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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

AZAPERONE

SUMMARY REPORT (2)

1. Azaperone is a neuroleptic sedative belonging to the class of butyrophenones. In veterinary medicine it is only used in pigs for a wide variety of indications (e.g. anti-agressiveness, obstetrics, stress, sedation and anaesthesia). Azaperone is available as a 4% injectable solution to be given strictly by a single, deep intramuscular injection at usual recommended doses ranging from 0.4 to 2 mg azaperone/kg bw, depending on the indication. Azaperone is not used in human medicine.

2. Azaperone is currently entered into Annex III of Council Regulation (EEC) No. 2377/90 as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Azaperone	Azaperol	All food producing species	100 µg/kg 50 µg/kg	Kidney Liver, muscle, fat	Provisional MRLs expire on 1.1.1998

3. Azaperone has a number of effects on the central nervous system. As other neuroleptics, azaperone is able to antagonize apomorphine and amphetamine-induced behavioural effects, mediated by brain catecholamines (especially dopamine). Therefore, it is thought to act by blocking dopamine receptors in the brain. Unlike the more antipsychotic neuroleptics, azaperone is a potent α-receptor blocker at lower doses, while dopamine receptors are blocked only at higher doses. Consequently, induction of sedation (reflected by palpebral ptosis) which is linked to anti-adrenergic properties, is obtained at therapeutic doses, while effects related to dopamine receptor blockade (such as catalepsy) are only present at higher doses.

   Besides the CNS-effects, azaperone also affects the reproductive organs. As a dopamine D_2-antagonist, azaperone is known to inhibit the prolactin inhibiting factor at the hypothalamo-pituitary level, hereby producing an enhanced prolactin release from the pituitary gland. The increased serum prolactin levels further result in an increased progestative status of the female genital tract and an increased mammary gland stimulation.

4. Several pharmacological studies were carried out with rats, mice and dogs after single dose exposure to azaperone. In all studies the subcutaneous route of administration was used, except for one test in dogs, which also used the oral route. In these tests, azaperone showed a number of related neuroleptic properties, including reduced motor activity, cataleptic effects, decreased stress- or trauma-related mortality, blocking of apomorphine emesis, prevention of fatal effects of the catecholamines. The lowest, most relevant NOEL in these studies was 0.08 mg/kg bw for norepinephrine antagonism following subcutaneous administration to rats.

   Of the metabolites, only azaperol has some pharmacological activity. After intraperitoneal administration to mice, azaperol was 4 to 30 times less active than azaperone.

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5. After subcutaneous administration to rats at single doses ranging from 0.08 to 80 mg/kg bw, azaperone was rapidly absorbed and distributed to tissues, with highest levels in liver, kidney and heart and lower levels in lung, fat, muscle and brain. Peak levels in plasma and tissues occurred within 0.5-1 hour, and were followed by rapid elimination of unchanged azaperone (within 8 hours levels in plasma and tissues were 4-100 times lower than peak levels), and somewhat slower elimination of metabolites. Both after single oral and single subcutaneous administration of 1 mg/kg bw azaperone, rats excreted approximately 20% in urine and approximately 80% in faeces, mainly within the first 24 hours. Excretion was completed within 4 days. The liver was the main site for metabolism. *In vitro*, the main metabolic pathways were reduction of the butanone (resulting in the main metabolite azaperol, which can be re-oxidized to azaperone *in vivo*), hydroxylation of the pyridine (resulting in 5-OH azaperone and 5-OH azaperol), oxidative N-dealkylation and oxidative N-dearylation. The *in vivo* studies with rats revealed metabolites in urine and faeces originating from the same metabolic pathways as found *in vitro*. No qualitative differences in metabolism were demonstrated after either oral or subcutaneous administration.

6. After single intramuscular administration to pigs at a dose of 1 mg/kg bw, plasma levels of azaperone peaked within 30 minutes and showed a fast biphasical depletion with half-lives of 20 minutes between 30 and 60 min and of 2.5 hours thereafter. Azaperone was rapidly distributed to tissues (with highest levels in kidney, liver and lung and lower levels in fat, brain and muscle), followed by a high rate of metabolism and elimination. Excretion was with the urine (62 to 89%, for single intramuscular doses of 4 and 1 mg/kg bw, respectively) and to a lesser extent with the faeces (less than 1%-13%, for single intramuscular doses of 1 and 4 mg/kg bw, respectively), mainly between 8 and 24 hours. *In vitro*, the main metabolic pathways were reduction of the butanone, oxidative N-dearylation and hydroxylation of the pyridine. Oxidative N-dealkylation was of minor importance. The *in vivo* studies with pigs indicated that the metabolites found in urine and tissues (azaperone, azaperol, their 5-hydroxylated forms, glucuronides and depyridinated metabolites) originated from the same metabolic pathways as found *in vitro*. Despite quantitative differences, the metabolic pathway for azaperone in pigs resembled the one in rats.

7. Acute oral toxicity studies were performed in mice, rats, guinea pigs and dogs, resulting in LD_{50}-values of 385, 245, 202 and greater than 20 mg/kg bw, respectively. Following subcutaneous administration LD_{50}-values in mice, rats and dogs were 179, 450 and greater than 40 mg/kg bw, respectively. Acute toxicity signs consisted of palpebral ptosis, sedation, tremors and occasionally vomiting and clonic seizures.

8. Several repeated-dose oral toxicity studies were performed in rats (0, 100, 400 or 1600 mg/kg feed for 15 weeks, 6, 12 or 18 months) and dogs (0, 1.25, 5 or 20 mg/kg bw/day for 13 weeks or 24 months). In rats also a repeated dose subcutaneous toxicity study was available (0, 2.5, 10 or 40 mg/kg bw/day for 13 weeks). In these studies, general toxic effects on body weight and organ weights (thymus in rats and dogs, liver and heart in dogs) were noted at higher doses than the effects that could be attributed to the pharmacological activity of azaperone. These latter effects, mainly a dose-related sedation and prolactin-mediated changes in the female genital tract, mammary gland and pituitary gland, resulted in an overall LOEL of 1.25 mg/kg bw as found in the 24-month dog study.

9. Tolerance studies in which pigs were intramuscularly injected with azaperone at doses ranging from 0.54 to 40 mg/kg bw revealed sedation, a fall in blood pressure and in arterial CO_2 tension at all dose levels. A fall in body temperature and in cardiac output was noted at doses from 2 and 2.5 mg/kg bw, respectively, while doses from 5 mg/kg bw revealed salivation and accelerated breathing.

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10. Although an oral 3-generation study in rats was available, this study was considered inadequate to enable the potential for effects on reproduction and fertility to be fully assessed, as males were left untreated and females were dosed on gestation days 6-15 only. Recently, the applicant conducted a male fertility study in rats (0, 5, 20 or 80 mg azaperone/kg bw/day, daily from 74 days prior to mating until the day before positive sperm control) upon request of the Joint WHO/FAO Expert Committee on Food Additives (JECFA; see paragraph 16). The results of this study are not yet available.

11. Doses of 0, 2.5, 10 or 40 mg azaperone/kg bw/day in oral studies with mice (on days 6-15 of gestion), rats (on days 6-15 of gestation in one or three generations, or from day 16 of gestation throughout a 3-week lactation period), hamsters (on days 6-10 of gestation) and rabbits (on days 6-18 of gestation), and in subcutaneous studies with rats (on days 6-15 or days 1-21 of gestation), did not indicate embryotoxic or teratogenic effects that could be related to azaperone treatment. In dams, pharmacological effects were noted at almost all doses tested. The lowest NOEL for foetotoxicity was found in mice (10 mg/kg bw/day). The lowest NOEL for post-natal toxicity was found in rats (5 mg/kg bw/day).

12. Azaperone was tested for mutagenicity in *in vitro* tests (Ames test with *Salmonella typhimurium* and gene mutation test with mouse lymphoma cells) and in *in vivo* tests (micronucleus test with rats and dominant lethal test with mice). Some metabolites of azaperone, including the main metabolite azaperol, were also tested in the Ames test with *Salmonella typhimurium*. Positive results were only obtained for azaperone and some of its metabolites in *Salmonella typhimurium* TA98 and TA1538 (i.e. strains that detect frame-shift mutations), with metabolic activation. All positive results were obtained within the same laboratory. However, the increase in the number of revertants was low (factor 2-3). Besides, Ames tests using the same strains, but conducted in other laboratories, did not confirm these findings. Given the negative results in the gene mutation test in mammalian cells and in the *in vivo* tests, azaperone is considered not mutagenic.

13. No carcinogenicity studies were performed. As azaperone can be considered not mutagenic in mammals, and the chemical structure does not possess any structural alerts, no carcinogenicity studies are deemed necessary.

14. No specific studies on immunotoxicity were submitted. However, related parameters determined in the repeated-dose toxicity studies did not positively indicate an immunotoxic potential of azaperone.

15. A study with human psychotics revealed that three daily doses up to 2 mg had no effect on clinical signs. Three daily doses of 2.5 to 20 mg led to a dose-dependent sedation, with complaints of dizziness at 3x20 mg. Since the human subjects were given azaperone in divided doses and it is unclear whether the doses were additive over the course of the day, the NOEL for sedation in humans is taken to be 2 mg/day, equivalent to 0.03 mg/kg bw. However, this study was poorly documented, poorly controlled and observations were of subjective nature.

16. Azaperone has also been evaluated by JECFA in 1991 and 1994. Due to the shortcomings of the human study, JECFA was unwilling to use this study in establishing an ADI. JECFA therefore established an ADI of 0-3 µg/kg bw, based on an oral NOEL of 0.63 mg/kg bw for pharmacological activity in dogs and using a safety factor of 200. This ADI was set on a temporary basis as JECFA asked for additional data on the genotoxicity of metabolites of azaperone and on reproduction toxicity (particularly on male fertility). JECFA will re-evaluate azaperone in 1998.

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17. The CVMP agreed with JECFA that pharmacological effects are most relevant in establishing the ADI for azaperone, and that the study with humans is unsuitable for this purpose. However, CVMP did not consider the oral NOEL of 0.63 mg/kg bw in dogs to be the most relevant pharmacological NOEL, as it is likely that in the dog not the most sensitive parameter has been tested and the dog is probably not the most sensitive species tested.

    CVMP considered the NOEL of 0.08 mg/kg bw for norepinephrine antagonism following subcutaneous administration to rats to be the most relevant pharmacological NOEL. This NOEL could be used to establish an ADI for the oral route, because it was shown from a comparison between oral and subcutaneous administration that both routes are equally effective. Consequently, an ADI of 0.8 µg/kg bw was established, using a safety factor of 100.

    Now, after re-evaluation of all available data, it is concluded that this ADI can be confirmed.

18. Total radioactive residues were investigated in two pigs per time point at 2, 24, 48 and 72 hours after a single intramuscular administration of ^{3}H-labelled azaperone at a dosage of 4 mg/kg bw. Apart from injection sites, total radioactive residues were highest at all time points in kidney and liver (declining from 11019 and 3675 µg/kg at 2 hours, via 625 and 698 µg/kg at 24 hours and 204 and 441 µg/kg at 48 hours, to 124 and 228 µg/kg at 72 hours, respectively). Residues in fat (1217 µg/kg at 2 hours, declining to 104 µg/kg at 72 hours), skin (1324 µg/kg at 2 hours, declining to 37 µg/kg at 72 hours) and muscle (588 µg/kg at 2 hours, declining to 13 µg/kg at 72 hours) were relatively low. Total radioactive residues therefore showed a fast depletion between 0 and 24 hours after administration and a somewhat slower depletion thereafter. Metabolite identification revealed several metabolites in edible tissues besides azaperone: azaperol, 5-OH azaperone, 5-OH azaperol, glucuronidated metabolites and a complex pattern of depyridinated products. The latter were derived from breakdown of the unstable 5-OH metabolites. In all tissues, the major residual component was azaperol, followed by azaperone. There was, however, a large variation in the ratio of concentrations of azaperone and azaperol to total residues in different tissues and at different sampling times. In liver and kidney, the depyridinated metabolites also accounted for a substantial part of the total residues. Total radioactive residues in the injection sites were very high and variable, and declined from 173900 µg/kg at 2 hours, via 60400 µg/kg at 24 hours and 44400 µg/kg at 48 hours, to 5800 µg/kg at 72 hours. Residues in the injection sites consisted mainly of the parent compound (70-90% of total residues) and to a lesser extent of azaperol (5-20% of total residues).

19. Several cold residue studies in pigs were performed, using intramuscular administration of the commercial product at single doses of 0.4, 1, 2, 2.2 or 4 mg/kg bw and determination of residues of azaperone and azaperol at several time points after administration, ranging from 2 hours to 7 days. In the key study, residues of azaperone and azaperol were investigated in four pigs per time point at 1, 2, 3, 5 and 7 days after a single intramuscular administration of 2 mg azaperone/kg bw. In liver, kidney, muscle, fat and skin, mean residues of both azaperone and azaperol were already below 100 µg/kg within 1 day and below 50 µg/kg within 2 days after administration, after which they were undetectable (below 25 µg/kg). Residues in the injection site were much higher and variable: concentrations of azaperone were 6960-51900 µg/kg at day 1, 2290-71800 µg/kg at day 2, 164-11100 µg/kg at day 3, 4230-29600 µg/kg at day 5 and less than 25-155 µg/kg at day 7. Concentrations of azaperol in the injection site were 4-20 times lower than those of azaperone, and declined from 1290-8250 µg/kg at day 1, via 31-1680 µg/kg at day 3 to less than 25-45 µg/kg at day 7.

20. After re-evaluation of all available data, the sum of azaperone and azaperol is now considered most appropriate as the marker residue, as azaperone and azaperol are the only metabolites with pharmacological activity and the ADI is set for pharmacologically active residues only. Besides, azaperol can be reconverted to azaperone. Although azaperol is less pharmacologically active than azaperone, as a worst case scenario in order to adequately protect the consumer, azaperol is considered as potent as azaperone.

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21. The pharmacologically active compounds azaperone and azaperol deplete to undetectable levels within a fews days after administration in all edible tissues except the injection site. Nevertheless, in view of the CVMP policy on target tissues, MRLs are set for all edible tissues. The high and more persistent concentrations of azaperone and azaperol in the injection site contra-indicates the use of azaperone for transport of pigs to the slaughterhouse.

22. An HPLC/UV method has been presented for the determination of azaperone and azaperol in tissues of pigs. This analytical method was described according to ISO 78/2, and had a limit of quantification of 25 µg/kg for both azaperone and azaperol in all tissues.

Conclusions and recommendation

Considering that:

- the ADI of 0.8 µg/kg bw (equivalent to 48 µg for a 60 kg person) is set on the basis of pharmacological activity, hence for pharmacologically active components only;
- azaperone and azaperol are the only metabolites with pharmacological activity;
- azaperol can be reconverted to azaperone;

the Committee recommends the inclusion of azaperone in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Azaperone	Sum of azaperone and azaperol	Porcine	100 µg/kg	Muscle, skin+fat, liver, kidney	

This MRL will theoretically result in a daily intake of 50 µg. As this intake is largely overestimated (based on the worst case scenario that the pharmacological acitivity of azaperol is equal to that of azaperone) it is compatible with the ADI of 48 µg for a 60 kg person.

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