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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

LEVOMETHADONE

SUMMARY REPORT

1. Levomethadone (synonym (R)-6-(dimethylamino)-4,4-diphenyl-3-heptanone, CAS No 125-58-6) is a lipophilic basic synthetic opioid analgesic. Levomethadone is the (-)-R-enantiomer or the optical *l*-enantiomer of the racemic *dl*-methadone. Levomethadone is used in combination with fenpipramide (an atropine-like substance) for pain relief, anaesthesia premedication and surgical anaesthesia in horses. Intravenous doses of 0.1 to 0.15 mg/kg bw are recommended.

   In human medicine it is used as analgesic at oral doses of up to 7.5 mg/person or intravenous doses of 2.5 mg/person. Dosing may be repeated 3 to 4 times, if necessary. Levomethadone is also used in the substitution therapy of opiate addicted patients.

2. Levomethadone is the pharmacologically active principle of the racemic *dl*-methadone. Its sterical configuration is resembles that of morphine. Levomethadone, like morphine, acts by binding to the μ-opiate receptors. Its analgesic action is comparable to that of morphine, accompanied by sedation, euphoria, respiratory depression and miosis.

   Other substance-specific secondary effects include bradycardia, hypertension, constipation and antidiuresis. Tolerance to levomethadone develops after repeated administration as demonstrated by a decrease in analgesic response and respiratory depression. When taken for prolonged periods of time levomethadone can cause dependency. The pharmacological potency of levomethadone in comparison to *d*-methadone and the racemate varies from species to species and according to the nature of the measured effect. Levomethadone has been shown to be more potent by a factor of up to 3 compared to the racemate in inducing effects like miosis and respiratory depression in humans. The *d*-enantiomer possesses only 1/10 to 1/50 of the analgesic activity of the levomethadone. Studies in rats showed that single doses of intraperitoneally administered levomethadone affected operant behaviour (decreased response rates) and spontaneous locomotor activity in a dose-dependent manner. Doses with no observed effects obtained from dose-response curves were in the range of 0.1 to 0.3 mg/kg bw. Analgesic action in mice was found to begin at subcutaneous doses greater than 0.1 mg/kg bw. For the racemic mixture of methadone a biphasic effect on hepatic drug metabolism in mice has been observed: single doses (30 mg/kg bw) inhibited metabolism while repeated oral doses of (50 mg/kg bw, twice daily for 3 days) induced hepatic microsomal enzyme activity.

3. Pharmacokinetic data have been obtained mainly using racemic methadone. There is however some evidence that the pharmacokinetic behaviour of the racemate and the individual isomers is comparable. In humans and laboratory animals, methadone is well absorbed after oral administration (approximately 80%). In humans, after single doses of approximately 0.1 to 1 mg/kg bw peak plasma concentrations are found after 1 to 5 hours. Plasma protein binding is up to 90% and the volume of distribution is relatively large (3 to 4 l/kg). Highest tissue concentrations are found in liver and lung followed by kidney and brain. Methadone crosses the placenta and is also detected in milk. Elimination half-lives in plasma of healthy volunteers were variable and ranged between 8.5 and 47 hours. In horses receiving a single intravenous injection of 0.15 mg/kg bw of ^{14}C-labelled levomethadone the terminal half-life of elimination of radiolabelled levomethadone was estimated with about 35 to 40 hours.

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Methadone is extensively metabolised, mainly in the liver. More than 30 metabolites have been identified in human and rat urine and bile. The major metabolic pathway is by N-demethylation followed by cyclisation to form substituted pyrrolidines and pyrrolines. The primary metabolite in excreta of humans and rats was 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, which is further metabolised to 2-ethyl-5-methyl-3,3,-diphenyl-1-pyrroline. Both compounds are pharmacologically inactive. Two minor, pharmacologically active, metabolites, methadol and nor-methadol comprise only 2% of urinary and faecal metabolites and were reported to be derived only from *d*-methadone. After single doses in man, the parent compound comprises 66% of substances in the 24-hour-urine, but only 30 to 40% after maintenance administration. In rats, parent compound is found at lower amounts (up to 10% of the dose). Levomethadone is excreted both in urine and faeces. The excretion pattern between bile and urine can vary according to dose, differences in liver and kidney function, and pH of urine. The higher the dose, the more appears to be excreted via bile.

4. The short-term toxicity of levomethadone in dogs is characterised by LD_{50} values of 75 mg/kg bw after oral, 50 mg/kg bw after subcutaneous, and 26 mg/kg bw after intravenous administration. For rats, an intravenous LD_{50} of 17 mg/kg bw and an oral LD_{50} of 86 mg/kg bw were reported. In mice an oral LD_{50} of 70 mg/kg bw and an intraperitoneal LD_{50} of 35 mg/kg bw were found. In studies comparing the enantiomers, levomethadone was 2 to 4 times more toxic than the *d*-isomer and 1 to 1.5 times more toxic than the racemate.

5. Several repeated dose toxicity studies were available for methadone but most of them did not meet current standards. There were no specific studies on levomethadone.

   In rats, dietary doses of 0, 100, 500, 1000, 2500, 5000, or 10 000 mg/kg feed (approximately 5 to 500 mg/kg bw) for a period of 100 days (6 animals per sex and dose) led to high mortality in females of the two highest dose groups and in males of the highest dose group. Histopathological findings were pneumonia associated occasionally with patchy atelectases in the lungs. There was a decrease in body weight gain in all dose groups (only marginal at the lowest dose). No NOEL can be retained from this study due to effects at all dose levels.

   Subcutaneous injections of 4 mg/kg bw daily for 10 weeks to 12 rats led to reduced body weight gain, increased liver to body weight ratios and irritation at the injection sites.

   Several investigations in rats were reported, which aimed at the examination of specific endpoints like changes in neurotransmitters and brain development and effects on certain enzymes and their developmental changes. Subcutaneous doses were used, mostly 5 mg/kg bw or higher. Depending on the age of the animals at study begin (neonates, 10 days old, 60 days old or older) repeated doses of methadone led to decreased body weights, decreased brain weights, reduced ornithine decarboxylase activity and polyamine concentrations of the heart and partly the brain, increases in adrenal tyrosine hydroxylase, and dopamine-ß-hydroxylase in pups treated from the first days of live. Additionally, methadone, when delivered to the test animal via the milk of their mothers, significantly suppressed the number of a2-receptors of the cortex at one year of age. No NOELs could be elaborated in respect to these changes.

   In mice receiving dietary levels of methadone corresponding to approximately 0, 15, 30, or 60 mg/kg bw (10 animals per sex and dose) for 90 days, dose-related central nervous system stimulation (hyperactivity, noise sensitivity) in females and in high dosed males, together with increased fighting activity were observed. Female mice consumed more food at all dose levels, without significant body weight gain changes in comparison to control. Histopathology did not reveal compound related effects. No NOEL can be derived due to effects at all dose levels.

   Female mongrel dogs (2 animals per dose, no control group) were treated subcutaneously with daily doses of 2 or 5 mg/kg bw for 100 days. One of the 2 dogs receiving the higher dose died. Animals were depressed and 1 dog at 2 mg/kg bw and both at 5 mg/kg bw lost weight. Kidney and liver function stayed normal. A slight decrease of red blood cell count and haemoglobin concentration as well as slight hypochromic microcytic anaemia were observed in 1 animal receiving 2 mg/kg bw and in both at the higher dose. A dose-dependent hyperglycaemic response was noted. Histopathology revealed no substance-related effects. No NOEL can be derived, due to poor reporting and inadequate study design.

Rhesus monkeys (3 males, 1 female) received daily subcutaneous injections of methadone for 75 to 94 days at an initial dose level of 5 mg/kg bw, which was increased stepwise to the highest tolerated dose of 11 to 13 mg/kg bw by 24 to 26 days. The animals showed severe depression, peaking 90 minutes after administration and lasting for 4 to 5 hours. Mydriasis (in contrast to miosis seen in the dog, the rat and man), salivation, lacrimation, muscular weakness and severe respiratory depression were seen regularly. No NOEL can be derived from this study.

6. Long-term toxicity studies were performed in rats and dogs for which only short reports were available. Daily methadone doses of 0, 5, 10, or 20 mg/kg bw were administered per gastric intubation to rats for 80 weeks (animal numbers not given). A recovery period of 8 weeks was included. The doses were gradually increased to reach the final doses. Methadone was tolerated well, and up to 10 mg/kg bw no pharmacological or behavioural effect was noted. Beginning at 15 mg/kg bw some animals of the highest dose group exhibited stupor like states with muscle rigidity and loss of righting reflex. Only hepatocellular hypertrophy was found at the histopathological examination in several males and females of all treated groups, but disappeared within the recovery period. No NOEL can be derived due to inadequate reporting of the study.

   In a one-year toxicity study in dogs methadone was administered in capsules to groups of 8 male and 8 female dogs per dose group at daily maintenance doses of 0, 5, 10, or 20 mg/kg bw (doses were gradually increased to the final level). A recovery period of 7 weeks was included for 2 males and 2 females per dose group. Reduction of body weight gain was seen in all dose groups and was especially marked in females. Dose-related sedation was noted at all dose levels and lasted from 1 to 5 hours. Excessive salivation and emesis after dosing were seen in some animals receiving 10 mg/kg bw and more. At these levels some animals also showed hyperirritability, which was manifested by aggressive behaviour and frequent fighting, resulting in injuries. Serum alkaline phosphatase levels were slightly increased in 6 males dosed with 20 mg/kg bw, but values were within the normal range. Serum calcium was slightly elevated in females receiving the highest dose. Electrocardiograms were taken periodically during treatment and at 4 weeks of recovery. Dose-related changes (increased heart rate, increased ventricular complex) were seen at all dose levels. Changes subsided within the recovery period. At the highest dose the changes in the electrocardiogram were still discernible after 6 weeks recovery. No histopathological changes of the heart were noticed at sacrifice. After 6 and 12 months on treatment, no abnormalities in organ weights, pathology or histopathology were noted at any dose level.

7. No specific information on reproduction toxicity has been provided for levomethadone. Various data on reproductive effects have however been reported for methadone. It was found that methadone decreased libido, testosterone levels, weights of seminal vesicles and prostate in male rats given repeated subcutaneous doses of 5 to 10 mg/kg bw twice daily. Single parenteral doses on day 8 and 9 of gestation, respectively, cause congenital deformities, mainly of the central nervous system and the spinal cord, in hamsters (31 to 185 mg/kg bw*)* and mice (approximately 20 mg/kg bw*)*. These effects were counteracted by concomitant treatment with naloxone.

   In several studies in rats and rabbits, embryo- and foetotoxic effects of methadone were observed beginning at doses of 5 mg/kg bw in rats and of 10 to 20 mg/kg bw in rabbits when given orally on gestational days 6 to 16 or 18, respectively. Methadone did not have significant teratogenic effects in these studies. In other rabbit studies, however, a slight increase in incidence of malformations was seen beginning with the oral dose of 3 mg/kg bw. The effect was however not found to be correlated to the dose level. Delayed parturition has been observed in rat dams, receiving methadone. Doses without effects have not been identified in the studies presented.

   After pre- and/or postnatal exposure of rats to methadone by treatment of their dams the main changes seen in pups were body, heart and brain weight reduction, reduced levels of brain ornithine decarboxylase activity as well as synaptosomal uptake of 5-hydroxytryptamine, dopamine, and norepinephrine in the brains. Norepinephrine uptake in synaptic vesicles was diminished. Decreased adrenal catecholamine levels and adrenal tyrosine hydroxylase activity was noted. Some recovery occurred by 3 weeks of age. Reduced numbers of δ– and μ–opiate receptors in the hypothalamus and cerebral cortex and of cerebral a2–receptors were found at one year of age.

Pre- and postnatal treatment of female and male rats with methadone was reported to affect developmental parameters in the following generation, which itself was never exposed. Subcutaneous methadone treatment of pregnant rats resulted in a significant decrease in male foetal blood testosterone and D^4-androstenedione levels. Maximal response was reached at the lowest dose of 5 mg/kg bw with higher doses not leading to significantly more severe decreases. These effects were antagonised by administration of naloxone at the same time to the pregnant animals. No corresponding effects were seen in female foetuses.

8. No NOELs for repeated dose toxicity and developmental toxicity could be retained because effects were noted at all doses tested.

9. No specific data have been submitted on the mutagenicity of levomethadone. Information was only available for methadone. No mutagenic effects have been encountered in the *Salmonella*-microsomal assay with and without metabolic activation. Weak activity was reported in the *Escherichia coli* DNA repair assay at high concentrations. Borderline mutagenicity was reported for assays with L5178Y mouse lymphoma cells and more definite in *Neurospora crassa*. A sex-linked recessive lethal assay in *Drosophila melanogaster* gave negative effects, as did a test using forward gene conversion in *Saccharomyces cerevisiae*. A dominant lethal test in mice showed positive results at intraperitoneal doses of 1 to 6 mg/kg bw, while a comparable test in rats (prolonged administration via feed at daily doses of 10 and 20 mg/kg bw, given subcutaneously) was negative. On the other hand, paternal effects on pregnancy outcome of untreated female rats have been found after treatment of male rats some hours before mating. *In vivo* metaphase analysis of the chromosomes of mice treated with 5 to 50 mg/kg bw produced a positive response. There was a dose-related increase in chromatid breaks, centric fusion and gaps. A statistically significant increase in the percentage of micronuclei in polychromatic erythrocytes of mice treated orally at a dose level of 25 mg/kg bw, but not at 50 mg/kg bw was noted. Methadone did not induce significant numbers of chromosome aberrations in rat bone marrow cells when tested at doses of 7 to 72 mg/kg bw (route not given). Several cytogenetic studies have been performed in humans on maintenance methadone schedule. Equivocal effects were observed. No exact data for any of the assays were available. It can be concluded that methadone has shown some genotoxic activity in *in vitro* assays, it appeared to be mutagenic in *in vivo* assays in mice but not in rats. Final conclusions regarding the genotoxic potential cannot be drawn at present.

10. In a 2-year carcinogenicity study groups of 50 Fischer 344 rats per sex and dose were treated with methadone, as the hydrochloride, at dietary levels corresponding to 0, 16.1, or 28 mg/kg bw in males and 0, 45.8 or 88.1 mg/kg bw in females. High dosed males displayed hyperactivity and hypersensitivity, to which tolerance developed rapidly. Similar behaviour was sporadically observed in high dosed females. Males and females had depressed growth rates, which were significant in the second year of study. High dosed females showed significantly increased food consumption. Mortality was not significantly affected by any dose. Significantly increased incidence of non-neoplastic lesions like thyroid hyperplasia was noted in females of both dose groups and uterus hyperplasia in the low dosed animals. Increased incidence of adrenal angiectasis was noted in males of both treated groups. Eosinophilic cytoplasmatic changes in the liver were noted in high-dosed males and in females of both dose levels. No significant substance-related change in the incidence of any type of tumour or total tumour incidence was found in the rats.

    Groups of 50 male and 50 female B6C3F1-mice were treated with methadone in form of the hydrochloride at dietary levels resulting in 0, 15.4, or 60.3 mg/kg bw for a period of 2 years. Dose-related hyperactivity and fighting appeared in treated groups, mainly among males. Tolerance to central nervous system stimulation developed rapidly, but sporadic fighting occurred in 20% of low dose males throughout treatment. Food intake was increased in female mice at both dose levels whereas it was more common in males of the low dose level. Mortality was not significantly affected by any of the doses of methadone administered. No significant substance-related changes in the incidence of any type of tumour or total tumour incidence was found in treated animals, with the possible exception of a significant increase in pituitary adenoma in low dosed females. Significantly increased incidences of thyroid hyperplasia were noted in the high-dosed males and in females of both dose levels. The increased incidence of testicular degeneration did not reach significance.

It can be concluded that administration of methadone at the above dose levels and conditions to rodents did not lead to a significant increase in tumour incidence. No NOELs for any other effects could be derived, as the lowest doses showed effects.

11. Information on sensitising potential or other effects of levomethadone or methadone have not been submitted.

12. For humans the following observations were reported. The analgesic action of levomethadone has a duration between 4 and 8 hours. The maximum of the respiratory depression is observed at 4 hours post administration but some effect can be observed up to 75 hours. Miosis was noticed up to 82 hours after a single oral dose of 7.5 mg (approximately 110 µg/kg bw). Occasional deaths have been observed after repeated doses of about 1 mg/kg bw in drug addicts during the first days of maintenance programmes, which partly may be caused by a lack of opiate tolerance and/or by liver damage resulting in accumulation of methadone in blood and brain. Reduced libido and decreased serum testosterone levels (on average by 43%) are observed in humans maintained on methadone. Threshold doses have not been elucidated. In babies born to opiate-tolerant women given methadone at oral dose levels of 0.5 to 2 mg/kg bw, severe withdrawal symptoms, prolonged developmental retardation and behavioural changes are observed. A consistent effect in several of these studies has been small head circumference (below the 3rd percentile). Overt brain malformations appear to be rare or absent, but a threshold dose for physical and behavioural developmental disturbance has not yet been determined. In some studies a positive correlation between methadone dose per person and birth weight was observed. The mechanism of this effect stays obscure.

13. From the repeated dose toxicity studies no toxicological NOEL could be retained. However, pharmacological effects of levomethadone were observed at considerably lower doses than those tested in toxicology studies. Owing to the nature of the substance, it seems appropriate to base safety considerations on the pharmacological potential of the substance. First pharmacological effects of levomethadone are seen at doses as low as 100 µg/kg bw (e.g. analgesic action, operant behaviour, locomotor activity) in rats and mice. In humans lowest oral doses of *dl*-methadone reported to be used therapeutically are even somewhat below these values and range from 40 to 80 µg/kg bw (2500 to 5000 µg/person). Keeping in mind that the *l*-isomer has a 2 to 3-fold greater potency than the racemate it can be estimated that a dose of approximately 1000 µg levomethadone per person could still exert some pharmacological action in naive, i.e. non-tolerant adults. As these figures appear to represent low effect doses, it was not possible to finally conclude on a pharmacological ADI.

14. A residue depletion study has been conducted in 3 horses given ^{14}C-levomethadone at the recommended dose of 0.15 mg/kg bw. Animals were slaughtered 24 hours (2 horses) and 72 hours (1 horse) post dose. At 24 hours highest total residue concentrations were observed in the internal organs liver (352 µg equivalents/kg) and kidney (117 µg equivalents/kg). Fat (9 to 2 µg equivalents/kg; subcutaneous or retroperitonal) and muscle (6 µg equivalents/kg) contained comparatively low concentrations of total residues. At 72 hours after dosing residue concentrations were 224 µg equivalents/kg in liver, 27 µg equivalents/kg in kidney, and in fat and muscle close to or at the limit of quantification (1.9 µg equivalents/kg).

15. Total drug derived residues in the standard food package amounted to approximately 40 µg at 24 hours and 20 µg at 72 hours post dose. However, these figures represent total radiolabelled residues. The major metabolites of levomethadone were found to be pharmacologically inactive. It can therefore be assumed that only a fraction (i.e. parent drug) of these total residues are relevant to consumer safety. Although levomethadone residues were found in liver up to 72 hours after treatment, it was noted that horse liver is not normally considered a food commodity in the European Union.

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:

- levomethadone is intended for intravenous treatment of horses only,
- levomethadone is used in a small number of individual animals for single treatments,
- levomethadone is extensively metabolised and major metabolites have been found to be pharmacologically inactive in experimental animals and humans;

the Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for levomethadone in horses 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
Levomethadone	Equidae	For intravenous use only