The European Agency for the Evaluation of Medicinal Products

EMEA/MRL/542/98-FINAL
March 1999

# COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

## RUTA GRAVEOLENS

## SUMMARY REPORT

1. *Ruta graveolens* L., synonyms bitter herb or garden rue, is a plant of the family *Rutaceae*. The homeopathic mother tincture is prepared according to the German Homeopathic Pharmacopoeia (HAB) by ethanolic extraction of the fresh aerial parts of *Ruta graveolens* at beginning of flowering. *Ruta graveolens* is intended for oral or parenteral use in food-producing species in homeopathic preparations containing concentrations of 1 part per 1000. The use follows the principles of homeopathic therapy where animals are diagnosed on basis of the individual pattern of clinical signs. The recommended maximum parenteral dose is 10 ml for large animals. The corresponding oral preparations of 1:1000 in form of tablets, globuli or drops are reported to contain somewhat lower amounts of plant extract than the injectable form. Treatment may be repeated but a fixed dose regimen is not common in homeopathy.

   In human homeopathic medicine mainly 1:10 and higher dilutions are used. The use of mother tincture and 1:10 dilutions in pregnancy is contraindicated. Phytotherapeutic use of the plant or its essential oil is no longer recommended. *Ruta graveolens* and its essential oil are also used as spice and flavouring agent in food production. Actual amounts of use are not known.

2. *Ruta graveolens* is characterised by a particularly wide spectrum of different plant constituents. More than 100 compounds have been identified up to now. These belong to four major classes of substances which may be present in variable amounts in the aerial parts of the plant:
   - alkaloids of the quinoline, quinolinone (e.g. graveoline), furoquinoline (e.g. dictamnine, γ-fagarine, skimmianine), pyranoquinoline (e.g. rutalinium) and acridone (e.g. furacridone) type;
   - coumarins like coumarin, dicoumarins, furanocoumarins (e.g. bergaptene), pyranocoumarins and coumarin-naphtochinone-compounds;
   - flavonoids (e.g. rutin);
   - essential oil (e.g. 2-nonanone, 2-nonylacetate, 2-undecyl acetate).

   The alkaloid and coumarin fraction was estimated with 0.4 to 1.4% of total plant constituents and the essential oils with 0.2 to 0.4% in the leaves. Quantitative data for individual constituents were limited.

3. Various pharmacological effects have been observed or postulated for *Ruta graveolens* extracts or fractions and isolated constituents thereof: *Ruta graveolens* alkaloid extracts have shown spasmolytic effects in several *in vitro* animal models (e.g. histamine- or acetylcholin-induced contraction in the isolated ileum of rats. On the other hand some alkaloids were found to induce contractions in the isolated uterus and to increase adrenergic effects in the seminal vesicles of Guinea pigs. Analgetic, antiphlogistic and anti-inflammatory action of certain ingredients has been postulated. A calcium blocking effect by furocoumarins as well as $K^+$-channel blocking effects by natural extracts of *Ruta graveolens* in nerve membranes has been reported. A slight anthelmintic effect of the essential oil, possibly proportional to the concentration of nonylmethyl ketone was described. The oil was reported to be devoid of contractile effects on cat uterus.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Switchboard: (+44-171) 418 8400 Fax: (+44-171) 418 8447
E_Mail: mail@emea.eudra.org http://www.eudra.org/emea.html

4. Specific studies on the pharmacokinetics of *Ruta graveolens* or its constituents were not available. After oral administration of the alkaloid fraction at doses of 50 mg/kg bw to rats the maximum blood level was obtained after 30 to 60 minutes.

5. Only limited data on acute toxicity of *Ruta graveolens* were available. For the essential plant oil acute toxicity was low with oral $LD_{50}$ values slightly above 2 g/kg bw in mice and above 5 g/kg bw in rats. The dermal $LD_{50}$ was found to be higher than 5 g/kg bw in rabbits. Deaths have been reported in humans after oral use of oil or plant extracts from *Ruta graveolens* for the purpose of abortion (details not available).

6. No information on repeated dose toxicity of *Ruta graveolens* or its constituents was available.

7. There is some evidence that *Ruta graveolens* extracts can have antiimplantation or abortifacient properties but results were not always consistent and details concerning parts of the plant or the constituents involved in these effects were limited: a decrease of implantation rate by approximately 90% has been observed in rats after intramuscular application of 40 mg and 80 mg/kg bw of an ethanolic extract of dry matter of the plant on day 1 *post coitum* or after oral application of 1 ml/kg bw of a cold infusion on day 4. Other investigators found antifertility effects in pregnant rats only after administration of very high doses of extracts, corresponding to 8 g of crude plant/kg bw and above. No effects were seen in Syrian hamsters. Repeated oral administration of approximately 1 ml/kg bw of the essential oil fraction leads to abortion in pregnant guinea pigs, possibly induced by general toxicity. Hot water extracts of the plant have been used orally in form of tea infusions as abortifacient in humans (details are not available). The *Ruta graveolens* essential oil has been tested in adequate oral teratology studies in rats and mice at doses up to 820 and 970 mg/kg bw, respectively, and was found to be without significant maternotoxic, embryotoxic, or teratogenic effect in these species.

8. There are numerous published studies on mutagenic properties and phototoxicity/mutagenicity of *Ruta graveolens*. Most investigations used specific isolated plant constituents: Acridone alkaloids of the leaves (e.g. rutacridone) have been reported to show mutagenic properties in the *Salmonella*-microsomal assay with metabolic activation possibly through formation of the metabolite rutacridone epoxide. The alkaloid dictamnine was reported to cause frame-shift-mutations in *Escherichia coli* WP2 at dark conditions. The alkaloid arborinine was found to inhibit DNA synthesis in HL-60-cells. Dictamnine, γ-fagarine, or skimmianine showed mutagenic activity with metabolic activation in *Salmonella typhimurium* TA100. The potent mutagen in the *Salmonella*-microsomal assay, isogravacridonchlorine, has only been observed in dried roots of *Ruta graveolens* (approximately 0.001%). *Ruta graveolens* alcoholic tincture (1:5) was reported to be mutagenic in *Salmonella typhimurium* TA98 without metabolic activation but with lesser effects after metabolic activation. This tincture also showed photomutagenic properties in the arginine auxotrophic green algae *Chlamydomonas reinhardtii* under the influence of UV-A light. These effects were attributed to some furocoumarins (e.g. bergaptene, psoralene, imperatorin) and furoquinolines (e.g. dictamnine, γ-fagarine, skimmianine). In human lymphocytes, an increase of sister chromatid exchange rate has been reported after incubation with 25 µg *Ruta graveolens* ethanol tincture (1:5) per ml. Increased sister chromatid exchange was also observed for the alkaloid γ-fagarine at 0.1 µg/ml.

   Overall, the published data provide indication of mutagenic properties of several *Ruta graveolens* constituents but assays for gene mutation, clastogenicity or aneugenic effects in mammalian cells were lacking or too limited to adequately conclude on a genotoxic hazard connected with extracts of the plant. On the other hand, some of the furocoumarins present in *Ruta graveolens* have been reported to inhibit the mutagenic effect of other ingredients of the tincture as for instance dictamnine and rutacridone. The oil *of Ruta graveolens* was reported to be inactive in mutagenicity tests in *Salmonella typhimurium* and *Saccharomyces cerevisiae.*

9. Carcinogenicity studies with *Ruta graveolens* were not available. In general, furocumarin derivatives (e.g. bergaptene) have been shown to increase the incidence of squamous cell carcinoma and other skin cancers like melanoma in human patients treated over long periods against psoriasis.

10. The leaves of *Ruta graveolens* were found to inhibit the growth of several bacterial species. Minimum inhibitory concentration (MIC) (in 50% of the strains/isolates per bacterial species investigated) values were about 40 to 100 µg/ml against *Bacillus subtilis* and *Staphylococcus aureus*. The MIC of acridonepoxides, rutacridonepoxide, and hydroxyrutacridonepoxide on the other hand, range between 0.1 and 1 µg/ml against different bacteria and between 1 and 5 µg/ml against different fungi.

11. *Ruta graveolens* is a photosensitiser in humans. Topical as well as oral exposure may lead to severe photodermatitis, mainly due to the content of furocoumarins. The essential oil is a local irritant. In the European Union the sum of furocoumarins, (e.g. psoralene, bergaptene) is not allowed to exceed 1 mg/kg in sun protection and bronzing products. Specific information on immunotoxicity of *Ruta graveolens* was not available.

12. It was not possible from the available information to establish a complete pharmacological and toxicological profile including NOELs and an ADI for *Ruta graveolens* extracts.

13. Risk assessment for *Ruta graveolens* may be based on a combination of worst-case assumptions: In the absence of data, it is assumed that all plant constituents are completely soluble in the mother tincture. The1:1000 dilution then would contain maximally 0.1% (e.g. 1 mg/ml) of plant material. Using an arbitrarily high figure of 10% for the total bioavailable plant fractions of possible pharmacological or toxicological concern (i.e. the essential oil, alkaloids, coumarins, flavonoids) a maximum intravenous dose (10 ml) would amount to 1 mg of these compounds in large animals (2 µg/kg bw, 500 kg bw). Assuming no metabolism or excretion, this dose could lead to an amount of 2 µg of residues in a standard portion of edible tissues. However, in the absence of further information an extrapolation of probable residues is not possible for milk.

14. This calculated amount of *Ruta graveolens* residues in food derived from treated animals is considered insubstantial compared to actual consumer exposure from use of *Ruta graveolens* as spice and flavouring agent in normal food production. In the European Union, *Ruta graveolens* herb may be used in certain food commodities as for instance baked goods, frozen dairy products, soft candy or non-alcoholic beverages. The level of use is up to 1900 µg/kg. *Ruta graveolens* oil is tolerated at concentrations of 630 to 9000 µg/kg in certain beverages and food products. *Ruta graveolens* and its essential oil have been approved for GRAS status (Generally Recognised as Safe) in 1974 by the United States Food and Drug Administration (FDA) and may be added to human food as flavouring agents (up to 2000 and 10 000 µg/kg, respectively). Both substances are also allowed in the United States in animal feedstuffs at the same levels.

**Conclusions and recommendation**

Having considered that:

- *Ruta graveolens* is used in veterinary homeopathy only as a highly diluted extract not exceeding one part per thousand prepared according to homeopathic pharmacopoeias,
- *Ruta graveolens* is used in a small number of individual animals, for infrequent and non-regular treatment in accordance with the principles of homeopathic therapy,
- the animals are unlikely to be sent for slaughter during or immediately after treatment,
- *Ruta graveolens* may be added at mg/kg levels as spice and flavouring agent in production of a variety of human food commodities;

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

| Pharmacologically active substance(s) | Animal species | Other provisions |
|---|---|---|
| *Ruta graveolens* | All food producing species | For use in homeopathic veterinary medicinal products prepared according to homeopathic pharmacopoeias, at concentrations in the products not exceeding one part per thousand only.<br>Not for use in animals from which milk is produced for human consumption. |