The European Agency for the Evaluation of Medicinal Products
*Veterinary Medicines and Inspections*

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# COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

## AZAGLY-NAFARELIN

## SUMMARY REPORT

1. Azagly-nafarelin ([D-Nal(2)$^6$, aza-Gly$^{10}$] luteinizing hormone releasing hormone) is a synthetic analogue of the hypothalamic gonadotrophin releasing hormone (GnRH). Azagly-nafarelin is identical in structure to the native fish gonadotrophin releasing hormone decapeptide sequence, with the exception of a 3-(2-naphthyl) alanine moiety replacing glycine at position 6, and the replacement of the amide group with an aza group on the glycine molecule at position 10. In veterinary medicine, azagly-nafarelin is indicated for the induction and synchronisation of ovulation in female *Salmonidae*. The compound is administered once per breeding season by intraperitoneal injection at a dose rate of 32 µg/kg bw.

2. Azagly-nafarelin mimics the action of native gonadotrophin releasing hormone by stimulating the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland in the target species. This release of follicle-stimulating hormone and luteinizing hormone is responsible for the subsequent production of gonadal sex steroids. A study performed in pre-pubertal male wistar rats demonstrated that the subcutaneous administration of 0.1 µg azagly-nafarelin/kg bw resulted in significant increases in the plasma concentrations of both luteinizing hormone (13.0 versus 1.33 ng/ml in the control group at 1.5 hours post treatment) and testosterone (6.1 versus 0.2 ng/ml in the control group at 2 hours post treatment). Azagly-nafarelin also induced significant elevations in plasma luteinizing hormone concentrations when administered to broodstock Rainbow trout by the oral and intraperitoneal routes. Whilst the mean plasma luteinizing hormone concentrations in the control group varied between 5 and 7.5 ng/ml over the 30-hour test period, fish treated with azagly-nafarelin by the intraperitoneal route (dose rate of 40 µg/kg bw) exhibited mean plasma luteinizing hormone concentrations of approximately 5, 50, 140 and 45 ng/ml at 0, 6, 12 and 24 hours post-treatment. Treatment by the oral route at a dose rate of 150 µg/kg (with or without trypsin inhibitor), resulted in highest mean luteinizing hormone concentrations of between 20 and 30 ng/ml at 12 to 30 hours post treatment.

3. No data were presented on the secondary pharmacodynamics of azagly-nafarelin. However, such data was not considered necessary in view of the lack of secondary pharmacodynamic effects of other GnRH analogues.

4. The pharmacokinetics of azagly-nafarelin was investigated in a study in 2-year old broodstock Rainbow trout. A single dose of either 10, 40 or 80 µg azagly-nafarelin/kg bw was administered intraperitoneally as an aqueous solution to 10 fish per group, whilst a single dose rate of 40 µg azagly-nafarelin/kg bw was also administered intravenously to a similar sized group. The water temperature was maintained at 12°C. A total of 15 blood samples were collected from each fish at different time points over a 6-day period post treatment, and plasma azagly-nafarelin concentrations in plasma were assayed by radioimmunoassay (limit of quantification of 0.05 ng/ml). Following intravenous administration, mean concentrations of azagly-nafarelin in plasma of 709 ng/ml were recorded at 15 minutes post treatment. At the 4-hour time point post treatment, the mean plasma concentration had declined to 51 ng/ml, with a subsequent further decline in the mean plasma concentration to 2.5 ng/ml at the 24-hour time point post treatment.

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The terminal half-life was 4.37 hours, and the $AUC_{0-\infty}$ was 1.186 ng·h/ml. The mean residence time was 2.87 hours, the clearance rate was 0.0361 l/h/kg, whilst the volume of distribution was 0.210 l/kg. Following intraperitoneal injection of azagly-nafarelin at 10, 40 or 80 µg/kg bw, the mean $C_{max}$ was 129, 272, 256 ng/ml, whilst the mean $t_{max}$ was 1.98, 1.33 and 1.5 hours, respectively. The terminal half-life was 3.89, 4.90 and 5.86 hours, whilst the mean residence time was 6.52, 6.84 and 7.26 hours for the 10, 40 and 80 µg/kg bw groups, respectively. The $AUC_{0-\infty}$ was 836, 1.170 and 1.508 ng·h/ml for the 10, 40 and 80 µg/kg bw groups, respectively.

Plasma azagly-nafarelin concentrations were assayed following the administration of azagly-nafarelin to broodstock Rainbow trout at dose rates of either 100 or 150 µg/kg bw by the oral route (with or without a trypsin inhibitor), and a dose rate of 40 µg/kg bw by the intraperitoneal route. The water temperature was maintained at 17°C. A total of 5 blood samples were collected at the different time points over a 30-hour test period. Plasma azagly-nafarelin concentrations were measured by radioimmunoassay, but the limit of quantification of the analytical technique was not specified. Following intraperitoneal injection, the mean plasma azagly-nafarelin concentration peaked at 25 ng/ml at 6 hours post treatment. The highest mean plasma concentrations recorded in the oral high-dose groups were 1.6 ng/ml at 12 hours post treatment (150 µg/kg bw plus trypsin inhibitor) and 1.2 ng/ml at 6 hours post treatment (150 µg/kg bw without trypsin inhibitor). The peak plasma concentration for the 100 µg/kg bw plus trypsin inhibitor group was only 0.4 ng/ml. Overall it was concluded that the substance was quickly depleted from plasma.

The oral bioavailability of azagly-nafarelin was assessed in a GLP-compliant study in pre-pubertal male wistar rats. Following an initial pilot study, two groups of rats were dosed orally at rates of 25 and 100 µg azagly-nafarelin /kg bw on a single occasion. Two additional groups received a single subcutaneous injection of azagly-nafarelin at dose rates of 25 and 100 ng/kg bw. A control group received vehicle only by the oral route. A total of 54 rats per group were employed. Concentrations of azagly-nafarelin in plasma were not assayed in this study. Instead, the plasma concentrations of luteinizing hormone and testosterone were measured by radioimmunoassay. The limit of quantification of the radioimmunoassay technique for luteinizing hormone was 0.8 ng/ml, whilst the corresponding value for the testosterone assay was 0.2 ng/ml. The mean plasma luteinizing hormone concentration in the control group varied between 1.33 and 1.75 ng/ml over the 6-hour test period. The highest mean concentration of luteinizing hormone recorded in the high-dose subcutaneous group was 13.0 ng/ml at the 1.5 hour time point post treatment, whilst the highest mean luteinizing hormone concentrations recorded in the oral low-dose, oral high-dose and subcutaneous low-dose groups were 2.38 (at 2 hours post treatment), 2.45 (at 2 hours post treatment) and 3.03 ng/ml (at 0.5 hours post treatment), respectively. The mean plasma testosterone concentration in the control group varied between 0.2 and 0.6 ng/ml over the same 6-hour test period. The highest mean concentration of testosterone recorded in the high-dose subcutaneous group was 6.1 ng/ml at the 2 hours post treatment, whilst the highest mean testosterone concentrations recorded in the oral low-dose, oral high-dose and subcutaneous low-dose groups were 1.02 (at 5 hours post treatment), 1.06 (at 1.5 hours post treatment) and 1.78 ng/ml (at 1 hour post treatment), respectively.

The AUD (area under the data) for the high-dose subcutaneous group was 5-8 times higher for mean testosterone concentration and 3-4 times higher for mean luteinizing hormone concentration when compared with all other groups (both treated and control). When the results for hormone concentrations over time were statistically analysed, no significant differences could be determined between the control group and both oral-dose groups for either hormonal response. The oral bioavailability of azagly-nafarelin was considered to be low.

5. No data on the acute toxicity of azagly-nafarelin were presented. However, such data was not considered necessary as acute toxic effects of azagly-nafarelin were considered unlikely.

6. A GLP-compliant repeat-dose oral toxicity study was performed in the rat. Following an initial pilot study, groups of rats (5 animals of either sex per dose group) were administered azagly-nafarelin by oral gavage for 28 days at dose rates of 0, 25, 250 and 2500 µg/kg bw. No test-treatment related clinical signs or mortality were observed during this study. Slight, though non-statistically significant, increases in food intake and body weight gain were observed at different time points in both males and females treated at the highest dose rate. Statistically significant reductions in the plasma concentrations of sodium and chloride were evident in some animals treated at dose rates of more than or equal to 250 µg/kg bw, whilst significant reductions in plasma potassium and tryglyceride concentrations were also evident at the highest dose rate. Cytological examination of vaginal smears obtained from females during the last two weeks of the study demonstrated that animals treated at the 2500 µg/kg bw dosage level exhibited a reduction in the average number of oestrous cycles and a slight increase in the average duration of each cycle (by approximately 1 day).

   Statistically significant reductions in the absolute weights of the testes, epididymus, seminal vesicles and prostate gland were evident in the high-dose male group. In addition, there was a statistically significant reduction in the relative weight of the seminal vesicles in the mid-dose male group (250 µg/kg bw). Female rats exhibited statistically significant increases in ovarian weights, and significant reductions in uterine weights at the 2500 µg/kg bw dosage level. The increased thymic weights reported in both males and females were not statistically significant.

   The major changes noted on post-mortem examination related to alterations in those organs that also exhibited changes in their weights, as detailed above. An increased incidence of pseudopregnancy was evident in females treated at the 2500 µg/kg bw dosage level. Changes were reported on histopathological examination of various reproductive tissues in both male and female rats treated at the 2500 µg/kg bw dose rate (note: histopathology was only performed on macroscopic lesions in the low- and mid-dose groups). Examination of the testes in high-dose male rats revealed evidence of slight to marked vacuolation of sertoli cells, with a reduction in the number of round and tailed spermatids, spematocytes, tubules lined by sertoli cells only, as well as a variable degree of germ cell degeneration. Oligospermia and hyposecretion of the prostate and seminal vesicles were observed in all male rats treated at this highest dosage rate. Female rats treated at the 2500 µg/kg bw dosage level exhibited evidence of dilatation of the uterine lumen, endometrial epithelial atrophy, abnormal mucification of the vaginal epithelium and an increase in the number of corpora lutea, which correlated with the enlargement in ovarian size. No treatment-related effect on the number of antral follicles per ovary was evident. A mild degree of lymphoid cell hyperplasia was evident in the thymus of some rats treated at the highest dose level. Based on the presence of a statistically significant reduction in the relative weight of the seminal vesicles in male rats treated at the 250 µg/kg bw dosage level, a NOEL of 25 µg/kg bw was retained from the results of this study. As the NOEL value retained in this study was approximately the same as the recommended therapeutic dose in *Salmonidae*, this study served to underline the low oral bioavailability of azagly-nafarelin.

7. A tolerance study was performed in the Rainbow trout. Azagly-nafarelin was administered on a single occasion by intraperitoneal injection at dose rates of 20, 40, 80 and 120 µg/kg bw. These dose levels correspond to slightly greater than 0.5, 1, 2 and 3 times the recommended therapeutic dose. There was no mortality following treatment. Treatment did not appear to have any adverse effects on behaviour, nor was there any significant increase in either internal or external lesions in the fish. No significant difference in fecundity was evident between treated and control fish. The egg quality did display a tendency to decrease at the two highest dose levels. Treatment with azagly-nafarelin did not induce any weight loss in the test fish. Histopathological examination of a representative number of fish per group did not reveal any evidence of treatment-related lesions in various internal organs.

8. No data on the reproductive toxicity or teratogenic potential of azagly-nafarelin were presented, other than the information contained in the 28-day repeat dose oral toxicity study in the rat that was previously reported upon. No additional data was considered necessary.

9. No studies on the mutagenic or carcinogenic potential of azagly-nafarelin were presented. In light of the absence of any structural alerts for this family of compounds, no studies were deemed necessary.

10. No information was available on the immunotoxicity of azagly-nafarelin. However, such data was not deemed necessary, as azagly-nafarelin is unlikely to possess any immunotoxic effects.

11. No information was available on the microbiological properties of residues of azagly-nafarelin. However, bearing in mind the structure of the molecule, it is not expected that any antimicrobial activity would be present.

12. Using the NOEL value of 25µg/kg bw from the repeat-dose oral toxicity study conducted in the rat, and applying a safety factor of 100, allows for the establishment of an ADI of 0.25 µg/kg bw (i.e. 15 µg/person) for azagly-nafarelin.

13. No data on residue depletion studies following the administration of azagly-nafarelin to *Salmonidae* were presented. However these data were regarded as not necessary because the oral bioavailablity of azagly-nafarelin was concluded to be low and treated fish are unlikely to be sent for slaughter during or immediately after treatment.

14. No data were available in relation to the residues of azagly-nafarelin in fish eggs.

**Conclusions and recommendation**

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

- an ADI of 0.25 µg/kg bw (i.e. 15 µg/person) was established for azagly-nafarelin,
- the oral bioavailablity of azagly-nafarelin is low,
- in *Salmonidae*, azagly-nafarelin was quickly depleted from plasma,
- treated fish are unlikely to be sent for slaughter during or immediately after treatment;

the Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for azagly-nafarelin 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 |
|---|---|---|
| Azagly-nafarelin | *Salmonidae* | Not for use in fish from which eggs are produced for human consumption |