



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

2-PYRROLIDONE

SUMMARY REPORT

1. 2-Pyrrolidone (CAS 616-45-5) is the lactam of gamma-aminobutyric acid. It is used as solvent and excipient in veterinary pharmaceutical products. This application relates to the use of 2-pyrrolidone in specific formulations of antibiotics for single or repeated parenteral administration to cattle, pigs, and sheep. The maximum concentration of 2-pyrrolidone in the products covered by the application is 40% (w/v) and the maximum dose is 40 mg/kg bw. 2-Pyrrolidone is also used in veterinary drug formulations for horses, cattle, swine, sheep, goats and poultry at higher parenteral doses up to 75 mg/kg bw.

In human medicine 2-pyrrolidone is used mainly as vehicle for dermally administered drugs as for instance antimycotics, in order to enhance and accelerate absorption. For this purpose it is often mixed (2:3) with N-methyl-2-pyrrolidone.

2-Pyrrolidone is a normal component of certain food items. The natural content of 2-pyrrolidone in plant foodstuffs was reported to be in the range of 0.1 to 2.2 mg/kg (e.g. prunes, orange juice, tomatoes) and up to 20 mg/kg in certain processed food (e.g. tomato pastes and sauces).

2. In mammals 2-pyrrolidone is associated with the metabolism of glutamic acid, putrescine and gamma aminobutyric acid. There is evidence that 2-pyrrolidone, as a cyclic form of gamma aminobutyric acid, is a precursor of gamma aminobutyric acid in the central nervous system. 2-Pyrrolidone is able to cross the blood-brain barrier and has been shown to be hydrolysed enzymatically to gamma aminobutyric acid *in vivo*. This conversion however appears to be effectively regulated by homeostatic mechanisms preventing uncontrolled gamma aminobutyric acid formation. A relatively large intravenous dose of 200 mg/kg of [²H₆]-pyrrolidone given to mice was shown not to alter brain steady state levels of gamma aminobutyric acid at 30 minutes post dosing. Endogenous concentrations of 2-pyrrolidone in human, dog, rat and mouse plasma, cerebrospinal fluid or brain was reported to be in the range of 5 to 30 µg/l, but considerably higher amounts in brain and cerebrospinal fluid were also described in some investigations (up to 3500 µg/kg).
3. Various, partly inconsistent results have been reported in respect to possible pharmacological effects of 2-pyrrolidone. While anticonvulsive properties were asserted for oral dose levels of 150 to 200 mg/kg bw in mice (ED₅₀), in other studies in mice only low anticonvulsive and electroencephalographic effects were observed following parenteral administration of doses of up to 1000 mg/kg bw. Reduction of motility, enhancement of meperidine-induced analgesia in rabbits or chlorpromazine-induced catalepsy in mice and reduction of body temperature in mice have been reported at parenteral doses beginning at about 37 to 75 mg/kg bw. Other effects, such as weakening of conditioned reflexes appear to require higher doses of 180 to 370 mg/kg bw in rodents. Clear conclusions on a pharmacological NOEL cannot be drawn. It appears however, that doses of 2-pyrrolidone needed to produce measurable pharmacological effects are equivalent or higher than the maximum clinical doses of 40 mg/kg bw used in the target animals.

4. Pharmacokinetic data were provided for rats and the target species pig and cattle. Following intramuscular injection to rats of 25 mg/kg bw of ^{14}C -labelled 2-pyrrolidone, the absorption and distribution was rapid with maximum concentrations of 33.1 mg/l blood at 1 to 3 hours after dosing. This concentration declined rapidly to about 4 mg/l after 8 hours. Approximately 40% of the dose was recovered in urine within the first day and only about 1% on the second day. About 10% of the dose was exhaled as CO_2 within 8 hours. Only negligible amounts were found in faeces. In urine, trace amounts of 2-pyrrolidone and gamma-amino butyric acid were found together with the metabolite 5-hydroxy-2-pyrrolidone. Quantitative values were not available. In another study, urinary elimination amounted to about 60% of the dose within 24 hours following intramuscular injection to rats of 23 mg/kg bw of ^3H -2-pyrrolidone. Loss of the tritium label appeared to be significant (15% of the dose within 7 hours). Data on oral pharmacokinetics of 2-pyrrolidone were very limited. In a study using oral gavage dosing of a combination of ^{14}C -N-methyl-2-pyrrolidone and 2-pyrrolidone (3:2 w/w) at dose levels of 112 mg and 75 mg/kg bw, 2-pyrrolidone was reported to be nearly completely absorbed and did not undergo significant first pass metabolism. Maximum plasma levels of the parent compound for the dose of 75 mg/kg bw were 25 to 30 mg/l after 2 hours.

In the target species swine and cattle, maximum plasma concentrations of radioactivity were reached rapidly (1 to 4 hours post dose) following single intramuscular doses of 40 mg/kg bw of ^{14}C -2-pyrrolidone. The volume of distribution appeared to be equivalent to the body water. Initially, plasma radioactivity in swine consisted mainly of unchanged 2-pyrrolidone (50 to 60 mg/l). This concentration declined rapidly to less than 0.05 mg/l within 12 hours, while the metabolite concentration of the oxidation product succinimide (formed via 5-hydroxy-2-pyrrolidone) increased to approximately 20 mg/l. In contrast, in calves plasma concentrations consisted primarily of unchanged 2-pyrrolidone over a 12-hour period (about 50 mg/l declining to 30 mg/l). Succinimide concentrations remained low in cattle (2.3 mg/l at 12 hours). Excretion of the dose in calves and swine was predominantly via urine (about 40% within 3 days) with very little radioactivity in faeces (3% and 6%). Approximately 80% of urinary radioactivity was identified as unchanged 2-pyrrolidone and the major metabolites 5-hydroxy-2-pyrrolidone and succinimide. Total excretion did not make up the balance and there was indication of significant incorporation of radioactivity into endogenous metabolic intermediates like amino acids, fatty acids, urinary CO_2 and urea. Expiration of $^{14}\text{CO}_2$ was not investigated.

5. 2-Pyrrolidone is reported to be of low acute toxicity in mammals with oral LD_{50} values of above 6500 mg/kg bw in rats and guinea pigs, 800 mg/kg bw after intravenous injection in rabbits, 3000 and 3700 mg/kg bw after subcutaneous injection in rats and mice, respectively.
6. GLP-compliant repeated dose toxicity studies were not provided. Two subacute intravenous tolerance studies in rats (5 animals/sex/group) and dogs (3 animals/sex/group) at daily dose levels of 0, 112 and 446 mg/kg bw given for 5 or 6 days revealed no clinical signs with the exception of slight intravascular haemolysis in rats. Insignificantly lowered rectal temperature (by 0.3 to 0.6°C) and slightly decreased red blood cell count (the latter relative to the baseline levels only but not to control group values) were the only changes found in dogs. In 3-month toxicity studies in rats (10 animals/sex/group) or dogs (3 animals/sex/group) receiving doses of 0, 5, 20 or 100 mg/kg bw of 2-pyrrolidone via drinking water or food, respectively, no adverse effects on body weight, food consumption, clinical parameters or histopathology were observed. The study design however, did not include any effective doses and, being pre-GLP, did not meet other current requirements. Therefore, no definite NOELs could be retained.

No long term oral toxicity study was available. This information was not considered necessary as 2-pyrrolidone is of endogenous origin.

7. Tolerance studies in target animals were available. No untoward effects with the exception of slight irritation at the injections sites were seen in animals receiving intramuscularly or intravenously up to threefold the therapeutic dose.

8. No adequate information on the effects of 2-pyrrolidone on reproduction and no multigeneration study was available. This information was not considered necessary as 2-pyrrolidone is of endogenous origin.
9. In a GLP-compliant oral gavage teratogenicity study in rats at dose levels of 0, 190, 600 or 1900 mg/kg bw of 2-pyrrolidone (25 dams, treated from day 6 to 15 of gestation) maternotoxic effects (reduced body weights) were reported at 600 and 1900 mg/kg bw. Increased numbers of malformations were observed only at the highest dose level; e.g. anal atresia combined with acaudia or microcaudia (5 pups of 5 litters), absent inominate artery (2 pups of 2 litters), dilatation of lateral ventricles (4 pups of 2 litters) and overall incidence of foetuses with minor skeletal anomalies. NOELs of 190 and 600 mg/kg bw were retained for maternal toxicity and embryotoxicity/teratogenicity, respectively. In a non-GLP oral teratology study in mice, at doses of 1279 mg/kg bw and 3199 mg/kg bw given from day 11 to 15 of gestation (12 to 14 animal/group), only decreased weight of foetuses at the high dose level were observed. Intraperitoneal daily doses of 770 mg/kg bw and 1930 mg/kg bw (12 to 14 animal/group) given over the same period led to increased numbers of resorptions in both treatment groups. No definite conclusions on teratogenic effects can be drawn. No teratogenicity study was available in a non-rodent species.
10. Several pre-GLP mutagenicity studies examining gene mutations in bacteria (Ames tests performed at concentrations up to about 100 mg/plate of 2-pyrrolidone with and without metabolic activation using the *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100) and chromosomal mutations (mitotic recombination and gene conversion in *Saccharomyces cerevisiae*) were performed. At high concentrations (higher than 20000 µg/ml) 2-pyrrolidone was found to induce aneuploidy in *Saccharomyces cerevisiae*. Cytogenetic analysis of bone marrow cells of mice *in vivo* and of human lymphocytes *in vitro* have been reported to show negative results. The studies were in short summary form only with detailed data lacking. In a GLP-conform cytogenetic study using human lymphocytes no clastogenic effects or aneuploidy nor polyploidy were observed at concentrations up to 3500 and 6000 µg/ml without and with metabolic activation, respectively. Gene mutation activity in mammalian cell systems was not tested. Overall, data did not provide evidence for mutagenic risk and the molecule is not related to known mutagenic substances nor does it appear to possess alerting molecular structures.
11. No carcinogenicity studies of 2-pyrrolidone were available. This information was not considered necessary as 2-pyrrolidone is not genotoxic.
12. No information on immunotoxicity of 2-pyrrolidone has been provided. No signs of an immunotoxic effect have been noted in any of the acute or subchronic toxicity studies.
13. No data on antimicrobial activity of 2-pyrrolidone or effects on human gut flora and technical processing of food were provided. This information appears not to be necessary for the type of compound.
14. A definite ADI cannot be proposed for 2-pyrrolidone. Though pharmacological data indicated that measurable effects are to be expected at doses equivalent or above the maximum applied doses only (40 mg/kg bw), no clear NOEL could be derived. Doses with no effect in oral repeated dose toxicity studies in rats and dogs appeared to be as high as 100 mg/kg bw, but GLP requirements were not met by the studies. Teratogenicity studies in rats showed embryotoxic/teratogenic effects at high maternotoxic doses only, but appropriate studies in non-rodent species are lacking.

15. Four pigs (26 to 29 kg) were injected intramuscularly with 40 mg/kg bw of ¹⁴C-labelled 2-pyrrolidone. One animal each was slaughtered on day 1 and 7 after dosing and two animals were slaughtered on day 21. The highest total residue concentrations on day 1 were found in liver (11.78 mg/kg), followed by kidney (7.89 mg/kg), then injection site and non-injection site muscle (4.85 and 4.64 mg/kg, respectively) and finally fat (0.99 mg/kg). In all tissues except fat, total residue concentrations declined to less than 1.0 mg/kg at 21 days after dosing. In fat, a concentration of 1.02 mg/kg was still detected on day 21. Unchanged 2-pyrrolidone was found only in liver on day 1 after dosing (0.06 mg/kg). Unchanged 2-pyrrolidone at concentration above 0.05 mg/kg was not detected in any kidney, fat muscle and injection site muscle sample of day 1 to 21 after dosing.

The succinimide concentrations on day 1 after dosing were around 3.0 mg/kg in liver, kidney, and muscle (injection site muscle and non-injection site muscle) and 0.52 mg/kg in fat. At later time points (day 7 and 21), no succinimide concentrations above 0.1 mg/kg could be detected in any tissues.

16. Three calves (50 to 127 kg) were injected intramuscularly with 40 mg/kg bw of ¹⁴C-labelled 2-pyrrolidone and slaughtered after 1, 7 and 21 days. The highest total residue concentrations on day 1 were found in kidney (23.9 mg/kg) and liver (23.7 mg/kg), followed by fat (17.2 mg/kg), and finally injection site and non-injection site muscle (12.2 and 12.1 mg/kg respectively). In all these tissues except fat, concentrations declined to less than 1.0 mg/kg by day 21 after dosing. For fat on day 21, a relatively high total residue concentration of 4.22 mg/kg was reported. The concentrations of unchanged 2-pyrrolidone on day 1 were in the range of 3.0 to 3.5 mg/kg for liver, kidney and muscle (injection site and non-injection site muscle). In fat, a concentration of unchanged 2-pyrrolidone of 1.39 mg/kg was found. On the days 7 and 21, the concentrations of unchanged 2-pyrrolidone were in all tissues below the limit of quantification of the analytical method (0.05 mg/kg). The succinimide concentrations on day 1 after dosing were around 4.5 mg/kg in liver, kidney, and muscle (injection site muscle and non-injection site muscle). These concentrations are somewhat higher than those of unchanged 2-pyrrolidone. In fat, the succinimide concentration was 3.1 mg/kg on the first day after treatment. At later time points (day 7 and 21), no succinimide concentrations above 0.1 mg/kg could be detected in any tissues.
17. Provided residue data for target species revealed that a worst case estimate of intake of 2-pyrrolidone from foodstuff from animals treated at the dose of 40 mg/kg bw is calculated to be 1.6 mg per person per day. This value is lower than the estimate intake due to natural occurring concentrations of 2-pyrrolidone in plant derived foodstuff.

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:

- 2-pyrrolidone is a normal component of the diet in humans,
- 2-pyrrolidone is of endogenous origin;
- 2-pyrrolidone is rapidly metabolised after oral and parenteral administration in mammalian species;

the Committee considers that there is no need to establish an MRL for 2-pyrrolidone and recommends 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
2-Pyrrolidone	All food producing species	At parenteral doses up to 40 mg/kg bw