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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ISOFLURANE

SUMMARY REPORT

1. Isoflurane is a liquid halogenated hydrocarbon used as a general anaesthetic when vaporized and administered by inhalation. In the veterinary field, isoflurane is used in horses as a general anaesthetic. In horses, the concentrations of isoflurane used for anaesthesia induction are 3-5%, whilst maintenance of anaesthesia is usually achieved with 1.5-2.5%.

2. Isoflurane acts as a central nervous system depressant, and depresses respiration as evidenced by an elevated blood CO_2 pressure.

3. In a non-GLP experiment in rats, isoflurane administered via the inhalatory route at a dose level of 2% for 4 and 10 hours, induced no changes in urinary volume or osmolarity, but there was a slight increase in the mean peak serum inorganic fluoride levels. Mean peak serum inorganic fluoride levels were 6.5 µmole/l following 4 hours exposure and 7.3 µmole/l following a 10 hour isoflurane exposure. In a similar study, Fischer 344 male rats were anaesthetised with isoflurane (2%) for 4 hours. Maximum excretion of ionic fluoride in urine occurred in the first 24 hours post-anaesthesia whilst non-ionic fluoride peaked during the second 24 hours post-anaesthesia. Values returned to preanaesthetic levels on day 3 post-anaesthesia except for ionic fluoride which remained slightly elevated for a further period of two days. Thin Layer Chromatography (TLC) examination of urine samples indicated that the non-ionic fluoride metabolite was trifluoroacetic acid. No pharmacokinetic parameters were presented in any of the studies.

4. A non-GLP metabolism study was carried out in 3 Hormel miniature pigs. Animals were exposed by inhalation to isoflurane concentrations ranging from 0.5% to 9.6% for either 20 or 24 hours. Results indicate non hepatic clearance.

5. Published data showed that isoflurane is defluorinated in adult horses after inhalation. In neonatal horses, isoflurane is not significantly metabolised after inhalation. In this study, isoflurane metabolism was measured in 8 adult and 13 neonatal horses. Adult horses exposed to isoflurane for 8.4 MAC/h (Minimum Alveolar Concentration/h) (MAC=1.31% in horses) had significantly elevated serum fluoride levels ($p<0.0001$) with a mean peak level of 3.8 µmole observed 5 hours post-anaesthesia. In these animals, serum fluoride levels returned to the normal values by 24 hours following the end of the exposure. Neonates exposed to 2.1 MAC/h showed no significant change of serum fluoride levels.

6. Published data showed a similar pharmacokinetic behaviour for the volatile anaesthetics sevoflurane, halothane and isoflurane in pigs. The tissues/gas partition coefficients were similar and indicated a fat tissue affinity (fat/blood partition coefficient was 64±9, 51±6 and 57±9 respectively). Furthermore, elimination kinetics of sevoflurane and halothane were studied in rats. Data obtained showed an anaesthetic elimination (β-elimination) time constants for adipose tissue of 241±53 and 246±51 minutes for sevoflurane and halothane respectively. In horses serum fluoride levels returned to normal by 24 hours following the end of the exposure to isoflurane; this indicates that isoflurane behaves similarly to sevoflurane and halothane and it is rapidly eliminated.

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7. The acute inhalatory toxicity of isoflurane was determined in mice, rats and dogs in non-GLP studies.

   LC_{50} values in mice after inhalation exposure (3 hours at isoflurane concentrations ranging from 0.83% to 1.89%) were 1.69% for males and 1.68% for females (at a temperature of 35ºC). In rats, a similar study (3 hour period at an isoflurane concentration from 1.11% to 1.92%) was performed and LC_{50} values for males were determined to be 1.88% and for females 1.53% at 23ºC. At 35ºC all tested animals (male or female) died at 1.72% isoflurane. A study was carried out in dogs after inhalation exposure of 2.25% alveolar concentration of isoflurane for a period of 4 hours. No significant adverse effects were observed.

   In mice (strain, age and weight not specified) the LD_{50} value after intraperitoneal injection was 6.74 g/kg bw.

   No data on acute oral toxicity have been provided.

8. Toxicity after repeated inhalation exposure was investigated in mice, guinea pigs, rabbits, rats, dogs and monkeys. During a 9-week exposure period in mice (isoflurane concentration was 0.02, 0.1 and 0,5% for 4 hours per day), no significant effects were observed.

   In rats, no alterations were found in body, liver and kidney weights, serum glutamate oxaloacetate transaminase (GOT) and hematocrits, liver and kidney histological examination nor hepatic cytochromes (b_5 and P_{450}) after repeated administration of sub-anaesthetic dosages (less than 0.5%) for up to 30 weeks. A NOEL of 0.13% was established.

   No significant effects were observed in guinea-pigs, dogs or monkeys after repeated inhalatory administration (at dosages of 0.15%, 1-1.5% and 1-1.5%, respectively).

   No repeated dose oral toxicity studies have been provided.

9. Because good tolerance has been shown in clinical trials done by a number of investigators, no further studies on tolerance in the target species will be needed.

10. Isoflurane did not show any significant adverse effects on either male or female fertility or on foetal development when administered via the inhalation route in sub-anaesthetic dosage (up to 0.4% in mice and 1.6% in rats). No reproductive oral toxicology studies have been submitted.

11. Pregnant female mice were exposed to isoflurane by inhalation at doses of 0.006%, 0.06% and 0.6% for 4 hours per day on days 6 to 15 of pregnancy. Only the highest dose level reduced bodyweight in the dams, decreased skeletal ossification and increased the incidence of hydronephrosis. The higher concentration (0.6%) was also foetotoxic and maternotoxic. In these conditions a NOEL of 0.06% was established. Studies carried out in rats (not in GLP compliance) give reassurance that isoflurane is not teratogenic in this species. The NOEL for foetotoxicity is greater than 0.4% isoflurane. Teratology studies carried out in pregnant rabbits (2.28% to 2.34%) did not show any treatment-related effect. No data about the possible teratogenic effect of isoflurane by the oral route have been submitted.

12. Several series of Ames tests carried out with isoflurane (0.01-30%) indicated the lack of mutagenic activity. Negative results were also obtained in two more *in vitro* tests; a UDS assay in rat isolated hepatocytes, where isoflurane (0.005-0.500 mg/ml) did not produce any DNA damage and in a Syrian hamster embryo test where no cell transformation occurred using isoflurane (0.005 to 1 mg/ml).

    Isoflurane (50 to 500 mg/kg feed) was not mutagenic in two *in vivo* mutagenicity tests (chromosomal aberrations of Chinese hamster bone marrow test and mammalian spot test carried out in pregnant mice).

    Furthermore, there are reports in the literature of several tests performed with isoflurane in Chinese hamster ovary and lung cells, and in human lymphocytes, where this compound did not increase the frequency of sister chromatid exchanges.

    It can be concluded that isoflurane did not show any mutagenic activity.

13. A carcinogenicity study in mice was insufficient because the treated and control mice were treated differently, control animals were from different experiments and the feed was contaminated. In a second study, groups of 82-90/sex/dose Swiss Webster mice were used. They were exposed (inhalation) 4 hours per day, 5 days per week for 78 weeks with 0-0.4% isoflurane. No dose-related trend in the incidence of any tumour type was detected. A third carcinogenicity study in rats was well conducted and reported. The exposure schedule was 6 hours per day, 5 days per week with isoflurane concentrations up to 0.1% for a minimum of 104 consecutive weeks. There was no evidence to suggest any influence of isoflurane on the incidence of any tumour type at inhaled concentrations less than or equal to 0.1%.

    The negative results in the mutagenicity assays and the negative data in a carcinogenicity study in mice and the study in rats clearly indicate that isoflurane does not have carcinogenic potential.

14. No studies have been carried out with isoflurane to show any potential immunotoxicity. However, in the case of this compound, these studies are not necessary because no evidence of immune system damage has been reported in the literature.

15. No studies have been carried out with isoflurane to show any potential alteration in the human gut flora. However, in the case of this compound, these studies are not necessary.

16. In human medicine, isoflurane has been extensively used as a general inhalation anaesthetic agent for more than 15 years. During the use of isoflurane no significant toxic effects have been reported in patients or operating theatre personnel. There was no evidence of association between exposure to isoflurane and liver damage or other organ damage in humans. The duration of the pharmacological activity in humans after the end of isoflurane exposure is very short (15-25 minutes).

17. Having regard to the absence of mutagenic and carcinogenic potential, the low toxicity shown by isoflurane and the occasional use in a limited number of animals that are unlikely to be sent to slaughter soon after treatment, it is concluded that further toxicity studies are not necessary. Isoflurane is unlikely to cause any toxic effect in humans.

18. While limited data concerning the metabolism of isoflurane in horses were provided, this data showed that isoflurane was rapidly eliminated. Therefore, it is concluded that further toxicity studies are not necessary.

19. Taking into account the rapid elimination and the pattern of use, it was concluded that consumers were most unlikely to encounter residues of isoflurane in meat.

Conclusions and recommendation

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

- isoflurane is used in a small number of individual animals, for an infrequent and non-regular treatment,
- the animal treated is unlikely to be sent for slaughter immediately after treatment;
- isoflurane is rapidly and extensively excreted,
- isoflurane is a volatile compound (boiling point 47-50ºC), so the residues that could be present in food are likely to disappear within the cooking process,
- the misuse of isoflurane is unlikely to occur;

the Committee considers that there is no need to establish an MRL for isoflurane 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
Isoflurane	Equidae	For use as anaesthetic only

The Committee recommends to Member States to apply a withdrawal period of 2 days following the use of the compound in horses should animals be slaughtered for human consumption following anaesthesia.