C. RUBIA TINCTORUM, MORINDA OFFICINALIS AND ANTHRAQUINONES

1. Exposure Data

1.1 Origin, type and botanical data

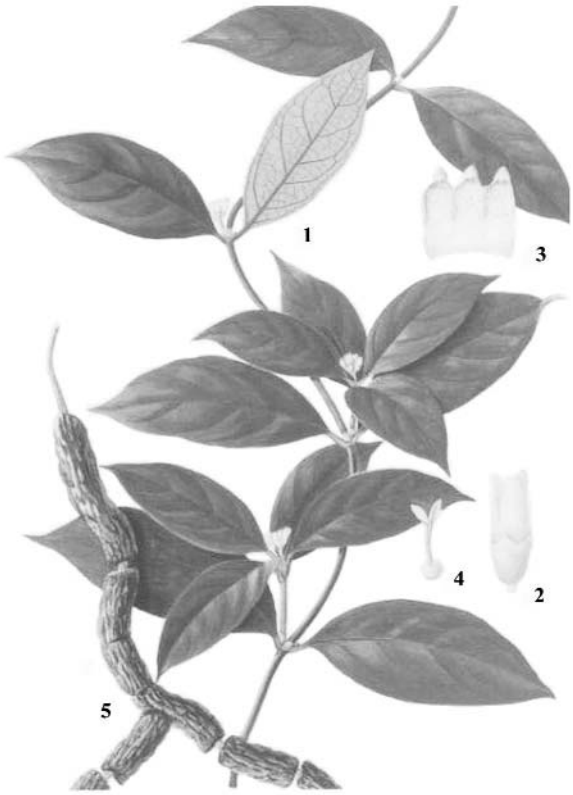
1.1.1 Rubia tinctorum *L.* (*Rubiaceae*)

The medicinal part of *Rubia tinctorum* is the dried root (Blumenthal *et al.*, 1998) (common names: madder, dyer's madder (Felter & Lloyd, 2002)). The small yellowish-green flowers are in loose, leafy, long-peduncled terminal or axillary cymes. The margin of the calyx is indistinct, 4- to 5-sectioned and has a tip that is curved inward. There are five stamens and an inferior ovary. The fruit is a black, pea-sized glabrous, smooth drupe containing two seeds. The perennial plant grows to a height of 60 to 100 cm. The pencil-thick rhizome creeps widely underground. The stem is quadrangular with backward-turning prickles at the edges. The stems are at times so thin that they are more descendent than erect. The leaves are in whorls, in fours below, in sixes above. They are oblong to lanceolate with one rib and protrudingly reticulate beneath. The plant is indigenous to southern Europe, western Asia and North Africa, and is cultivated elsewhere (Medical Economics Co., 2000).

1.1.2 Morinda officinalis *How* (*Rubiaceae*) (Figure 1)

The medicinal root of *M. officinalis* is cylinder-shaped (with oblate circumference of the section) and slightly curved. The diameter of the root is usually 0.5–2 cm. The surface, with vertical wrinkles and transverse crackles, is yellowish-gray or dark gray. In some roots, the bark is thicker, violet or light violet in colour, and easy to separate from the xylem. The xylem, with a diameter of 1–5 mm and with slightly dentate margin, is hard and yellowish-brown or whitish-yellow in colour. Morinda root is odourless, sweet and slightly sour in taste. The fleshy root is used for medicinal purposes.

Figure 1. *Morinda officinalis* How



From Qian (1996)
1. flower twig; 2, flower; 3, stamen in dissected flower; 4. pistil; 5, root

1.2 Use

The roots of *R. tinctorum* contain a red colouring matter which is used for dyeing. Additionally, extracts from *R. tinctorum* are used for the treatment of kidney and bladder stones (Westendorf *et al*., 1990; Blömeke *et al*., 1992). Plants containing 1-hydroxy-anthraquinone have been widely used for pharmaceutical purposes such as treatment of kidney and bladder stones, as a laxative mixture, and as a mild sedative (Brown & Brown, 1976; Mori *et al*., 1990; Wang *et al*., 1992). Madder root has reportedly also been used medicinally for menstrual and urinary disorders (Medical Economics Co., 1998, 2000). The roots of *M. officinalis* have been used as a Chinese natural medicine for tonic and analgesic purposes.

Anthraquinone glycosides are the active principles of plant-derived laxatives such as senna, cascara, frangula, rhubarb and aloe. These five plant-derived laxative substances are not included in the present review, because there are no published reports on their

potential carcinogenicity. 1,8-Dihydroxyanthraquinone, the aglycone moiety of the laxative ingredient of senna, was formerly marketed as a laxative under the trade name Dantron®, but human drug products containing Dantron® (see IARC, 1990) were withdrawn from commerce in the United States in 1987 after it was shown to cause intestinal tumours in experimental animals.

1.3 Chemical constituents

Anthracene derivatives are widely distributed in the plant kingdom. Especially in the dicotyledons, many families, such as the Hypericaceae (*Hypericum*), Polygonaceae (*Rheum*, *Rumex*, *Polygonum*), Rhamnaceae (*Rhamnus*) and Rubiaceae (*Rubia*, *Morinda*, *Galium*), are rich in anthracene derivatives. In the monocotyledons, only the family Liliaceae (*Aloe*) contains this class of chemicals. About 90% of these compounds occur as derivatives of 9,10-anthracenedione (anthraquinones) with several hydroxy and other functional groups, such as methyl, hydroxymethyl and carboxy groups. Hydroxyanthraquinones are the active principles of many phytotherapeutic drugs.

1.3.1 Rubia tinctorum

Compounds found in *Rubia tinctorum* include purpurin (oxyalizarin; 1,2,4-trihydroxyanthraquinone), mollugin (6-hydroxy-2,2-dimethyl-2*H*-naphtho[1,2-*b*]pyran-5-carboxylic acid, methyl ester), 1-hydroxy-2-methylanthraquinone, 2-ethoxymethylanthraquinone, rubiadin (1,3-dihydroxy-2-methylanthraquinone), 1,3-dihydroxyanthraquinone, 7-hydroxy-2-methylanthraquinone, lucidin (1,3-dihydroxy-2-hydroxymethylanthraquinone), 1-methoxymethylanthraquinone, 2,6-dihydroxyanthraquinone, lucidin-3-*O*-primeveroside [6-(β-D-xylosido)-D-glucoside], alizarin (1,2-dihydroxyanthraquinone), lucidin-*O*-ethyl ether, 1-hydroxy-2-hydroxymethylanthraquinone 3-glucoside, 2-hydroxymethylanthraquinone 3-glucoside, 3,8-dihydroxy-2-hydroxymethylanthraquinone 3-glucoside, ruberythric acid (alizarin primeveroside; alizarin glycoside), quinizarin and iridoid asperuloside (Schneider *et al*., 1979; Kawasaki *et al*., 1992; Derksen *et al*., 1998; El-Emary & Backheet, 1998; Marczylo *et al*., 2000).

A number of compounds have been characterized from the roots of *R. tinctorum* (the source of commercial madder colour) by various analytical methods. Among these compounds are alizarin, ruberythric acid, purpurin, lucidin, rubiadin, mollugin, 1-hydroxy-2-methylanthraquinone, tectoquinone (2-methylanthraquinone), nordamnacanthal (1,3-dihydroxy-2-anthraquinonecarboxaldehyde), 1-hydroxy-2-methoxyanthraquinone, 1,3-dihydroxy-2-ethoxymethylanthraquinone, scopoletin (7-hydroxy-6-methoxycoumarin) and the glucosides and/or the primeverosides of these compounds (Kawasaki *et al*., 1992; Westendorf *et al*., 1998; Medical Economics Co., 2000). The majority of the anthraquinones present in the plant itself or in plant extracts are glycosides (Blömeke *et al*., 1992; Westendorf *et al*., 1998).

1.3.2 Morinda officinalis

A number of compounds have been isolated from *M. officinalis*. Anthraquinones found in the plant include 1,6-dihydroxy-2,4-dimethoxyanthraquinone, 1,6-dihydroxy-2-methoxyanthraquinone, methylisoalizarin, methylisoalizarin-1-methyl ether, 1-hydroxy-2-methoxyanthraquinone, 1-hydroxy-2-methylanthraquinone, physcion, 1-hydroxy-anthraquinone, 2-methylanthraquinone, 2-hydroxy-3-hydroxymethylanthraquinone, rubiadin and rubiadin-1-methyl ether. Terpenoids found include asperuloside tetra-acetate, monotropein, morindolide and morofficinaloside. Glucosides found include nystose, 1F-fructofuranosylnystose, inulin-type hexasaccharide and heptasaccharide. β-Sitosterol, 24-ethylcholesterol, a ketone (officinalisin) and several amino acids have also been found (Li *et al.*, 1991; Yang *et al*., 1992; Yoshikawa *et al*., 1995; Zheng & Dong, 1997; Yao *et al*., 1998).

1.4 Sales and consumption

No data were available to the Working Group.

1.5 Component(s) with potential cancer hazard (1-hydroxyanthraquinone; 1,3-dihydroxy-2-hydroxymethylanthraquinone (lucidin))

1.5.1 *Nomenclature*

1-Hydroxyanthraquinone

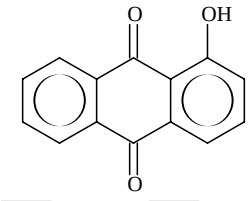
Chem. Abstr. Serv. Reg. No.: 129-43-1
Chem. Abstr. Serv. Name: 1-Hydroxy-9,10-anthracenedione
Synonyms: Erythroxyanthraquinone; α-hydroxyanthraquinone

1,3-Dihydroxy-2-hydroxymethylanthraquinone (lucidin)

Chem. Abstr. Serv. Reg. No.: 478-08-0
Chem. Abstr. Serv. Name: 1,3-Dihydroxy-2-(hydroxymethyl)-9,10-anthracenedione
Synonyms: Henine

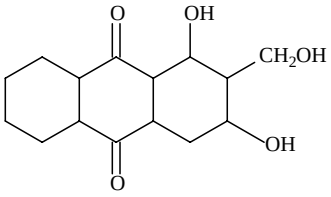
1.5.2 *Structural and molecular formulae and relative molecular mass*

1-Hydroxyanthraquinone



$C_{14}H_8O_3$ Relative molecular mass: 224.21

Lucidin



$C_{15}H_{10}O_5$ Relative molecular mass: 270.24

1.5.3 *Chemical and physical properties of the pure substance*

1-Hydroxyanthraquinone

(*a*) *Description*: Fine yellow crystals (Buckingham, 2001)
(*b*) *Melting-point*: 195–196 °C (Buckingham, 2001)

Lucidin

(*a*) *Description*: Yellow crystals (Buckingham, 2001)
(*b*) *Melting-point*: 330 °C (Buckingham, 2001)
(*c*) *Dissociation constant*: pK_a, 8.11 (20 °C, water) (Buckingham, 2001)

1.5.4 *Analysis*

A method using reversed-phase high-performance liquid chromatography (HPLC) has been described that allows the separation of 13 naturally occurring naphthoquinones and anthraquinones, including 1-hydroxyanthraquinone. The separation was achieved under isocratic and gradient conditions (Steinert *et al.*, 1996).

The following method has been used to determine hydroxyanthraquinones in experimental laboratory animal diet containing madder. Hydroxyanthraquinones were isolated

by Soxhlet extraction with ethyl acetate, evaporation of the solvent, and dissolution in methanol. Separation of the hydroxyanthraquinones was accomplished by HPLC with spectrometric detection at 410 nm (Westendorf *et al*., 1998).

Lucidin content in *R. tinctorum* crude drug powder has been determined using reversed-phase HPLC with methanol/acetic acid as the mobile phase and detection at 225 nm (Wang *et al*., 1997).

An HPLC method with isocratic elution has been developed for the separation of anthraquinones with particular attention to the detection of lucidin in commercially available sources of *R. tinctorum* aglycones (Bosáková *et al*., 2000).

A reversed-phase HPLC method has been developed for the simultaneous characterization of anthraquinone glycosides and aglycones in extracts of *R. tinctorum*. The anthraquinones, including lucidin, are separated on a reversed-phase column with a water–acetonitrile gradient as eluent and measured with ultraviolet detection at 250 nm (Krizsan *et al*., 1996; Derksen *et al*., 1998).

1.5.5 *Production*

1-Hydroxyanthraquinone has been synthesized by diazotization of 1-aminoanthraquinone and heating the diazonium salt with concentrated sulfuric acid. After dilution with water, the precipitated crude product was diluted with acetone and purified by preparative thin-layer chromatography (TLC) in toluene:ethyl formate:formic acid (75:24:1) (Blömeke *et al*., 1992).

A general method for synthesis of anthraquinones, including 1-hydroxy-anthraquinone, has been developed. The anthraquinones were obtained under mild conditions from *ortho*-dicarboxylic acid chlorides and suitable aromatic substrates via a Friedel–Crafts process (Sartori *et al*., 1990).

1-Hydroxyanthraquinone has also been prepared in 96.4% yield by reacting 1-nitroanthraquinone with sodium formate in dimethylformamide at 130 °C for 17 hours (Michalowicz, 1981).

Lucidin has been synthesized from nordamnacanthal (Prista *et al*., 1965).

Available information indicates that 1-hydroxyanthraquinone is manufactured by one company each in China and Japan (Chemical Information Services, 2001).

1.5.6 *Use*

Anthraquinones are the largest group of naturally occurring quinones. Both natural and synthetic anthraquinones have been widely used as colourants in food, drugs, cosmetics, hair dyes and textiles (Brown & Brown, 1976; Mori *et al*., 1990). 1-Hydroxyanthraquinone can be used as an intermediate in the production of dyes and drugs (Imaki & Fukumoto, 1988). The Working Group was not aware of any commercial use of purified lucidin.

1.5.7 *Occurrence*

1-Hydroxyanthraquinone has been isolated from the roots of *Rubia cordifolia*, *Morinda officinalis* and *Damnacanthus indicus*, from the heartwood of *Tabebuia avellanedae* and the herb *Cassia occidentalis* (Brown & Brown, 1976; Mori *et al*., 1990; Wang *et al.*, 1992; Yang *et al.*, 1992; Steinert *et al*., 1996; Buckingham, 2001). 1-Hydroxyanthraquinone has also been identified as a metabolite of alizarin primeveroside, found in *Rubia tinctorum*, when alizarin primeveroside was given orally to rats (Blömeke *et al*., 1992).

Lucidin has been identified in plants from *Rubia* (*R. tinctorum, R. iberica*), *Coprosma (C. lucida, C. rotundifolia, C. acerosa)*, *Morinda (M. citrifolia, M. umbellata)*, *Galium* (*G. fagetorum, G. mollugo, G. dasypodum*), *Hymenodictyon* (*H. excelsum*) and *Commitheca* (*C. liebrechtsiana*) species (Prista *et al*., 1965; Burnett & Thomson, 1968a,b; Zhural'ov & Borisov, 1970; Thomson & Brew, 1971; Murti *et al*., 1972; Leistner, 1975; Briggs *et al*., 1976; Hocquemiller *et al*., 1976; Bauch & Leistner, 1978; Inoue *et al*., 1981; Zhural'ov *et al*., 1987; Buckingham, 2001).

1.5.8 *Human exposure*

Thousands of patients in European countries have been treated chronically in the past, against kidney stones, with madder root preparations (*R. tinctorum*) at high doses. The daily amount of lucidin ingested by these patients was calculated to be 3–10 mg. Under certain circumstances, the daily lucidin intake may even reach several hundred milligrams (Westendorf *et al*., 1998).

2. Studies of Cancer in Humans

Laxatives based on naturally occurring anthraquinone derivatives

Herbs containing anthraquinone derivatives (rhubarb, senna, frangula, cascara, aloe) are used as laxatives. A meta-analysis of 11 studies dealing with colorectal cancers and laxative use showed that use of laxatives carried an increased risk (odds ratio, 1.5; 95% CI, 1.3–1.6) for colorectal cancer (Sonnenberg & Müller, 1993). A more recent and very large study among nurses in the USA found no association between colorectal cancer and laxative use (Dukas *et al.*, 2000). The relevance of this evidence to the carcinogenicity of anthraquinone-containing herbs used as laxatives is unknown because it is uncertain whether the use of laxatives in general is an adequate proxy measure for the use of anthraquinones.

The following studies mentioned anthraquinones specifically in evaluating laxative use as a risk factor for cancer. Also reported below are studies that used melanosis coli as a marker of exposure, because this in turn may reflect consumption of anthraquinones (see Section 4.2).

2.1 Case–control studies

2.1.1 *Gastrointestinal cancer*

Boyd and Doll (1954) analysed data collected in a previous inquiry, which detailed histories of purgative use for 2249 patients. People whose history of taking purgatives extended over a continuous period of at least five years were considered as 'purgative users'. After excluding patients suffering from gastrointestinal diseases other than cancer, there remained 614 patients with gastrointestinal cancers (387 cancers of the large bowel and 227 cancers of the stomach) and 1313 control patients (647 cancers of the lung and 666 patients with non-cancer non-gastrointestinal diseases). A history of regular (at least once per week) use of purgatives was reported for 222/614 [36.2%] of the patients with gastrointestinal cancer and 343/1313 [26.1%] of the control patients. Use of cascara was reported by [6%] of cases and [5.4%] of controls. Use of senna was reported by [6.2%] of cases and [2.6%] of controls and when exposure was restricted to chronic users (regularly, at least once per week), the percentages were [5.2%] of cases and [1.7%] of controls ($p = 0.04$). The difference between cases and controls was similar for separate consideration of cancer of the large bowel.

Siegers *et al.* (1993) studied retrospectively 3049 patients and prospectively 1095 patients who underwent endoscopic examinations in Lübeck, Germany. In the retrospective study, melanosis coli was found in 3.1% of patients without other abnormalities, in [2.8%] of patients with colitis or diverticulosis, in 8.6% of patients with adenomas and in 3.9% of patients with carcinomas. In the prospective study, melanosis coli was found in 6.9% in patients without other abnormalities, in [4.5%] in patients with colitis or diverticulosis, in 9.8% of patients with adenomas and in 18.6% of patients with carcinomas. The relative risk for colorectal cancer among subjects with melanosis coli was 3.0 (95% CI, 1.2–4.9). Among 33 of the patients who had both melanosis coli and adenoma or carcinoma, all but two acknowledged abuse of anthranoid laxatives for 10–30 years. [The Working Group noted that detection of melanosis coli requires colonoscopy and that indication of colonoscopy may result in selection bias of the controls.]

Kune (1993) analysed laxative use in 685 colorectal cancer patients in Australia, diagnosed over a 12-month period (1980–81), compared with 723 age- and sex-matched community-based controls. Use of laxatives containing anthraquinones was reported by [13.9%] of the cases of colorectal cancers and [14.1%] of controls. Comparing cases and controls using laxatives containing anthraquinones with patients and controls using no laxatives, the relative risk for colorectal cancer related to the use of laxatives containing anthraquinones was 1.01 [confidence intervals were not reported].

In a retrospective analysis of 2229 consecutive patients having undergone a colonoscopy in Erlangen, Germany, between 1985 and 1992, the presence of colorectal cancer was not associated with melanosis coli or laxative use: colorectal cancer was present in 2.7% ($n = 60$) of all patients, 2.9 % ($n = 3$) of the 102 patients with melanosis coli (relative risk, 1.1; 95% CI, 0.35–3.4) and in 3.1% ($n = 9$) of the 286 patients with anthranoid laxative use (relative risk, 1.1; 95% CI, 0.48–2.3) (Nusko *et al.*, 1997). [The Working

Group noted some discrepancies with data from the same study reported by Nusko *et al.* (1993).]

Nusko *et al*. (2000) performed a prospective case–control study in Erlangen of the association between anthranoid laxative use and risk for development of colorectal adenomas or carcinomas. The study included a total of 202 patients with newly diagnosed colorectal carcinomas, 114 patients with adenomatous polyps and 238 patients (controls) with no colorectal neoplasms who had been referred for total colonoscopy between 1993 and 1996. For each subject, the use of anthranoid preparations was assessed by standardized non-blind interview after colonoscopy, and melanosis coli was studied by histopathological examination. Use of anthranoid laxatives was reported by 33/238 controls, 16/114 adenoma patients and 29/202 carcinoma patients. Anthranoid use did not confer any significantly elevated risk for development of colorectal adenomas (unadjusted odds ratio, 1.0; 95% CI, 0.5–1.9) or carcinomas (unadjusted odds radio, 1.0; 95% CI, 0.6–1.8). 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 that for carcinomas was 0.93 (95% CI, 0.5–1.7). Duration of anthranoid laxative use, when included in the logistic regression as a continuous variable, was not significantly associated with colorectal carcinoma ($p = 0.41$). Macroscopic and high-grade microscopic melanosis coli were not significantly associated with the development of adenomas or carcinomas.

2.1.2 *Urothelial cancer*

Bronder *et al.* (1999) reported on 766 cases of urothelial cancers (98% confirmed by histology) in Berlin, Germany, between 1990 and 1994. A control group (1:1) was obtained by sampling, from the West Berlin Population Registry, persons of German nationality who had lived in Germany for at least 20 years and matched with the patients for sex and age. Through a standardized questionnaire completed by 648 patients and 647 controls, social class was recorded as well as consumption of analgesics, laxatives and tobacco. After adjustment for tobacco use and social class, the risk of urothelial carcinoma was increased in laxative users. Use of contact laxatives was reported by 63 urothelial cancer patients versus 29 controls (odds ratio, 2.5; 95% CI, 1.5–4.2) and 13 renal pelvis and ureter cancer patients versus two controls (odds ratio, 9.3; 95% CI, 1.1–83.3). For different laxatives, the corresponding figures (urothelial cancer patients versus controls) were: chemical and anthranoid laxatives, five versus two (odds ratio, 2.7; 95% CI, 0.47–16); anthranoid laxatives alone, 37 versus 20 (odds ratio, 2.0; 95% CI, 1.1–3.7); aloe, 16 versus 11 (odds ratio, 1.6; 95% CI, 0.66–3.7); senna, 26 versus 13 (odds ratio, 2.4; 95% CI, 1.1–5.0); and rhubarb, eight versus four (odds ratio, 2.6; 95% CI, 0.68–9.6). [The Working Group noted that no results for laxatives adjusted for use of analgesics were presented.]

3. Studies of Cancer in Experimental Animals

3.1 1-Hydroxyanthraquinone

3.1.1 *Oral administration*

Rat: A group of 30 male ACI/N rats, 1.5 months of age, was fed 1% 1-hydroxyanthraquinone in CE-2 diet throughout the experimental period of 480 days. Thirty control rats were fed basal diet. Rats that survived more than 280 days developed various tumours of the intestine (25/29). These comprised caecal adenomas (10/29) or adenocarcinomas (5/29) and colonic adenomas (12/29) or adenocarcinomas (11/29). No such tumours were diagnosed in control rats. In addition, neoplastic nodules and hepatocellular adenomas (12/29) and forestomach papillomas and glandular stomach adenomas (5/29) were observed. No such tumours were observed in control animals (Mori *et al*., 1990).

A group of 27 male ACI/N rats, six weeks of age, was fed 1.5% 1-hydroxyanthraquinone in the diet for 48 weeks. A second group (14 rats) was also given 16 mg/L indomethacin in the drinking-water for the experimental period. Fifteen control rats were fed basal diet. Rats fed with 1-hydroxyanthraquinone had incidences of 12/27 large intestinal neoplasms (adenomas and adenocarcinomas) and 14/27 forestomach tumours (papillomas). In rats fed 1-hydroxyanthraquinone and treated with indomethacin, these incidences were 0/14 and 2/14, respectively. Untreated animals and rats given indomethacin alone had no neoplasms in the large intestine and forestomach (Tanaka *et al*., 1991).

3.1.2 *Administration with known carcinogens*

Rat: Two groups of male and female ACI/N rats, six weeks of age, were given weekly intraperitoneal injections of 25 mg/kg bw methylazoxymethanol acetate for two weeks. One group (21 males and 20 females) was subsequently fed 1% 1-hydroxyanthraquinone until the end of the experiment (44 weeks). The second group treated with methylazoxymethanol acetate (19 males and 19 females) was fed basal diet. A third group (17 males and 20 females) received 1-hydroxyanthraquinone alone and a control group (16 males and 22 females) was fed basal diet only. The incidences of caecal tumours (males, 15/17; females, 11/15) and colon tumours (males, 17/17; females, 14/15) in the group treated with methylazoxymethanol acetate and 1-hydroxyanthraquinone were significantly higher ($p < 0.05$) than those of the groups treated with methylazoxymethanol acetate alone (caecal tumours: males, 0/19; females, 3/15; colon tumours: males, 8/15; females, 11/15) or 1-hydroxyanthraquinone alone (caecal tumours: males, 1/17; females, 0/17; colon tumours: males, 0/17; females, 1/17). The incidence and multiplicity of caecum and colon tumours in the first group were significantly greater than the combined results

for those of second and third groups, suggesting that 1-hydroxyanthraquinone acted synergistically with methylazoxymethanol acetate (Mori *et al.*, 1991).

3.2 1,3-Dihydroxy-2-hydroxymethylanthraquinone (lucidin)

No data on the carcinogenicity of lucidin in rodents were available to the Working Group, but this compound is an active principle of *Rubia tinctorum* used as a herbal medicine.

Rat: Groups of 15–20 male and female ACI rats, weighing 150–200 g, were fed a standard diet containing 0, 1 or 10% madder root (*Rubia tinctorum*) for 780 days. This root contained lucidin (0.34%) but also large amounts of alizarin (0.67%; 1,2-dihydroxyanthraquinone) and the primeverosides of both compounds. All surviving animals were killed and necropsied at 780 days. In the groups receiving 10% madder root diet, 2/16 males and 3/17 females developed hepatocellular adenomas, whereas none were observed in the controls or the 1% madder root group of either sex. In addition, renal tubule-cell adenomas were observed in 1/16 males and 2/16 females in the 10% madder root group and a renal tubule-cell carcinoma in 1/14 males in the 1% madder root group. No renal tumours were seen in the control groups (Westendorf *et al.*, 1998). [The Working Group noted the small number of animals used in this study and that the madder root contained large amounts of other compounds such as alizarin.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

1-Hydroxyanthraquinone

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

In an early study, 1-hydroxyanthraquinone was found to be absorbed when continuously administered to rats [strain not specified] by stomach tube (50 mg suspended in aqueous gum arabic), and its metabolites were identified by paper chromatography. Urine and faeces were collected during 48 h after treatment and extracted with diethyl ether. Of the 1-hydroxyanthraquinone originally present, 2.49% and 0.74% were converted into alizarin (1,2-dihydroxyanthraquinone), in urine and faeces, respectively. The alizarin was then excreted after sulfation and glucuronidation (Fujita *et al.*, 1961).

1,3-Dihydroxy-2-hydroxymethylanthraquinone (lucidin)

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

No direct information was found on the absorption, distribution, metabolism or excretion of ludicin in experimental animal systems. However, a report has appeared on the oral administration of lucidin 3-*O*-primeveroside to rats, which resulted in the excretion in the urine of lucidin and (to a lesser extent) rubiadin (1,3-dihydroxy-2-methylanthraquinone) (Blömeke *et al.*, 1992).

4.2 Toxic effects

Chronic ingestion of laxatives of the anthracene group such as cascara, senna, frangula, aloe or rhubarb is considered to be an etiological factor in melanosis coli, a condition in which colonic macrophages accumulate a dark pigment with the staining characteristics of lipofuscin. In 14 patients submitted to repeated proctoscopies, Speare (1951) produced melanosis 11 times by prescribing cascara sagrada for 4–14 months and eliminated it nine times by withdrawing the laxative for 5–11 months. Steer and Colin-Jones (1975) studied histological patterns of rectal biopsies from seven patients with melanosis coli three months after they ceased to take anthraquinone-containing purgatives. A decrease was observed in the number of macrophages infiltrating the mucosa, as well as a reduction in the intensity of the acid phosphatase reaction.

The effect of anthraquinone glycosides on cell proliferation in the sigmoid colon of patients *in vivo* has been studied. Twenty-four hours before colonoscopy with a sigmoid biopsy, nine patients were given an oral dose of 1 mL/kg of a syrup containing 2.0 mg/mL of the anthraquinone glycosides sennosides A and B. Proliferative activity of epithelial cells was determined by incubating the biopsy specimens with the thymidine analogue 5-bromo-2′-deoxyuridine (BrdU) and then visualizing BrdU-labelled cells immunochemically. Proliferative activity was expressed as the labelling index, i.e. the number of labelled nuclei divided by the total number of nuclei multiplied by 100 (%). Data from these nine subjects were compared with those of 10 subjects who had a normal colonoscopy and 14 patients with sporadic colonic neoplasms who had not received anthraquinone laxatives before the colonoscopy. The labelling index (% ± SD) was significantly higher in the group given the sennosides (26.4 ± 6.1) than in normal patients (5.7 ± 1.8) or in patients with a colonic neoplasm (8.7 ± 2.6; $p < 0.005$). Furthermore, the reduction of the number of cells per crypt, observed as a reduced crypt height in the treated group, suggests that compensatory cell proliferation may occur as well (Kleibeuker *et al.*, 1995).

1-Hydroxyanthraquinone

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

Little information is available on the toxic effects of 1-hydroxyanthraquinone in experimental animals, and no acute toxicity studies have been reported. A group of ACI/N rats fed 1.5% 1-hydroxyanthraquinone in the diet for 48 weeks showed inflammatory changes of various degrees (colitis, ulcerative colitis or melanosis coli) in the large bowel, but no clinical sign of toxicity (Tanaka *et al.*, 1995).

1-Hydroxyanthraquinone was fed in the diet (0.5%, 1%, 2%, 4%) to male Fischer 344 rats for seven days. Induction of cell proliferation in the intestines was analysed by BrdU labelling. At the high dose, 1-hydroxyanthraquinone induced cell proliferation in the caecum and the proximal colorectum, but there was little evidence of intestinal cytotoxicity (Toyoda *et al.*, 1994).

1-Hydroxyanthraquinone is a constituent of the heartwood of *Tabebuia avellanedae* (Burnett & Thompson, 1967; Steinert *et al.*, 1996) and the roots of *Morinda officinalis* and *Damnacanthus indicus* (Yang *et al.*, 1992). No reports documenting toxicity of any of these three plants appear to have been published.

1,3-Dihydroxy-2-hydroxymethylanthraquinone (lucidin)

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

No information was available on general toxic effects of lucidin. However, this compound is a known constituent of the roots of *Rubia tinctorum* (madder) (Burnett & Thomson, 1968a), and one study has been published on the acute and subacute toxicity of madder root. A 14-day toxicity test was conducted on an aqueous extract of madder root administered by gavage to $(C57BL/6 \times C3H)F_1$ mice. The maximum tolerated dose was between 3500 and 5000 mg/kg bw. A subacute toxicity test was then performed on 62 mice of each sex with madder root extract incorporated into their diet at 0, 0.3, 0.6, 1.25, 2.5 or 5% for 90 days. All mice tolerated these doses well, and none showed clinical signs of toxicity or adverse effects on body weight gain. Histopathological examination showed retention cysts of the kidneys and epidermal vaginal cysts in a few of the treated and control mice. It was concluded that dietary exposure to madder root at the doses tested had no significant acute or subacute toxic effects on mice (Ino *et al.*, 1995). [The Working Group noted that this study is more germane to the toxicity of any glycoside forms of lucidin present than to that of the aglycone itself.]

4.3 Reproductive and developmental effects

1-Hydroxyanthraquinone and 1,3-dihydroxy-2-hydroxymethylanthraquinone (lucidin)

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

No data were available to the Working Group.

4.4 Genetic and related effects

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems* (for references see Table 1)

1-Hydroxyanthraquinone

1-Hydroxyanthraquinone induced frameshift mutations in *Salmonella typhimurium* TA1537 in the absence of metabolic activation; addition of liver homogenate led to a reduction of the mutagenic activity. In strains TA98 and TA100, no mutagenic effects were seen. The induction of unscheduled DNA synthesis in rat hepatocytes *in vitro* was studied independently by two groups and a positive response was found in both studies.

A few studies were available on genotoxic effects of alizarin, a metabolite of 1-hydroxyanthraquinone. Alizarin was weakly mutagenic in *S. typhimurium* TA1537 in the presence of S9 and in rat hepatocyte DNA-repair assays, but it was consistently inactive in transformation experiments with C3H/M2 mouse fibroblasts and in *Hprt* mutation assays with Chinese hamster V79 cells (Westendorf *et al.*, 1990).

1,3-Dihydroxy-2-hydroxymethylanthraquinone (lucidin)

A positive response was obtained with lucidin in *Salmonella*/microsome reverse mutation assays in strains TA100, TA102, TA104, TA1537, TA1538 and TA98. The highest activity was seen in the frameshift strain TA1537 and in the base substitution strain TA100. The compound was mutagenic in these assays in the absence of metabolic activation and addition of liver homogenate had no major effect on the mutagenic potency. Lucidin also gave positive results in unscheduled DNA synthesis experiments with rat liver cells, it caused gene mutations in Chinese hamster V79 cells (without activation) and it transformed mouse fibroblasts. With polynucleotides *in vitro* the compound binds to DNA bases. The only data from an in-vivo study indicated that DNA adducts were present in various organs of mice fed lucidin (2 mg per day per animal for

Table 1. Genetic and related effects of hydroxyanthraquinones and their derivatives

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
1-Hydroxyanthraquinone				
Salmonella typhimurium TA100, TA98 reverse mutation	–	–	100 µg/plate	Blömeke *et al*. (1992)
Salmonella typhimurium TA1537, reverse mutation	+	+[c]	30 µg/plate	Blömeke *et al*. (1992)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	11.2	Kawai *et al*. (1986)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	50	Blömeke *et al*. (1992)
1,3-Dihydroxy-2-hydroxymethylanthraquinone (lucidin)				
Salmonella typhimurium TA100, TA98, reverse mutation	+	+	10 µg/plate	Yasui & Takeda (1983)
Salmonella typhimurium TA100, reverse mutation	+	+	0.5 µg/plate	Westendorf *et al.* (1988)
Salmonella typhimurium TA104, TA98, reverse mutation	+	+	5 µg/plate	Westendorf *et al.* (1988)
Salmonella typhimurium TA100, reverse mutation	+	+	0.5 µg/plate	Poginsky (1989)
Salmonella typhimurium TA100, reverse mutation	+	+	5 µg/plate	Kawasaki *et al.* (1992)
Salmonella typhimurium TA102 reverse mutation	+	+	10 µg/plate	Westendorf *et al*. (1988, 1990)
Salmonella typhimurium TA1535 reverse mutation	–	?	10 µg/plate	Westendorf *et al*. (1988)
Salmonella typhimurium TA1537, reverse mutation	+	+	0.5 µg/plate	Westendorf *et al*. (1988, 1990)
Salmonella typhimurium TA1538 reverse mutation	+	+	10 µg/plate	Westendorf *et al*. (1988)
Salmonella typhimurium TA98 reverse mutation	+	+	5 µg/plate	Poginsky (1989)
Salmonella typhimurium TA98, reverse mutation	+	+	30 µg/plate	Kawasaki *et al*. (1992)
Drosophila melanogaster, somatic mutation, wing-spot test	–		1% in feed	Marec *et al.* (2001)
Unscheduled DNA synthesis, rat primary hepatocytes, *in vitro*	+	NT	6.3	Westendorf *et al*. (1988)
Unscheduled DNA synthesis, rat primary hepatocytes, *in vitro*	+	NT	12.5	Poginsky (1989)
Unscheduled DNA synthesis, rat primary hepatocytes, *in vitro*	+	NT	10	Blömeke *et al*. (1992)
Gene mutation, Chinese hamster lung V79 cells *Hprt* locus *in vitro*	+	NT	10	Westendorf *et al*. (1988)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, Chinese hamster lung V79 cells *Hprt* locus *in vitro*	+	NT	10	Poginsky (1989)
Cell transformation, C3H/M2 mouse fibroblasts	+	NT	5	Westendorf *et al*. (1988)
DNA strand breaks, V79 Chinese hamster fibroblasts *in vitro* (alkaline elution)	+	NT	20	Westendorf *et al.* (1988)
DNA binding, primary rat hepatocytes *in vitro*, ^{32}P-postlabelling	+	NT	40	Poginsky *et al*. (1991)
Binding to adenine and guanine *in vitro*, HPLC, FAB-MS analysis	+	NT	0.2	Kawasaki *et al*. (1994)
Binding to poly [d(A-T)] and polydC*polydG *in vitro*, ^{32}P-postlabelling	+		200 000	Poginsky *et al.* (1991)
Binding to DNA from male Parkes mouse liver, kidney, duodenum and colon *in vivo*, ^{32}P-postlabelling	+		2 mg/day for 4 days; feed	Poginsky *et al*. (1991)

[a] +, positive; –, negative; NT, not tested; ?, equivocal; FAB, fast atom bombardment; MS, mass spectrometry

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day

[c] Lower response in the presence of S9

four days). In hepatic tissue, 1.16 ± 0.2 adducts per 10^8 nucleotides were detected; slightly higher adduct levels were found in kidneys and duodenum, namely 2.19 ± 1.2 and 1.91 ± 0.6, respectively (Poginsky *et al.*, 1991). The only genotoxicity assay in which lucidin gave a negative response was the *Drosophila* wing-spot test.

4.5 Mechanistic considerations

1-Hydroxyanthraquinone

There is some evidence that 1-hydroxyanthraquinone is genotoxic. In rats, induction of cell proliferation *in vivo* was seen in the caecum which was paralleled by enhanced ornithine decarboxylase activity (Mori *et al*., 1992). In a combination experiment with methylazoxymethanol acetate (given by single intraperitoneal injection) and 1-hydroxyanthraquinone (1% in diet; 40 weeks), the level of tumour necrosis factor α was increased when rats were exposed to the combination compared with the groups that received either compound alone. It was postulated that this might account for the synergistic carcinogenic effect of these compounds (see Section 3.2.2) (Yoshimi *et al.*, 1994). In studies of mutations in cancer-related genes from tumours of rats exposed to methylazoxymethanol acetate and 1-hydroxyanthraquinone, specific mutations were found in the gene encoding β-catenin but not in other genes such as *apc* (Suzui *et al*., 1999).

1,3-Dihydroxy-2-hydroxymethylanthraquinone (lucidin)

Lucidin is genotoxic *in vitro* and *in vivo* (Westendorf *et al*., 1988). Data on carcinogenic activity of this compound were not available, but it has been shown that madder root (*Rubia tinctorum*) — which contains lucidin — causes tumours in liver and kidneys of rats. The DNA-adduct pattern from colon, liver and kidneys of rats showed one adduct that was also found when deoxyguanosine-3′-monophosphate was incubated with lucidin *in vitro*. This supports the assumption that lucidin is involved in the carcinogenic effects of *Rubia tinctorum* (Westendorf *et al.*, 1998). It has been hypothesized that lucidin is activated by phase II enzymes through substitution of the hydroxymethyl group, followed by hydrolytic cleavage, leading to the formation of a DNA-reactive methylene metabolite or carbenium ion (Poginsky *et al*., 1991).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Hydroxyanthraquinones are constituents of a number of plant species including *Rubia tinctorum* (madder root) and *Morinda officinalis* that are used in traditional herbal medicines. 1,3-Dihydroxy-2-hydroxymethylanthraquinone (lucidin) occurs in *R. tinctorum* in the form of glycoside conjugates, and 1-hydroxyanthraquinone similarly occurs in *M. officinalis*.

5.2 Human carcinogenicity data

Herbs containing anthraquinone derivatives

Herbs containing anthraquinone derivatives are used as laxatives and several studies have reported data relating indices of use of such laxatives to cancer outcomes. In an early British study, patients with gastrointestinal cancer reported higher past chronic use of senna than did patients with other diseases. Three German case–control studies on colorectal cancer using melanosis coli as an exposure indicator gave conflicting results. An Australian case–control study on colorectal cancer which assessed self-reported use of anthraquinone laxatives as an index of exposure found no excess risk. A study of urothelial cancer in Germany found elevated relative risks in relation to several types of laxative, including those containing anthraquinones. For all of these case–control studies, it is difficult to exclude bias and confounding from dietary habits, constipation or use of analgesics. Except for two studies (the Australian colorectal cancer and the German urothelial cancer studies), selection of controls is also a major concern.

5.3 Animal carcinogenicity data

1-Hydroxyanthraquinone was tested for carcinogenicity by oral administration in three studies in rats and induced adenocarcinomas of the large intestine in two studies.

No carcinogenicity tests have been carried out with 1,3-dihydroxy-2-hydroxymethylanthraquinone (lucidin) *per se*, although the herb madder root (*Rubia tinctorum*) (which contains this compound among others) was tested by oral administration in rats. Madder root caused an increase in hepatocellular adenomas and adenomas and carcinomas of the renal cortex in males and females in a single experiment.

5.4 Other relevant data

1-Hydroxyanthraquinone is metabolized by rats and excreted as alizarin (1,2-dihydroxyanthraquinone). When given orally to rats, it induced inflammatory changes in the colon. It is mutagenic in bacteria and causes unscheduled DNA synthesis in rat liver cells *in vitro*.

Lucidin primeveroside is hydrolysed in rats to the aglycones 1,3-dihydroxy-2-hydroxymethylanthraquinone (lucidin) and rubiadin, which are excreted in urine. Lucidin is mutagenic in bacteria, mutagenic and genotoxic in cultured mammalian cells and forms DNA adducts in mice.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of laxatives containing anthraquinone derivatives.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1-hydroxyanthraquinone.

There is *limited evidence* in experimental animals for the carcinogenicity of madder root (*Rubia tinctorum*).

Overall evaluation

1-Hydroxyanthraquinone is *possibly carcinogenic to humans (Group 2B).*

Madder root (*Rubia tinctorum*) is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

Bauch, H.J. & Leistner, E. (1978) Aromatic metabolites in cell suspension cultures of *Galium mollugo* L. *Planta med*., **33**, 105–123

Blömeke, B., Poginsky, B., Schmutte, C., Marquardt, H. & Westendorf, J. (1992) Formation of genotoxic metabolites from anthraquinone glycosides present in *Rubia tinctorum* L. *Mutat. Res*., **265**, 263–272

Blumenthal, M., Busse, W.R., Goldberg, A., Gruenwald, J., Hall, T., Riggins, C.W. & Rister, R.S., eds (1998) *The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines*, Austin, TX/ Boston, MA, American Botanical Council/Integrative Medicine Communications

Bosáková, Z., Peršl, J. & Jegorov, A. (2000) Determination of lucidin in *Rubia tinctorum* aglycones by an HPLC method with isocratic elution. *J. high Resol. Chromatogr*., **23**, 600–602

Boyd, J.T. & Doll, R. (1954) Gastro-intestinal cancer and the use of liquid paraffin. *Br. J. Cancer*, **8**, 231–237

Briggs, L.H., Beachen, J.F., Cambie, R.C., Dudman, N.P.B., Steggles, A.W. & Rutledge, P.S. (1976) Chemistry of *Coprosma* genus. Part XIV. Constituents of five New Zealand species. *J. Chem. Soc. Perkin Trans. I*, 1789–1792

Bronder, E., Klimpel, A., Helmert, U., Greiser, E., Molzahn, M. & Pommer, W. (1999) [Analgesics and laxatives as risk factors for cancer of the efferent urinary tract — Results of the Berlin Urothelial Carcinoma Study.] *Soz. Präventivmed.*, **44**, 117–125 (in German)

Brown, J.P. & Brown, R.J. (1976) Mutagenesis by 9,10-anthraquinone derivatives and related compounds in *Salmonella typhimurium*. *Mutat. Res.*, **40**, 203–224

Buckingham, J., ed. (2001) *Dictionary of Natural Products on CD-ROM*, Boca Raton, FL, Chapman & Hall/CRC Press

Burnett, A.R. & Thomson, R.H. (1967) Naturally occurring quinones. Part X. The quinonoid constituents of *Tabebuia avellanedae* (Bignoniaceae). *J. chem. Soc.(C)*, 2100–2104

Burnett, A.R. & Thomson, R.H. (1968a) Naturally occurring quinones. XV. Biogenesis of the anthraquinones in *Rubia tinctorum* (madder). *J. chem. Soc. C*, 2437–2441

Burnett, A.R. & Thomson, R.H. (1968b) Anthraquinones in *Morinda umbellata*. *Phytochemistry*, **7**, 1421–1422

Chemical Information Services (2001) *Directory of World Chemical Producers 2001.2 Edition*, Dallas, TX [CD-ROM]

Derksen, G.C.H., Van Beek, T.A., De Groot, A. & Capelle, A. (1998) High-performance liquid chromatographic method for the analysis of anthraquinone glycosides and aglycones in madder root (*Rubia tinctorum* L.). *J. Chromatogr. A.*, **816**, 277–281

Dukas, L., Willett, W.C., Colditz, G.A., Fuchs, C.S., Rosner, B. & Giovannucci, E.L. (2000) Prospective study of bowel movement, laxative use, and risk of colorectal cancer among women. *Am. J. Epidemiol.*, **151**, 958–964

El-Emary, N.A. & Backheet, E.Y. (1998) Three hydroxymethylanthraquinone glycosides from *Rubia tinctorum*. *Phytochemistry*, **49**, 277–279

Felter, H.W. & Lloyd, J.U. (2002) *King's American Dispensatory. Rubia-Madder* (http://ftp.oit.unc.edu/herbmed/eclectic/kings/rubia.html)

Fujita, M., Furuya, T. & Matsuo, M. (1961) Studies on the metabolism of naturally occurring anthraquinones. I. The metabolism of 1-hydroxyanthraquinone and 2-hydroxyanthraquinone. *Chem. pharm. Bull.*, **9**, 962–966

Hocquemiller, R., Fournet, A., Bouquet, A., Bruneton, J. & Cave, A. (1976) *Commitheca liebrechtsiana* (Rubiaceae). *Plant Med. Phytother.*, **10**, 248–250

IARC (1990) *IARC Monographs on the Evaluation of Carcinogenic Risk to Humans*, Vol. 50, *Pharmaceutical Drugs*, Lyon, IARC*Press*, pp. 265–275

Imaki, S. & Fukumoto, Y. (1988) [Process for the preparation of hydroxyanthraquinone derivatives as intermediates for dyes and drugs.] [Patent No. JP 63091347] *Jpn. Kokai Tokkyo Koho* (in Japanese)

Ino, N., Tanaka, T., Okumura, A., Morishita, Y., Makita, H., Kato, Y., Nakamura, M. & Mori, H. (1995) Active and subacute toxicity tests of madder root, natural colorant extracted from madder (*Rubia tinctorium*), in (C57BL/6 × C3H)F_1 mice. *Toxicol. ind. Health*, **11**, 449–458

Inoue, K., Nayeshiro, H., Inoue, H. & Zenk, M. (1981) Quinones and related compounds in higher plants. Part 12. Anthraquinones in cell suspension cultures of *Morinda citrifolia*. *Phytochemistry*, **20**, 1693–1700

Kawai, K., Mori, H., Sugie, S., Yoshimi, N., Inoue, T., Nakamaru, T., Nozawa, Y. & Matsushima, T. (1986) Genotoxicity in the hepatocyte/DNA repair test and toxicity to liver mitochondria of 1-hydroxyanthraquinone and several dihydroxyanthraquinones. *Cell Biol. Toxicol.*, **2**, 457–467

Kawasaki, Y., Goda, Y. & Yoshihira, K. (1992) The mutagenic constituents of *Rubia tinctorum*. *Chem. pharm. Bull*., **40**, 1504–1509

Kawasaki, Y., Goda, Y., Noguchi, H. & Yamada, T. (1994) Identification of adducts formed by reaction of purine bases with a mutagenic anthraquinone, lucidin: mechanisms of mutagenicity by anthraquinones occurring in *Rubiaceae* plants. *Chem. pharm. Bull*., **42**, 1971–1973

Kleibeuker, J.H., Cats, A., Zwart, N., Mulder, N.H., Hardonk, M.J. & de Vries, E.G.E. (1995) Excessively high cell proliferation in sigmoid colon after an oral purge with anthraquinone glycosides. *J. natl Cancer Inst.*, **87**, 452–455

Krizsan, K., Szokan, G., Toth, Z.A., Hollosy, F., Laszlo, M. & Khlafulla, A. (1996) HPLC analysis of anthraquinone derivatives in madder root (*Rubia tinctorum*) and its cell cultures. *J. liq. Chromatogr. relat. Technol*., **19**, 2295–2314

Kune, G.A. (1993) Laxative use not a risk factor for colorectal cancer: Data from the Melbourne colorectal cancer study. *Z. Gastroenterol.*, **31**, 140–143

Leistner, E. (1975) [Isolation, identification, and biosynthesis of anthraquinones in cell suspension cultures of *Morinda citrifolia*.] *Planta med*., **Suppl**., 214–224 (in German)

Li, S., Ouyang, Q., Tan, X., Shi, S. & Yao, Z. (1991) [Chemical constitutents of *Morinda officinalis How*.] *Zhongguo Zhong Yao Za Zhi*, **16**, 675–675 (in Chinese)

Marczylo, T., Arimoto-Kobayashi, S. & Hayatsu, H. (2000) Protection against Trp-p-2 mutagenicity by purpurin: Mechanism of in vitro antimutagenesis. *Mutagenesis*, **15**, 223–228

Marec, F., Kollárová, I. & Jegorov, A. (2001) Mutagenicity of natural anthraquinones from *Rubia tinctorum* in the *Drosophila* wing spot test. *Planta med*., **67**, 127–131

Medical Economics Co. (1998) *PDR for Herbal Medicines*, 1st Ed., Montvale, NJ, pp. 660–661, 1103

Medical Economics Co. (2000) *PDR for Herbal Medicines*, 2nd Ed., Montvale, NJ, pp. 80–81, 490

Michalowicz, W.A. (1981) *Preparation of Hydroxyanthraquinones* (US Patent No. 4,292,248), Charlotte, NC, American Color & Chemical Corporation

Mori, H., Yoshimi, N., Iwata, H., Mori, Y., Hara, A., Tanaka, T. & Kawai, K. (1990) Carcinogenicity of naturally occurring 1-hydroxyanthraquinone in rats: Induction of large bowel, liver and stomach neoplasms. *Carcinogenesis*, **11**, 799–802

Mori, Y., Yoshimi, N., Iwata, H., Tanaka, T. & Mori, H. (1991) The synergistic effect of 1-hydroxyanthraquinone on methylazoxymethanol acetate-induced carcinogenesis in rats. *Carcinogenesis*, **12**, 335–338

Mori, H., Mori, Y., Tanaka, T., Yoshimi, N., Sugie, S., Kawamori, T. & Narisawa, T. (1992) Cell kinetic analysis of the mucosal epithelium and assay of ornithine decarboxylase activity during the process of 1-hydroxyanthraquinone-induced large bowel carcinogenesis in rats. *Carcinogenesis*, **13**, 2217–2220

Murti, V.V.S., Seshadri, T.R. & Sivakumaran, S. (1972) Chemical components of *Rubia iberica*. *Indian J. Chem*., **10**, 246–247

Nusko, G., Schneider, B., Müller, G., Kusche, J. & Hahn, E.G. (1993) Retrospective study on laxative use and melanosis coli as risk factor for colorectal neoplasma. *Pharmacology*, **47** (Suppl. 1), 234–241

Nusko, G., Schneider, B., Ernst, H., Wittekind, C. & Hahn, E.G. (1997) Melanosis coli — A harmless pigmentation or precancerous condition? *Z. Gastroenterol*., **35**, 313–318

Nusko, G., Schneider, B., Schneider, I., Wittekind, C. & Hahn, E.G. (2000) Anthranoid laxative use is not a risk factor for colorectal neoplasia: Results of a prospective case control study. *Gut*, **46**, 651–655

Poginsky, B. (1989) [*Occurrence and Genotoxicity of Anthracene Derivatives in* Rubia tinctorum *(L)*] (PhD Thesis), Hamburg, University of Hamburg (in German)

Poginsky, B., Westendorf, J., Blömeke, B., Marquardt, H., Hewer, A., Grover, P.L. & Phillips, D.H. (1991) Evaluation of DNA-binding activity of hydroxyanthraquinones occurring in *Rubia tinctorum* L. *Carcinogenesis*, **12**, 1265–1271

Prista, L.N., Roque, A.S., Ferreira, M.A. & Alves, A.C. (1965) Synthesis of lucidin and lucidin 1-methyl ether starting from nordamnacanthal and damnacanthal. *Garcia Orta*, **13**, 45–50 [consulted as abstract CA66:46240]

Quian, X.-Z. (1996) *Colour Pictorial Handbook of Chinese Herbs*, Beijing, The Peoples Medical Publishing House

Sartori, G., Casnati, G., Bigi, F. & Foglio, F. (1990) A new methodological approach to anthraquinone and anthracyclidone synthesis. *Gazz. Chim. ital*., **120**, 13–19

Schneider, H.J., Unger, G., Rossler, D., Bothor, C., Berg, W. & Ernst, G. (1979) [Effect of drugs used for the prevention of urinary calculi recurrence on the growth and metabolism of young experimental animals.] *Z. Urol. Nephrol*., **72**, 237–247 (in German)

Siegers, C.P., Von Hertzberg-Lottin, E., Otte, M. & Schneider, B. (1993) Anthranoid laxative abuse — A risk for colorectal cancer? *Gut*, **34**, 1099–1101

Sonnenberg, A. & Müller, A.D. (1993) Constipation and cathartics as risk factors of colorectal cancer: A meta-analysis. *Pharmacology*, **47** (Suppl. 1), 224–233

Speare, G.S. (1951) Melanosis coli. Experimental observations on its production and elimination in twenty-three cases. *Am. J. Surgery*, **November**, 631–637

Steer, H.W. & Colin-Jones, D.G. (1975) Melanosis coli: Studies of the toxic effects of irritant purgatives. *J. Pathol.*, **115**, 199–205

Steinert, J., Khalaf, H. & Rimpler, M. (1996) High-performance liquid chromatographic separation of some naturally occurring naphthoquinones and anthraquinones. *J. Chromatogr.*, **A723**, 206–209

Suzui, M., Ushijima, T., Dashwood, R.H., Yoshimi, N., Sugimura, T., Mori, H. & Nagao, M. (1999) Frequent mutations of the rat β-catenin gene in colon cancers induced by methylazoxymethanol acetate plus 1-hydroxyanthraquinone. *Mol. Carcinog*., **24**, 232–237

Tanaka, T., Kojima, T., Yoshimi, N., Sugie, S. & Mori, H. (1991) Inhibitory effect of the non-steroidal anti-inflammatory drug, indomethacin on the naturally occurring carcinogen, 1-hydroxyanthraquinone in male ACI/N rats. *Carcinogenesis*, **12**, 1949–1952

Tanaka, T., Suzui, M., Kojima, T., Okamoto, K., Wang, A. & Mori, H. (1995) Chemoprevention of the naturally occurring carcinogen 1-hydroxyanthraquinone-induced carcinogenesis by the non-steroidal anti-inflammatory drug indomethacin in rats. *Cancer Detect. Prev*., **19**, 418–425

Thomson, R.H. & Brew, E.J.C. (1971) Naturally occurring quinones. XIX. Anthraquinones in *Hymenodictyon excelsum* and *Damnacanthus major*. *J. chem. Soc. C*, 2001–2007

Toyoda, K., Nishikawa, A., Furukawa, F., Kawanishi, T., Hayashi, Y., Takahashi, M. (1994) Cell proliferation induced by laxatives and related compounds in the rat intestine. *Cancer Lett.*, **83**, 43–49

Wang, S.X., Hua, H.M., Wu, L.J., Li, X. & Zhu, T.R. (1992) [Anthraquinones from the roots of *Rubia cordifolia* L.] *Yaoxue Xuebao*, **27**, 743–747 (in Chinese)

Wang, G.L., Tian, J.G. & Chen, D.C. (1997) [Study on chemical composition of *Rubia cordifolia* L. and *R. tinctorum* L. II. Quantitative determination of alizarin and lucidin by reversed-phase HPLC.] *Yaowu Fenxi Zazhi*, **17**, 219–221 [consulted as abstract: *Analytical Abstracts*, **60**, H199] (in Chinese)

Westendorf, J., Poginsky, B., Marquardt, H., Groth, G. & Marquardt, H. (1988) The genotoxicity of lucidin, a natural component of *Rubia tinctorum* L., and lucidinethylether, a component of ethanolic *Rubia* extracts. *Cell Biol. Toxicol.*, **4**, 225–239

Westendorf, J., Marquardt, H., Poginsky, B., Dominiak, M., Schmidt, J. & Marquardt, H. (1990) Genotoxicity of naturally occurring hydroxyanthraquinones. *Mutat. Res.*, **240**, 1–12

Westendorf, J., Pfau, W. & Schulte, A. (1998) Carcinogenicity and DNA adduct formation observed in ACI rats after long-term treatment with madder root, *Rubia tinctorum* L. *Carcinogenesis*, **19**, 2163–2168

Yang, Y.J., Shu, H.Y. & Min, Z.D. (1992) Anthraquinones isolated from *Morinda officinalis* and *Damnacanthus indicus*. *Yao Xue Xue Bao (Acta pharm. sin.)*, **27**, 358–364

Yao, Z.Q., Guo, Q. & Huang, H.H. (1998) [Officinalisin, a new compound isolated from root-bard of medicinal Indian mulberry (*Morinda officinalis*).] *Chinese trad. Herb. Drugs*, **29**, 217–219 (in Chinese)

Yasui, Y. & Takeda, N. (1983) Identification of a mutagenic substance in *Rubia tinctorum* L. (madder) root, as lucidin. *Mutat. Res.*, **121**, 185–190

Yoshikawa, M., Yanaguchi, S., Nishisaka, H., Yamahara, J. & Murakami, N. (1995) [Chemical constituents of Chinese natural medicine, *Morindae Radix*, the dried roots of *Morinda officinalis* How.: Structures of morindolide and morofficinaloside.] *Chem. pharm. Bull.*, **43**, 1462–1465 (in Japanese)

Yoshimi, N., Sato, S., Makita, H., Wang, A., Hirose, Y., Tanaka, T. & Mori, H. (1994) Expression of cytokines, TNF-α and IL-1α, in MAM acetate and 1-hydroxyanthraquinone-induced colon carcinogenesis of rats. *Carcinogenesis*, **15**, 783–785

Zheng, H.Z. & Dong, Z.H. (1997) [Radix Morindae officinalis]. In: [*Modern Study of Traditional Chinese Medicine*], Vol. II, Xue Yuan Press, 1234–1245 (in Chinese)

Zhuravl'ov, M.S. & Borisov, M.I. (1970) [Anthraquinones of *Galium dasypodum*]. *Farm. Zh. (Kiev)*, *25*, 76–79 (in Ukrainian)

Zhuravl'ov, M.S., Shtefan, L.M. & Luchkina, T.V. (1987) [Anthraquinones of *Galium fagetorum*. II.] *Khim. Prir. Soedin*, 908 [consulted as abstract: CA108:147204] (in Russian)