitely (81).

II.3.3.5 Drug abuse

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

Müller-Lissner 2005 (82) concludes 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 the investigations that do not support such damage are well done. The cited references (Smith B 1968 (83); Riemann JF et al. 1980 (84) and 1982 (85); Berkelhammer C et al. 2002 (86); Meisel JL et al. 1977 (87); Pockros PJ et al. 1985 (88)) show abnormalities observed in humans (damage to enteric nerves, smooth muscle atrophy; distension or ballooning of axons, reduction of nerv-specific cell structures and increase in lysosomes, and sometimes a total degeneration of whole nerve fibers; short-lived superficial damage to the mucosa). They are uncontrolled observations and therefore the author concludes that the cause of these damages can also be the constipation itself or pre-existing changes of unknown etiology.

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 EO et al. 1990 (89)) does not support the hypothesis that anthraquinone containing laxatives are able to provoke relevant degenerative changes in the colonic nerve tissue. But this investigation was only conducted in 11 matched pairs only.

For safety concerns we inform the patients that if stimulating laxatives are taken for longer than a brief period of treatment, this may lead to impaired function of the intestine and dependence on laxatives.

Controversial discussion is done whether chronic use of anthranoid-containing laxatives promotes the development of colorectal carcinomas.

Siegers C-P et al. 1993 (90) 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% in patients with no abnormality seen on endoscopy, 9.8% ($p=0.068$) in patients with adenomas and 18.6% in 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 anthranoid laxative abuse if the pseudomelanosis coli in patients with no abnormality is calculated with 1 %.

Kune GA et al. 1988 (91) and Kune GA 1993 (92) reported of 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 community 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 aetiologic 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 ($p=0.02$) was found with the risk of colorectal cancer 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 G et al. 1993 (93) tested whether in these patients laxative use or the endoscopially 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 A and Muller AD 1993 (94) performed a meta-analysis, since individual case-control studies have failed to resolve the question whether constipation and use of cathartics represent significant risk factors of colorectal cancer. The analysis of 14 previously (from 1954 to 1988) published case-control studies revealed statistically significant risks for colorectal cancer associated with both constipation and use of cathartics, the pooled odds ratios 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 ratio than various dietary components, such as fat, meat, alcohol, and low-vegetable or low-

residue diets, the authors concluded that their risks reflects the confounding influence of underlying dietary habits.

Loew D et al. 1994 (95) 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 pseudomelanosis coli (PMC) test was used as an indicator of exposure to anthranoid-containing 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</=0.197).

Jacobs EJ et White E 1998 (96) examined the associations of colon cancer with constipation and use of commercial laxatives in a case-control study (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 G et al. 2000 (97) performed a prospective case control study at the University of Erlangen to investigate the risk of anthranoid-containing 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 M et al. 2003 (71) describe a case of melanosis coli, which occurred in a 39-year-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. From a practical point of view, the authors discourage from use of anthranoid-containing laxatives, although they stated that "the role of the short-term use of the laxative in the development of this patient's adenoma is highly speculative" because he clearly was at risk for developing colonic neoplasm considering his long-standing ulcerative colitis in association with primary sclerosing cholangitis and the use of immunosuppressive medication after liver transplantation. Furthermore it would remain controversial whether melanosis coli is associated with an increased risk for colorectal cancer because of controversial results of several investigations.

Roberts MC et al. 2003 (98) conducted a population-based, case-control study with equal representation by blacks. Constipation, defined as fewer than three reported bowel movements per week, was associated with a greater than two-fold risk for 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 do not explicitly mention anthraquinone-containing laxatives. They mentioned the group "stimulants, fibers, natural remedies, stool softeners, oils, osmotic agents, enemas, suppositories, and unknown". unknown". In particular they mention phenolphthalein and magnesium.

Nilsson SE et al. 2004 (99) 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 might indicate an increased risk of cancer, and cardiovascular and neurological diseases. The homocysteine level depends on the supply of folate and cobalamine, and constipation and/or laxative treatment might compromise this supply. 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 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.

Jae Sik Joo et al. 1998 (100) 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 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 conclude 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.

II.3.3.6 Safety in special populations and situations

When rhubarb preparations are administered to incontinent adults, pads should be changed more frequently to prevent extended skin contact with faeces because of the experiences in children wearing napkins.

Patients with kidney disorders should be aware of possible electrolyte balance.

II.3.3.6.1 Drug interactions

Patients taking cardiac glycosides, antiarrhythmic medicinal products, medicinal products inducing QT-prolongation, diuretics, corticosteroids or liquorice root, have to consult a doctor before taking rhubarb preparations concomitantly.

Chronic use or abuse of rhubarb preparations may lead to hypokalaemia like the abuse of all anthranoid-containing laxatives. This hypokalaemia and the increased loss of potassium may

interfere with cardiac glycosides, antiarrhythmic medicinal products, and medicinal products inducing QT-prolongation. Concomitant use with diuretics, corticosteroids and liquorice root may aggravate electrolyte imbalance.

II.3.3.6.2 Use in pregnancy and lactation

There are no recent investigations available.

Like mentioned above (69), 95 pregnant women suffering from constipation were treated with a combination preparation containing rhubarb. Most of them were in the third trimenon. 12 women were gynaecologically treated because of a threatening abortion. Only one of these women miscarried. No information about the state of the new-borns is given in the publication.

In theory, it is possible that reflex stimulation might occur, involving not only the colon but also uterine muscles and then might lead to the development of hyperaemia in the pelvic region and to miscarriage as a result of neuromuscular stimulation of uterine muscles. Therefore anthranoid-containing laxatives were misused as an abortifacient agent. Animal experiments demonstrated that placental passage of rhein is small.

Experimental data, mainly in vitro tests showed a genotoxic risk of several anthranoids (e.g. emodin, chrysophanol, and physcion). In vitro assays overestimate the potential hazard from exposure and must be reevaluated by in vivo experiments. The NOAELs for emodin defined by Jahnke GD are twice the decimal power and above the maximum daily dose of hydroxyanthracene derivatives (30 mg).

In vivo studies of the crude senna herbal substance in rat hepatocytes (chromosome aberration test, mouse spot test, in vivo/in vitro UDS (unscheduled DNA synthesis); showed no evidence of any genetic effects (Heidemann 1993 (49)).

Rhubarb and also senna mainly contain dianthron-glycosides, cascara and also frangula and aloe mainly contain 10-glycosyl-anthrones, anthrachinon- and anthron-glycosides. The risk of hydrolysis in aglycones and consequently systemic absorption in the gastrointestinal tract is higher for the anthrachinon- and anthron-glycosides than for the dianthron-glycosides due to the different chemical structures. The amount of aglycones represents the possible genotoxic risk. Even for this reason cascara, frangula and aloe seem to be less appropriate for sensitive patient groups like pregnant women than senna and rhubarb (103).

On the other hand there only exist older preclinical data which refer to a special extract of senna pods containing 1.4 to 3.5 % of anthranoids, corresponding to 0.9 to 2.3 % of potential rhein, 0.05 to 0.15% of potential aloe-emodin and 0.001 to 0.006% of potential emodin or to isolated active constituents, rhein or sennosides A and B. No cytotoxic, toxic, embryotoxic or genotoxic effect could be found in Chromosome Aberration Assay in Bone Marrow Cells of the Rat, Micronucleus Test in Rats, Mouse Spot Test. Therefore the monographs on senna recommend for such a specified senna extract that the use is to be avoided just during the first trimester but with the advice that the further use during pregnancy should only be an intermittent and just if other actions like behavioural modification, dietary changes and use of bulk forming agents fail. Use in pregnancy cannot be recommended for all other senna preparations (we refer to the senna assessment report).

Such data are not available for rhubarb or any preparation thereof. Therefore use during pregnancy cannot be recommended. Furthermore other actions like behavioural modification, dietary changes and use of bulk forming agents should be the first actions taken during pregnancy to treat constipation.

Use during lactation is not recommended, as there are insufficient data on the excretion of metabolites in breast milk, too. Investigations with a “standardised senna laxative” (Agiolax®), which also contains Plantago ovata seeds/husks as bulk substances, showed that

small amounts of active metabolites (rhein) are excreted in breast milk. A laxative effect in breast fed babies has not been reported (104).

Bright E et al. 1970 reported when rhubarb was taken by lactating mothers, the amount excreted in the milk was too small to affect the baby (105). There is no detailed information available.

II.3.3.7 Assessor's overall conclusions on clinical safety

Because of the possible genotoxic or tumourigenic risk in experimental investigations and the results of Siegers 1993 a pharmacovigilance actions for anthranoid-containing laxatives (60) were initiated in Germany 1996. The daily dose and the duration of administration were limited. Children, pregnant women and nursing mothers were excluded from the application of rhubarb containing laxatives.

The results of the more recent studies are inconsistent and the possibility of a carcinogenic risk of long-term use of anthranoid-containing laxatives cannot be assessed definitely. 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 assess the carcinogenic risk definitely.

In his review article van Gorkom BA 1999 (101) concluded that although the short-term use of anthranoid laxatives is generally safe, long-term use cannot be recommended.

In "Goodman & Gilman's The Pharmacological Basis of Therapeutics" (11th edition 2006) (102) the following conclusion is drawn: "Regardless of whether a definitive causal relationship can be demonstrated between the use of anthraquinone laxatives and colonic pathology, these agents should not be recommended for chronic or long-term use."

Taking all available data in consideration, the conditions determined in the German pharmacovigilance actions for anthranoid-containing laxatives (60) have to be maintained for the moment.

Within a period of 4 – 13 months long-term administration of anthranoid-containing medicinal products leads to 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 halting laxative administration.

Long-term stimulant laxative use may result in anatomic changes in the colon. For safety concerns we inform the patients that if stimulating laxatives are taken for longer than a brief period of treatment, this may lead to impaired function of the intestine and dependence on laxatives.

II.4 TRADITIONAL USE

Rhubarb was medically used in China long time ago. Rhubarb is already mentioned in an herbal book of the 27th century BC (106). The Chinese Materia Medica of 1998 (112) described the traditional use as follows: "Purging heat and loosing the bowels, used for retention of the feces and abdominal pain, fever with constipation and dysentery with inadequate discharge of the bowels; reducing heat in the blood and counteracting toxicity, used for hematemesis and epistaxis, inflammation of the eyes and swelling of the throat and gum due to heat in the blood; jaundice caused by damp-heat. Externally used for scalds and burns; eliminating blood stasis, traumatic injuries, hemorrhage from the upper gastrointestinal tract, appendicitis with abdominal pain, boils, sores and abscess."

In Europe Pedanios Dioskurides, a Greek physician and pharmacologist of the 1st century BC, mentioned the use of *Rheum rhaponticum* in his

'Materia Medica" for several gastrointestinal and urogenital disorders, for periodic fever and for bites of poisonous animals (107).

Paracelsus (1494 – 1541) used rhubarb as a laxative and as a purgative for the gall. Lonicerus also indicated the use as a purgative of the liver and for fever in his herbal book from 1564. The purgative effects were also described by Bock (1565) and Matthiolus (1626). Matthiolus also used rhubarb for dysentery with bloody diarrhoea, haematemesis and for hypermenorrhoea. The use as a laxative and purgative, but also the use as an antidiarrhoeal agent was confirmed by von Haller (1755) and by Weinmann (1745). Weimann also used rhubarb as a tonic (106).

Hecker (1814) favoured the use for diarrhoea more than for constipation. He also used rhubarb as a tonic and a styptic.The antidiarrhoeal and styptic effects are only achieved by small doses (0.1 -0.3 g), which shall inhibit fermentation process, gastric acid and mucus secretion and improve appetite (106). This is confirmed by Weiss 1974 (109) and Martindale 1967 and 1977, but the recommended dose in Martindale is 0.2 – 1 g: "Rhubarb is a mild anthraquinone purgative. It differs from other anthraquinone purgatives in that it exerts an adstringent action after purgation; with small doses the adstringent action predominates and rhubarb is therefore used as an adstringent bitter and occasionally in the treatment of diarrhoea." (110, 108)

Clarus (1860) (106) and Weiss 1974 (109) ascribed choleric properties to rhubarb.

Hager 1927 (111) indicated that rhubarb has a laxative effect when administered in repeated or in higher doses. Rhubarb is also used as a stomachic and to improve appetite, for gastrointestinal catarrh and liver and spleen diseases.

According to Madaus (106) rhubarb was used as a laxative, a stomachic and for liver and spleen diseases in Denmark. The bark of the root was used for cough and cold in Lithuania. In Austria rhubarb was used as a laxative and purgative and in Hungary also a laxative.

II.4.1.1 Assessor's overall conclusions on traditional use

The use of rhubarb as a laxative is mentioned in nearly all references. In consequence of the laxative properties the herbal substance was also used as a purgative. The use as a laxative is accepted as a well established use.

In former times purification often was the first step to treat a lot of diseases. Such a procedure is obsolete now. Furthermore there are no plausible pharmacological data for the purification of other organs than the bowel.

Due to the content of tannin agents rhubarb was also used for diarrhoea and dysentery and as a styptic. The dose administered shall be smaller than for the use as laxative but the data are not consistent.

Due to the possible risks traditional use cannot be accepted.

II.5 ASSESSOR'S OVERALL CONCLUSIONS

The postulated laxative effect is mainly based on the pharmacological data, experts' opinions (German monograph, ESCOP monograph, WHO monograph etc.) and clinical experiences. Clinical and pharmacological data obtained from other anthranoid-containing laxatives (we refer to the assessment report of *Cassia senna* L. et *Cassia angustiolia* Vahl, folium) and the 2 non-controlled investigations with Laxariston® support the efficacy of this also anthranoid-containing herbal substance for short term use in cases of occasional constipation. The current level of evidence for "the short term use of occasional constipation" can be identified as III.

The investigations concerning other indications than occasional constipation are insufficient to support further indications.

There are two investigations dealing with the treatment of gastrointestinal bleeding. The data available are limited and cannot be assessed definitely. A well-established use cannot be granted.

Use for topical treatment of *Herpes labialis* in a combination with sage needs further investigation whether the part of rhubarb is essential for the efficacy or not. Furthermore such a use does not fulfil the criteria for well-established use according to the Directive 2001/83/EC. This must be regarded as a new indication and all documents for a full application have to be submitted.

Due to the possible risks traditional use as described above cannot be accepted.

The conditions determined in the pharmacovigilance actions for anthranoid-containing laxatives have to be maintained for the moment because further investigations are needed to clarify the carcinogenic risk. The results of the more recent studies are inconsistent. However, a risk was also revealed for constipation itself and underlying dietary habits.

The use in children below the age of 12 years and during pregnancy and lactation is not recommended.

In consideration of the recommendations in the monograph the benefit-risk-ratio is positive and the short term use of occasional constipation can be regarded as safe.

III. ANNEXES

III.1 PROPOSED COMMUNITY HERBAL MONOGRAPHS FOR <NAME OF HERBAL SUBSTANCE(S), HERBAL PREPARATION(S) OR COMBINATIONS THEREOF[4][5]>

III.2 PROPOSAL FOR LIST ENTRY ON <HERBAL SUBSTANCE(S), HERBAL PREPARATION(S) OR COMBINATIONS THEREOF WITH TRADITIONAL USE>[6]

III.3 LITERATURE REFERENCES

[4] According to the 'Procedure for the preparation of Community monographs for traditional herbal medicinal products' (EMEA/HMPC/182320/2005 Rev.2)

[5] According to the 'Procedure for the preparation of Community monographs for herbal medicinal products with well-established medicinal use' (EMEA/HMPC/182352/2005 Rev.2)

[6] According to the 'Structure of the Community list of herbal substances, preparations and combinations thereof for use in traditional herbal medicinal products' (EMEA/HMPC/100824/2005 Rev.2)