

chloride (0.075 mg/kg) demonstrated no treatment-related effect on heart function or cardiovascular health (Ref. 222) at a dose 30 times the maximum daily absorbed dose expected from brushing and rinsing.

Sanguinaria extract was tested in a fertility/reproduction study in rats (Ref. 223), in developmental toxicity studies in rats and rabbits (5 to 400 mg/kg per day) (Refs. 224, 225, and 226), and in a perinatal/postnatal study in rats (5 to 60 mg/kg per day) (Ref. 227). The NOAEL level of sanguinaria extract was 25 mg/kg per day for development toxicity in rabbits, and 15 mg/kg per day for maternal toxicity. Sanguinaria extract had no effect on fertility, reproduction, or fetal and neonatal development in rats and rabbits at doses below those resulting in general toxicity in the adult animals.

Mutagenicity studies were conducted with both sanguinaria extract and sanguinarine chloride with *in vitro* methods using microorganisms and mammalian cells in culture and *in vivo* in mice. Weak positive responses were elicited only in the bacterial assay using *Salmonella typhimurium* (Ames assay) in the presence of metabolic activation (Ref. 228). Studies of sanguinaria extract were negative in the bacterial assay with *Escherichia coli* (Ref. 229), in an unscheduled DNA synthesis assay in rat primary hepatocytes (Ref. 230), and in a micronucleus cytogenetic assay in mice (Ref. 231). An Ames test for metabolites of sanguinaria extract in rat urine using *S. typhimurium* was negative. Studies of sanguinaria chloride were negative in other Ames assays with *S. typhimurium* (Ref. 232), and *Saccharomyces cerevisiae* (Ref. 233) with and without metabolic activation. Two mammalian cell assays (Ref. 234) with sanguinarine chloride, including a Chinese hamster ovary (CHO)-hypoxanthine-guanine phosphoribosyltransferase (HGPRT) forward gene mutation assay and unscheduled DNA synthesis assay in rat primary hepatocytes (Ref. 235) provided results that were equivocal or uninterpretable. Neither study, however, gave a positive mutagenic response. The CHO assay is historically difficult to conduct and interpret.

Long-term (90 to 98 weeks) carcinogenicity studies (Ref. 236) by gavage at dosages of 0 to 60 mg/kg per day sanguinaria extract in rats did not produce treatment-related preneoplastic or neoplastic lesions to suggest a carcinogenic effect. Dosage at 40 mg/kg per day did not produce toxicity and is considered the NOAEL dosage. A lifetime diet carcinogenicity study of sanguinaria extract was evaluated in rats (8 to 200 mg/kg per day) (Ref. 237). No

test related hematological, biochemical, or urological changes were observed at any dosage level. No test article related macro- or microscopic pathology changes were observed. A 200 mg/kg per day dosage level can be considered the NOAEL level.

Two controlled 13-week subchronic studies done in monkeys and dogs (Ref. 238) examining ocular toxicity provided no evidence that sanguinaria extract or sanguinarine chloride affected intraocular pressure or produced any other ophthalmologic changes.

Human exposure to sanguinarine with twice daily use of toothpaste and oral rinse has been estimated to be 0.056 mg/kg per day (Ref. 238). Comparison of doses tested in animal studies with human doses expected from use of toothpaste or oral rinse appears to support the use of sanguinaria extract at a significantly higher concentration than contained in currently marketed products.

Ten animal safety studies conducted between 1982 and 1984 were submitted for dentifrice formulas containing 300 to 2,000 µg/mL of sanguinaria extract. None of the studies tested the currently marketed toothpaste formula containing 750 µg/mL of sanguinaria extract. Acute oral toxicity was greater than 20 g/kg in rats for a toothpaste formula containing 300 µg/mL of sanguinaria extract, and 5 g/kg in rats for a formula containing 500 µg/mL of sanguinaria extract (Refs. 239, 240, and 241). Primary skin and eye irritation studies carried out in rabbits (Refs. 242 and 243) demonstrated mild irritation reaction when a toothpaste formula containing less than 750 µg/mL was tested. Mild mucosal irritation was observed when a toothpaste formula containing 300 µg/mL of sanguinaria extract was tested in cheek pouches of hamsters (Refs. 244 through 248).

Two clinical studies (Refs. 249 and 250) demonstrated only mild mucosal irritation in test subjects. No differences were noted in the severity of lesions between the test and control groups.

Eleven clinical studies of animal safety conducted between 1983 and 1987 (Ref. 251) were submitted. Because modification of the oral rinse formulation from pH 3.2 to pH 4.5 began in 1989, none of these studies provided animal safety data on the currently marketed oral rinse (pH 4.5).

Based on data on the oral rinse formula containing 450 to 1,000 µg/mL sanguinaria extract at a pH of 3.2, no mucosal irritation was noted in the hamster cheek pouch (Refs. 252 and 253) or albino guinea pig studies (Ref. 254). No signs of toxicity or pharmacological effects were observed in test animals when a rinse formula of

450 µg/mL sanguinaria extract at pH 3.2 was tested (Ref. 255).

Four human studies conducted between 1982 and 1985 evaluated the irritation and sensitization potential of dentifrice formulas containing sanguinaria extract using a repeated insult patch test design involving a 2-percent aqueous slurry (Refs. 256 through 259). These studies demonstrated no induction of irritation or allergic contact dermatitis. An exaggerated use study (Ref. 260) using an earlier formula (300 µg/g sanguinaria extract) demonstrated no irritation or sensitization in soft oral cavity tissues. Two 6-month studies on a toothpaste containing sanguinaria and sodium monofluorophosphate (Refs. 261 and 262) showed no adverse effects on oral hard or soft tissues. Soft tissue examinations included inspection of the lips, tongue, hard and soft palate, gingiva, mucobuccal fold areas, inner surface of the cheeks, and sublingual areas. Although testing of the microbial flora was inconclusive in one study (Ref. 261), sanguinaria did not promote overgrowth through the development of resistant microbial strains.

A 6-month, double-blind, randomized study using a dentifrice containing 0.075 percent sanguinaria extract (Ref. 263) showed no significant oral irritation or adverse reactions. A 1-week exaggerated use study showed that 18 of the 28 subjects experienced mucosal sloughing (Ref. 264).

Although nine human safety studies were presented, only one study (Ref. 265) tested the currently marketed oral rinse containing 300 µg/mL of sanguinaria extract at pH 4.5. However, this study tested the efficacy of the formula and was not designed to test the safety of the oral rinse. Three of the remaining eight studies showed that repeated application of the earlier oral rinse formula at pH 3.2 under a semioclusive patch test did not induce clinically significant irritation or evidence of induced contact dermatitis in humans (Refs. 266, 267, and 268). This earlier rinse formula gave no evidence of localized or generalized clinical manifestations in test subjects in two of the 7-day exaggerated use studies (Refs. 269 and 270). The Subcommittee concludes that sanguinaria extract at 0.03 to 0.075 percent concentration in an oral rinse or dentifrice dosage form is safe.

ii. *Effectiveness.* The Subcommittee reviewed controlled clinical studies ranging from 1 week to 6 months in duration. Three short-term studies (two 1 week and one 1 month) had equivocal results between the active and placebo toothpaste preparations. Of the three