COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

OXALIC ACID

SUMMARY REPORT

1. Oxalic acid (CAS No144-62-7) is a comparatively strong organic dicarboxylic acid intended for control of *Varroa destructor* in honey-bee colonies. It is administered by spraying, trickling or evaporation. The recommended dose is 1 to 3 g per hive. Treatment should be conducted as an autumn or winter-application in November/December in broodless honey-bee colonies.

2. The mechanism of acaricidal action against *Varroa destructor* has not been investigated in detail and is attributed partly to a sensitivity of this species to acid pH. Oxalic acid has no identified pharmacological or therapeutic properties in mammalian species. It is a constituent of plants where its physiological role is not precisely known. It has been suggested that it is involved in seed germination, storage and regulation of calcium, ion balance, detoxification, structural strength and insect repellence. It may also function as a pH regulator and might have antioxidant properties. Oxalic acid occurs in plants at various amounts from 5 mg/kg up to 200 g/kg dry weight. Oxalic acid is in use as a cleaner, as a bleaching and dying auxiliary and for other purposes in the industrial and domestic sector.

3. In mammals, oxalic acid is an end product of metabolism of natural components of some amino acids as well as glycolate and ascorbic acid. In humans, endogenous sources constitute approximately 30 - 70 % of the oxalic acid excreted daily *via* urine (about 20-30 mg). The faecal excretion mainly results from dietary oxalic acid intake. Dietary intake comprises on average 50 mg daily, with wide variations depending on the type of food. The natural oxalic acid levels in human tissues are in the range 0.6 to 4 mg/kg in blood, kidney, liver, muscle, brain and bone, the highest concentration being measured in kidneys and the lowest in brain.

4. After intravenous administration of small doses of ^{14}C-labelled oxalic acid to humans, the substance was mainly excreted as parent compound via the urine (more than 90 %) with only small amounts excreted via the faeces. The plasma elimination half-life was determined to be about 2 hours. Urinary excretion in overdosed rats was only about 65 %, possibly due to compromised kidney function. In overdosed rats more than 10 % of the administered dose was excreted via the faeces - partly as parent compound, and partly, after degradation by intestinal bacteria (e.g. *Oxalobacter formigenes*) as CO_2. In rats receiving unspecified intraperitoneal doses the parent substance has been reported to be retained in bones (more than 3% of dose). After oral administration *via* gavage or diet, the absorption was limited in rodents (less than 30 %) and humans (3-20 %). The degree of absorption was largely dependant on the percentage of oxalic acid soluble under different pH conditions in the intestinal tract, on the presence of free ionised calcium ions and other cations like magnesium and also on the presence of oxalate-degrading bacteria in the human gut. Increased absorption can occur (occasionally reaching 60 %) in certain diseases shortening or limiting transport *via* jejuno-ileal section of intestine, and fat malabsorption, facilitating calcium binding to fatty acids thereby enhancing oxalate absorption. Intestinal bacteria as *Oxalobacter formigenes* can degrade up to 40 % of the ingested oxalate.

5. In bees, ^{14}C-labelled oxalic acid was absorbed, distributed and metabolised after oral and topical administration. Twelve hours after topical application (by trickling) of ^{14}C-oxalic acid dihydrate to bees, ^{14}C-activity was detected in the haemolymph (peak concentration: 10 µg/g) and in all areas between the honey sac and rectum. In the haemolymph ^{14}C-activity decreased to a level of 2 µg/g within 72 hours and was no longer detectable in the intestines 22 and 31 days post-application. Radioactive CO_2 was detected in the air collected within the treated colonies. By-products of the evaporation of oxalic acid were examined in one study. After evaporation of oxalic acid crystals, on average 54 % was recovered. Approximately 1% of the evaporated oxalic acid was identified as formic acid. Neither formaldehyde nor acetaldehyde were detectable. It was concluded that half of the oxalic acid decomposes into carbon dioxide and water the other half vaporises to form fine drops and dust that precipitate in the hive.

6. Acute toxicity studies indicate that oxalic acid is of moderate to high toxicity by the oral route in mammalian species. The oral LD_{50} values determined for rats were 475 mg/kg bw for males and 375 mg/kg bw for females. For dogs and cats, the oral toxic doses were 1 g and 200 mg, respectively. After oral administration, the main target organ is the kidney with formation of crystals of calcium oxalate, associated with focal necrosis, mineralisation and impairment of kidney function. However, calcium depletion with sequelae of hypocalcaemia have also been reported. After intravenous administration of about 40 mg/kg bw to dogs 100 % of animals died shortly after administration. Oxalate binds to blood calcium and induces neurotoxicity and cardiac arrest. Oxalic acid is irritantating to skin, eye and the respiratory tract. Its dermal toxicity is low. No deaths were reported following the topical application of 20 g/kg bw oxalic acid to 3 rabbits.

7. In two studies groups of male and female rats were fed diets supplemented with 0, 25 and 50 g/kg (equivalent to 0, 2000 and 5000 mg/kg bw/day) oxalic acid for 70 days. The high dose levels depressed animal growth rate. This was accompanied by renal toxicity (increased water intake, increased kidney weight, abnormal gross appearance of kidneys at necropsy and stone formation) and reduced thyroid function. It is possible that the endocrine effects are not directly related to the compound but are the consequence of the poor health condition of the treated rats. In a non-invasive screening test for the detection of renal disease sodium oxalate administered subcutaneously to rats at 25, 50 and 75 mg/kg bw/day for 2, 3 and 1 weeks, respectively, caused mainly hematuria, with increase in the excretion into urine of white blood cells, epithelia, and casts. Histopathological examination of kidneys indicated a small number of oxalate deposits in animals treated with sodium oxalate. No tubular dilatation was present. These effects were indicative of a mild nephrotoxicity due to tubular obstruction following the administration of subcutaneous doses of oxalic acid greater than or equal to 25 mg/kg bw/day, for 5 days a week for 2 weeks. Studies provided on repeated dose toxicity exhibited several deficiencies with regard to current guidelines. It was not possible to retain a NOEL following repeated dose oral administration of oxalic acid to rats. Repeated dose toxicity studies in a second species are not available. No long-term repeated dose toxicity study has been performed apart from the 2 year carcinogenicity study.

8. Reproductive toxicity of oxalic acid was assessed in CD-1-mice in a fertility study which included a range finding module, the treatment of one generation in the continuous breeding system (FACB) and the assessment of fertility parameters of the first generation and developmental and fertility parameters in a second generation. In the range finding study, the lowest dose of 0.25 % already led to reduced water intake and at the two highest doses of 2.5 and 5% oxalic acid in drinking water most animals died. Dietary doses of 0, 0.05, 0.1 and 0.2 % were chosen for the main study corresponding to 89, 162 and 275 mg/kg bw/day (calculated in males only). Fertility and reproduction parameters were observed in the F0 animals for 18 weeks and in the last part reproductive performance was assessed in the offspring (F1). First generation pups were allowed to mature to about 74 days of age. Due to a lack of conspicuous effects in the first generation, only the highest dose and control group were continued for production of the second generation.

In the F0/F1 generations the number of litters in fertile pairs, live pup weight and prostate gland weight decreased significantly at 0.2 % in the diet, while all other parameters were unaffected. In the F1 generation the total number of live pups decreased, and prostate gland weight decreased significantly. Decreased water consumption was induced in the F1 0.1% and 0.2 % dose groups. Relative kidney weight of F2 females and the incidence of abnormal sperm in F2 males was increased. It can be concluded that oxalic acid is a weak reproductive toxicant (embryotoxicity) in CD-1 mice at a dose of 0.2% in drinking water corresponding to about 275 mg/kg bw/day.

The substance did not induce overt teratogenic effects or postnatal toxicity. No effect on blood calcium concentration was seen. Oxalic acid appeared to interfere with spermatogenesis in this study. It was not possible to derive a NOEL since data from the 0.05% and 0.1% dose level groups were not recorded for the second generation.

9. Two teratogenicity studies - one in rats, one in sheep - were provided. Neither study used valid protocols covering embryotoxic and teratogenic effects. In the rat study 10 females per dose group received oxalic acid at daily doses of 0, 159, 205 mg/kg bw per gavage under ether anaesthesia from day 7 post conception up to the day of parturition. Higher doses of 227 and 272 mg/kg bw had proven fatal within 7 days in a pilot study, while 136 mg/kg bw had led to marked vacuolation in cells of proximal tubules and tubular nephrosis in the pups. Neither gross malformation nor tubular nephrosis were observed in offspring in the main study, although the highest dose administered was well within the range of the effective low dose in the pilot study. No NOEL can be derived from this study. Female sheep (n=14 per dose, approximately 32 kg bw) were dosed either from breeding until mid-gestation (day 76) or from mid-gestation to lambing or from breeding to lambing with doses of 3, 6, 12 or 18 g oxalic acid mixed with normal diet. No information on oxalic acid content of the diet was given. At a dose of 18 g/sheep reduced food consumption and decreased body weight were observed. Renal oxalosis was observed only in lambs of mothers treated through the second part or through the whole pregnancy. No gross malformation was seen. Blood chemistry results (blood urea nitrogen, creatinine, calcium) showed no significant alterations. No NOEL can be derived. No final conclusion on teratogenic or embryotoxic effects can be drawn.

10. There are only limited data from non GLP studies and published reports to assess the genotoxic potential of oxalic acid. The results of the various Ames tests indicate that oxalic acid is clearly negative in the *Salmonella typhimurium* assay. Based on the data for the chromosome aberration test *in vitro* on mammalian Chinese hamster lung fibroblasts (CHL cells) oxalic acid is not considered to be a clastogenic compound. There was a weak positive result in the chromosome aberration test with plant root meristem cells but the relevance of this effect for mammals and human is unknown and the study was poorly documented. There are no studies which address the mutagenic potential of oxalic acid *in vivo*. However the negative results of the relevant *in vitro* studies and the data from a chronic toxicity study suggest that oxalic acid has no carcinogenic properties in rats. Therefore further mutagenicity studies are not required for a risk assessment of residues of the compound.

11. In a carcinogenicity study, Osborne-Mendel male and female rats were administered for 2 years a diet supplemented with oxalic acid (1000, 5000, 8000 and 12000 ppm corresponding to 50 to 600 mg/kg bw/day). Body weight and food consumption rate were examined at weekly intervals. Lung, heart, liver, spleen, pancreas, stomach, small intestine, kidney, adrenal, testis were microscopically examined and colon, bone marrow, leg bones, leg muscles, lymph nodes, uterus, ovary, thyroid and parathyroid were non systematically examined. There were no effects of the treatment on body weight, body weight gain and food consumption during the first 52 weeks of the experiment. There was no significant difference between the mortality rate (recorded at 18 and 24 months) of the rats at any dosage level in treated groups and in the controls. Pathological examination indicated a slight periportal hypertrophy of the hepatic cells. Tumours showed no difference in incidence among the various animal groups. Within the limitations of the study, which did not meet the criteria of current guidelines, there was no evidence of carcinogenicity in rats given approximately 50 to 600 mg/kg bw/day oxalic acid in the diet for 2 years. Considering also the negative results of the mutagenicity tests it can be assumed that oxalic acid is devoid of carcinogenic properties.

12. No information on the immunotoxic potential of oxalic acid and its salts are available. With the possible exception of transient reduction in relative thymus weight after subcutaneous administration of oxalic acid (75 mg/kg bw) to rats, there was no evidence for any immunotoxic effect. The effect on thymus weight may reflect the general toxic effect of the compound.

13. No studies were provided on intrinsic neurotoxic effects of oxalic acid or oxalates. Following overdosage, hypocalcaemia-induced neurotoxic effects - peripheral and CNS effects, including paraesthesia, tetany, seizures and depressed cardiac contractibility may be seen. However, in susceptible subpopulations, for example humans with severely compromised kidney function or patients with primary hyperoxaluria, hypocalcaemia-induced neurotoxic sequelae are to be expected even after moderate oxalic acid intake.

14. Anaerobic bacteria that metabolise oxalic acid (*Oxalobacter formigenes*) occur in the intestine of most animal species including humans. Numerous data suggest that bacteria degrading oxalate may increase in the intestine and metabolise oxalic acid to non-toxic substances. Studies on possible adverse effects of oxalic acid residues on dominant and subdominant bacterial species of the gut were not provided but are not required since oxalic acid at relatively high levels is found naturally in the intestine of healthy human subjects without influencing the normal barrier function of the human gut flora.

15. Intravenous doses of about 25 mg/kg bw oxalic acid inadvertently administered to human patients have led to kidney failure, cardiac arrest and death in spite of intensive care measures. High oral intake *via* a diet rich in oxalic acid has also occasionally led to severe poisoning and deaths. Oral fatal doses of oxalic acid were reported to range from 3 to 30 g/person. The susceptibility of individuals varies greatly, depending on prior kidney damage, certain intestinal disease states or genetic abnormalities such as primary hyperoxaluria. Determination of individual oxalate intake of 3 volunteers maintaining food records over 3 days suggests high average ingestion (152 ± 83 mg/day, range 44 to 351 mg/day) of normal consumers. On the basis of huge variations of oxalic acid content in the diet it may be assumed that occasional intake may reach and exceed 1 g/day, in particular in vegetarians. It is believed, that similar to rodents and ruminants, bacterial decomposition of oxalic acid in the human intestine may largely increase with high prolonged oxalate exposure *via* diet, leading to reduced bioavailability and consequently lowered toxicity. Therefore, adverse reactions are not common in humans in spite of daily intake occasionally reaching potentially dangerous levels. On the other hand, reduction of *Oxalibacter formigenes* fauna for instance by antibiotic treatment, may enhance the toxicity of ingested oxalic acid.

16. No adequate studies in laboratory animals and/or observations in humans were available which would allow conclusions to be drawn regarding NOELs, in particular for the relevant histological and functional alterations of the kidney induced by oxalic acid. Extrapolation of a safe dietary intake in humans on the basis of studies in laboratory animals is also hampered by the fact, that rodents appear to be much less sensitive to oral toxic effects of this substance than humans. There are additional problems caused by huge variation in genetic/physiological dispositions and vulnerability of human sub-populations.

The US Environmental Protection Agency concluded in 1999, that 0.14 mg oxalic acid or oxalate/kg/day over a 24-hour period represents the human allowable exposure from all sources to oxalic acid. The scientific basis for this conclusion is not available to the Committee, and, therefore, the relevance of this figure cannot be assessed. No safe dietary dose for oxalic acid can be established.

17. Oxalic acid is a natural constituent of honey and is found normally in the range of 1 mg/kg to 800 mg/kg, depending on the botanical source of the honey. A large number of publications was provided concerning possible excess residues of oxalic acid in honey as a result of treatment of bee hives with the substance. Investigations were mainly conducted as field trials and were not performed under GLP conditions. Nevertheless the investigations covered a realistic spectrum of treatment conditions and gave a useful overview of the possible residues of oxalic acid in honey (sum of free acid plus oxalates) as they would occur under conditions of good bee keeping practice, using recommended oxalic acid application methods like spraying, trickling and evaporation. In none of the investigations was a significant increase of natural oxalic acid in honey observed. Concentrations of oxalic acid in honey measured after recommended and off-label treatment conditions were in the range of 5 to 289 mg/kg which is well within the natural variability.

18. Oxalic acid is a ubiquitous substance in mammalian tissues and plants. It is endogenously produced in humans and excreted at amounts of about 25 mg daily via urine. Normal concentrations of oxalates in plants were in the range of 5 to 200,000 mg/kg dry weight. The daily intake of oxalic acid in the European diet can roughly be estimated in the range of 5 to 500 mg (exceeding 1000 mg in individual vegetarians). The daily intake of oxalic acid that would be expected in 20 g honey is in the range of 0.02 to 16 mg. Therefore, the theoretical oxalic acid intake in honey from either treated or non treated hives is negligible compared to the intake of the substance in daily food from other sources.

19. A fully validated analytical method was not provided but was considered not necessary for inclusion in Annex II of Council Regulation (EEC) No 2377/90.

Conclusions and recommendation

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

- oxalic acid is a substance of endogenous origin which occurs in all mammalian species and in plants,

- plant derived food constitutes the major source of dietary oxalic acid and the intake in European diets was estimated to be in the range of 5 mg to 500 mg/day occasionally exceeding 1000 mg/day,

- oxalic acid is occurring naturally in honey with an average content of approximately 200 mg/kg (range 1 mg/kg to 800 mg/kg) and no significant increase of the natural content was observed following treatment of bees,

- the theoretical intake of oxalic acid in honey from either treated or non treated hives is insignificant compared to the overall intake of oxalic acid in daily food from other sources;

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

Pharmacologically active substance	Animal species	Other provisions
Oxalic acid	Honey bees	