The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

SINAPIS NIGRAE SEMEN

SUMMARY REPORT

1. *Sinapis nigrae semen* (Mustard Seeds) are the seeds of *Brassica nigra* (L.). The drug contains glucosinolates: mainly sinigrin (allylglucosinolates, ca. 1 to 5%), which is enzymatically cleaved to allylisothiocyanate when grinding the seeds into powder and then rubbing with warm water as well as chewing. Volatile mustard oil contains approximately 92% of allylisothiocyanate. The seeds contain fatty oil (30 to 35%), proteins (approximately 40%), and phenyl propane derivatives: including among others sinapine (choline ester of sinapic acid, approximately 1%).

2. *Sinapis nigrae semen* is used orally in a veterinary medicinal products in combination with other substances in the content of 9% to induce rut and heat in cows, horses, pigs, sheep and goats. The dosage regime is 50 g of the product two times daily on two subsequent days for cows and horses, and 10 to 20 g of the product two times daily (depending on the weight of the animals) during three to four days for pigs, sheep and goats. The drug is used externally in an other veterinary product in an 1.2% isopropanolic tincture together with other hyperaemic drugs for relief of muscle- and tendonstrains in form of liniments and compresses in cattle, horses, sheep and goats.

   Sinapis nigrae semen is used externally in human traditional medicine in a mustard poultice for bronchial pneumonia and pleuritis, and in a mustard spirit (2%) as antirheumatic.

   Mustard containing the seeds of *Brassica nigra* is a normal component of the human diet.

3. *Sinapis nigrae semen* has antimicrobial and hyperaemic properties.

4. After oral administration of 25 mg/kg bw to rats and mice the main active substance allylisothiocyanate is nearly completely absorbed via the mucus membrane of the gastrointestinal tract within 15 minutes.

   Fifteen minutes after intravenous administration of 25 mg/kg bw of radioactive marked allylisothiocyanate main radioactivity was found in the urinary bladder of male rats and mice. The radioactivity in male animals was ten times higher than in female ones. The main metabolite in urine is N-acetyl-S-(N-allylthiocarbamoyl)-L-cystein. After oral and intravenous application of 25 mg/kg bw to rats and mice of radioactive labelled allylisothiocyanate the main compound is nearly completely excreted within 24 hours. Seventy to 80% are excreted via urine, about 15% via the lungs and about 5% via the faeces independent from the route of administration.

   If large amounts of mustard seed are fed to lactating cattle, milk gets a burning taste which shows that allylisothiocyanate is also excreted via milk.

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5. After administration of a single oral dose of 200 to 400 mg/kg bw allylisothiocyanate to rats and 100 to 800 mg/kg bw to mice retardation of growth and unspecific signs of toxicity appeared.

   Oral doses of 3 to 50 mg/kg bw allylisothiocyanate in mice and 25 to 400 mg/kg bw in rats showed depending on the dose a thickening of the mucous membrane of the stomach and the 50 mg/kg bw in mice additionally strong thickening of the wall of the urinary bladder. A single oral dose of 10 mg/kg bw allylisothiocyanate in rats minimises the reception of iodine in the thyroid glands up to 40% and after administration of 20 mg/kg bw of up to 60% respectively.

   The LD_{50} value for allylisothiocyanate is about 25 to 200 mg/kg bw in the rat.

6. Allylisothiocyanate can cause severe inflammation after dermal application to an extent where blisters and necrosis are possible.

7. Oral administration of 12 or 25 mg/kg bw allylisothiocyanate by gavage 5 days a week for 103 weeks in rats showed a dose depending reduction of body weight gain as well as a reduction of average life span. Increased hepatocellular vacuolation could be found in the liver of these animals. Application of 25 mg/kg bw allylisothiocyanate during 60 days led to a significant increase of weight of thyroid gland (42%) without a reduction of the radioactive iodine intake.

   In additional literature the following study was found reported: Allylisothiocyanate was given in doses of 0, 10, 20 or 40 mg/kg by oral intubation to male rats 5 days a week for up to 6 weeks. The highest dosage level caused a decrease in body weight, thymus weight, blood glucose and serum globulin levels. Increased liver and adrenal weights were found in all test groups. Histopathological changes were observed in the kidneys of animals at dosages of 20 and 40 mg/kg and in the livers of animals at the highest dosage level.

8. No data on reproduction parameters were submitted. Oral administration of up to 18.5 mg/kg bw allylisothiocyanate to pregnant CD-1-mice Wistar rats, Chinese hamsters and rabbits between day 6 and 15 of pregnancy showed no teratogenic effects. Even higher doses up to 120 mg/kg bw in rats showed no embryotoxicity. In one literature reference resorptions were reported for mice and rats.

9. Allylisothiocyanate showed significant effects in the Ames test only after prolonged incubation in the presence of liver cell homogenate and only against *S. typhimurium TA100*. Other bacterial strains showed no or only weak effects. In cell cultures of human lung cells or spinal cord cells of rats no chromosomal aberrations could be found.

10. Oral doses of 12 and 25 mg/kg bw allylisothiocyanate showed no significant tumour incidence in B6C3F1-mice when administered 5 times a week during 103 weeks. Male Fisher-344/N rats showed an increase of papillomas and epithelic hyperplasias in the urinary bladder in comparison to the female animals and the control group. Female rats showed an increase of subcutaneous fibrosarcomas. IARC (International Agency for the Research on Cancer) found that “there is limited evidence for the carcinogenicity of allylisothiocyanate to experimental animals”, no evaluation could be made for humans due to absence of epidemiological data.

11. No data on neurotoxicity and immunotoxicity were submitted.

12. When the drug is used at the therapeutic dosages recommended no health hazards or side effects in humans are known. Gastrointestinal disorders (and, rarely, kidney irritation) could occur following internal administration of higher doses, due to the mucus-membrane-irritating effect of the mustard oil. The drug possesses a minimal potential for skin sensitisation; contact allergies have been observed.

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13. Allylisothiocyanate, the main active constituent of *Senapis nigrae semen,* is also a constituent of cruciferous vegetables e.g. cabbage, cauliflower and Brussels sprout which are normal components of the human diet. Mean intake of sinigrin (precursor of allylisothiocyanate) in the UK in 1984 from cabbage, cauliflower and Brussels sprouts was estimated to be 9.3 mg/day. The intake is underestimated as not all cruciferous vegetables and mustard are considered.

Conclusions and recommendation

Having considered the criteria laid down by the Committee for the inclusion of substances in Annex II to Council Regulation (EEC) No. 2377/90 and in particular that:

- the active principles of *Sinapis nigrae semen* are contained in animal feed and in the human diet including mustard,
- allylisothiocyanate the main active constituent of *Senapis nigrae semen* is rapidly excreted,
- *Sinapis nigrae semen* is used only for occasional treatment of individual animals,
- animals are unlikely to be sent for slaughter immediately after treatment;

the Committee concludes that there is no need to establish an MRL for *Sinapis nigrae semen* and recommended its inclusion in Annex II to Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Sinapis nigrae semen	All food producing species	

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