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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

HYDROCHLOROTHIAZIDE

SUMMARY REPORT

1. Hydrochlorothiazide (6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide CAS No 58-39-5) is a benzothiadiazide derivative with diuretic and antihypertensive properties. The substance is intended for treatment of oedema in cattle. The recommended daily dose is 0.5 to 6 mg/kg bw for oral administration and 1 to 2 mg/kg bw for the intravenous route for a maximum of 3 days.

   The substance is also used in human medicine in a range of daily oral doses of 25 to 100 mg per person (0.4 to 1.7 mg/kg bw) .

2. Benzothiadiazide derivatives exert their diuretic effect at the distal convoluted tubule and collecting duct. Although the exact mechanism is not fully understood, there is some evidence that the effect of diuresis is due to the specific inhibition of the chloride-dependent sodium transportation in the distal tubule, resulting in a decreased reabsorption of sodium and excretion of urine with increased concentrations of sodium, chloride, magnesium and potassium ions. Furthermore, the reabsorption of water in the collecting ducts is inhibited. The weak inhibitory action of hydrochlorothiazide on the carbonic anhydrase in the proximal tubule has no therapeutic relevance.

3. In a pharmacokinetic study in cattle, 5 animals were treated orally with approximately 3.5 mg hydrochlorothiazide/kg bw and intravenously with 2 mg/kg bw. Plasma data after intravenous administration showed a triphasic kinetic with a short terminal elimination half-life of 5 hours. The mean volume of distribution was low (0.7 l/kg) indicating essentially extracellular distribution. The total body clearance was high (133.6 ml/h) with a very short mean residence time of approximately 1.5 hours. After oral administration hydrochlorothiazide could only be quantified in plasma of 1 of 5 cows. In humans, about 70% of an orally administered dose is absorbed. Peak plasma levels are reached approximately 2 to 3 hours after dosing. Hydrochlorothiazide is bound to plasma proteins to about 40%. The terminal elimination half-life is 9.5 hours. Hydrochlorothiazide does not undergo significant metabolism. About 4% of the dose was converted to 2-amino-4-chloro-m-benzenedisulfonamide. Urinary recovery of unchanged compound averages 70% after oral administration and over 90% after intravenous administration.

4. In mice the LD_{50} values of hydrochlorothiazide were reported to be 3080 mg/kg bw or greater than 8000 mg/kg after oral administration and 590 mg/kg bw after the intravenous route.

5. Short-term repeated dose toxicity studies were conducted in Fischer 344/N rats and B6C3F_1 mice (5 males and 5 females per dose and species) receiving diets containing 0, 3125, 6250, 12 500, 25 000 or 50 000 mg hydrochlorothiazide/kg feed for 15 consecutive days (approximately 160 to 2500 mg/kg bw in rats and 450 to 7150 mg/kg bw in mice). Mean body weight gains of both male and female rats and of male mice in all treated groups were 5 to 14% lower than those of the controls. Feed consumption in rats of the 25 000 and 50 000 mg/kg feed groups was reduced during the first week. A slight to moderate dose-dependent thymic haemorrhage was noted in 1 to 3 rats of each sex in the top three dose groups. Calculi were found in the urinary bladder in 1 mouse of each sex in the second highest dose group and in 2 mice of each sex in the top dose group. A NOEL could not be established.

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6. A 13-week toxicity study was carried out in $B6C3F_1$ mice. Groups of 10 males and 10 females were fed diets containing 0, 3125, 6250, 12 500, 25 000 or 50 000 mg/kg feed (approximately 445 to 7150 mg/kg bw). Death occurred in 7 males and 1 female receiving 50 000 mg/kg feed, in 2 males at 25 000 mg/kg feed, 3 females at 12 500 mg/kg feed and 1 male and 3 female at 3125 mg/kg feed before the end of the study. No deaths occurred in the groups receiving 6250 mg/kg feed, in males at 12 500 mg/kg feed and in females at 25 000 mg/kg feed as well as in controls. Except for the high dosed males, death of animals were considered not to be compound related (no increased mortality of $B6C3F_1$ mice occurred in a 2-year carcinogenicity study at doses of up to 5000 mg/kg feed). Mean body weights were 11% lower in high dosed male mice and up to 24% lower at the top three dose levels in female mice. Compound related effects included nephrosis and hydrochlorothiazide-calculi in the urinary bladder in the top three dose groups. Inflammation and/or epithelial hyperplasia in the urinary bladder were found in male mice of the top three dose levels and in female mice of the top four doses but not in the low dose group. Though the overall interpretation of this study is partly flawed by the death of several animals, it may be retained that substance related effects like increased nephropathy and urinary bladder lesions were not observed at a dose of approximately 445 mg/kg bw (3125 mg/kg feed).

   A 13-week repeated dose toxicity study was conducted in Fischer 344/N rats. Groups of 10 males and 10 females were fed diets containing 0, 3125, 6250, 12 500, 25 000 or 50 000 mg hydrochlorothiazide/kg feed (approximately 160 to 2500 mg/kg bw). No death occurred in any group. Final mean bodyweights were 7 to 16% lower in all dose groups. Mineralisation in the kidney was observed in all dosed rats. Nephrosis was found in 1 male and 5 females of the high dose group.

   Because of mineralisation in kidney at all dose levels, an additional 13-week oral toxicity study in rats (10 animal per sex and dose) was performed using considerably lower doses ranging from 250, 500, 1000, 2000 and 4000 mg/kg feed (12.5 to 200 mg/kg bw). Again, a dose related incidence of mineralisation in the kidney was observed in all dose groups but this effect was judged to be only minimal to mild at the lowest dose tested (4 males and 5 females at 250 mg/kg feed, 8 males and 8 females at 500 mg/kg feed, 10 males and 10 females at 1000, 2000, 4000 mg/kg feed). The dose of 12.5 mg/kg bw may be retained as a LOEL

   A 1-year oral toxicity study (interim kill of a 2 year feeding study) was carried out Fischer 344/N rats (10 animal per sex and dose) using dietary doses of 0, 250, 500 or 2000 mg/kg feed. One of 10 females of the high dose group died before the end. Nephropathy occurred in all dose groups and also in controls but the average severity appeared to be greater in the treated males and in female rats of the high dose group. Mild focal renal mineralisation was noted in the mid and high dosed males and all dosed females. A NOEL was not established.

7. In dogs, daily doses of 50 to 200 mg hydrochlorothiazide for up to 9 months have been reported to cause enlarged and hyperactive parathyroid glands (experimental details were not available).

8. Hydrochlorothiazide was administered in corn oil per gavage to pregnant CD Sprague Dawley rats (0, 100, 300, or 1000 mg/kg bw/day) and CD outbred albino mice (0, 300, 1000, or 3000 mg/kg bw/day) on days 6 through 15 of gestation. On day 20 of gestation 36 to 39 rat dams per dose group and on day 17 of gestation 20 to 27 mouse dams per dose group were evaluated.

   In rats dams clinical signs including piloerection, single day weight loss, alopecia, diarrhea, chromodacryorrhea were observed but were not dose-related. Maternal body weight on day 20 of gestation, maternal body weight gain during gestation and corrected maternal weight gain exhibited a decreasing trend. Maternal body weight during treatment decreased in the dosed groups and was significantly depressed at the high dose group. The relative maternal kidney weight of dosed rat dams was slightly (7-8%) increased above controls, but there was no evidence of a dose response relation. Apart from the effects, which are coherent with the clinical findings observed after the administration of high doses of hydrochlorothiazide, there were only mild signs of maternal toxicity in form of significantly depressed maternal body weight gain during gestation in the high dose rat dams.

   In mouse dams clinical signs including dehydration, single day weight loss, lethargy, piloerection were noticed. Lethargy and piloerection appeared to be dose-related. No effect on maternal weight gain or water consumption, gravid uterine weight, relative maternal liver weight, or relative maternal kidney weight was observed.

No dose related embryo- or foetal toxicity and no significant increases of malformations were found in rats and mice. It was concluded that hydrochlorothiazide does not produce teratogenic effects in the offspring of rats and mice at the highest doses tested. In mice, a NOEL for maternotoxicity can be set at 3000 mg/kg bw orally. The NOEL for maternotoxicity in rats can be set at 300 mg/kg bw based on decreased body weight gain during treatment in the high dose group.

The teratogenic potency of hydrochlorothiazide was studied in humans in a series of 50 282 women, 107 of which had been exposed to hydrochlorothiazide during the first trimester of pregnancy. There were nine malformed children in the exposed group, giving a non-significant standardised relative risk.

A 2-generation study was not provided but is not considered necessary owing to the long period of use in human medicine without indication of substance related developmental or teratogenic effects.

9. *In vitro* the mutagenic potency of hydrochlorothiazide was investigated in the *Salmonella typhimurium* gene mutation assay with and without metabolic activation using the strains TA 98, TA 100, TA 1535, TA 1537. Equivocal mutagenicity (weakly positive in 1 out of 3 experiments) was recorded only with the strain TA 98 without S9 mix. Furthermore two other reported *Slamonella*-microsomal assays in *Salmonella typhimurium* gave negative results. However, the products of hydrochlorothiazide resulting from reaction with nitrite in acetic acid solution were positive with or without metabolic activation in *Salmonella typhimurium* strain TA 98. The substance did not induce reversion in the arg^- strain Hs30R of *Eschericia coli,* whereas irradiation of the strain with near-ultraviolet light in the presence of hydrochlorothiazide caused mutation. In a reported spot test, hyrochlorothiazide induced nondisjunction and mitotic crossing over in *Aspergillus nidulans.* However, it must be noted that the assay was poorly documented. An alkaline elution assay in rat hepatocytes was negative. A mouse lymphoma assay was positive in the absence of exogenous metabolic activation at doses above 500 µg/ml. The observed effects were only discernible at cytotoxic concentrations. Hydrochlorothiazide induced sister chromatic exchanges in cytogenetic assays in Chinese hamster ovary cells, but no chromosomal aberrations with and without metabolic activation were detected. Chromosomal aberrations were also not found in Chinese hamster lung cells at doses up to 500 µg/ml with and without metabolic activation, but polyploidy was observed 48 hours after treatment.

   In vivo the mutagenic potential of hydrochlorothiazide was evaluated in a micronucleus test in Chinese hamsters (up to 4000 mg/kg bw), a spermatogonia assay and a dominant lethal test in mice (up to 4000 mg/kg bw). All assays were negative. An *in vivo* sister chromatid exchange assay in Chinese hamsters after treatment with hydrochlorothiazide was also negative. No induction of sex linked recessive lethal mutations was seen in germ cells of male *Drosophila melanogaster* following feeding or injection of 10 000 mg/kg hydrochlorothiazide.

   It was concluded that hydrochlorothiazide has equivocal mutagenic activity *in vitro*. The negative results of the *in vivo* tests indicate that hydrochlorothiazide and its metabolites do not represent a genotoxic hazard.

10. Carcinogenicity studies were conducted in rats and mice: In a reported carcinogenicity study groups of 24 male and female rats were fed with hydrochlorothiazide at 0 or 1000 mg/kg of diet for 104 weeks. The incidence and severity of chronic progressive nephropathy and of lesion secondary to chronic renal disease, polyarteritis and mural thrombosis were increased. No difference in overall tumour incidence or in incidence of tumours at any site was observed between treated and control rats.

A 2-year carcinogenicity study was conducted in Fischer 344/N rats. Groups of 50 males and 50 females received hydrochlorothiazide in the diet for 105 to 106 weeks, with average daily intakes of 0, 11, 23, or 89 mg/kg bw. Body weights were reduced to the same extent in all treated groups: Mean body weights of dosed male rats were 9 to 21% lower than those of controls after one week, and 8 to 25% lower in dosed females after 3 weeks. No significant difference in survival was observed. The severity of nephropathy was increased in dosed groups relative to controls. The incidence of nephric cysts (males: control 4%, low dose 39%, mid dose 54%, high dose 54%; females: 0%, 6%, 8%, and 6%, respectively) and hyperplasia of the transitional epithelium of the renal pelvis (males: 12%, 43%, 52%, 46% respectively; females: 20%, 80%, 80%, 80%, respectively) were also increased. Lesions secondary to the increased renal effects were also noted in the dosed rats. Parathyroid hyperplasia, fibrous osteodystrophy and mineralization of multiple organs all occurred with increased incidences in dosed rats. No increase in either overall incidence or in the incidence of tumours at any site was observed.

A further two year carcinogenicity study was carried out in $B6C3F_1$ mice. Groups of 50 males and 50 females were fed for 103 to 104 weeks with diets containing average daily intakes of 0, 280 or 575 mg/kg bw hydrochlorothiazide. Mean body weights of dosed and control animals were not significantly different. No significant differences in survival were observed. In the livers of male mice of the high dose group, the incidence of focal necrosis was higher than in animals of the control and low dose groups (12% versus 2%). Hepatocellular adenomas and combined hepatocellular adenomas/carcinomas (control 15%, low dose group 20%, high dose group 42%) occurred in male mice with a positive trend, which reached statistical significance at the high dose level. However, the overall incidence of spontaneous hepatocellular neoplasms in the control mice of the experiment was considered unusually low compared to a mean incidence of 30±7.6% (range 16 to 60%) seen in more than 2000 male control mice of the strain $B6C3F_1$ in contemporary 2-year studies. The tumor incidence in the high dosed males is also within this range. This may emphasise that the higher incidence in the high dose group is not attributable to a clear substance-related tumourigenic activity. No other histological differences in liver tissue of the different study groups were reported. In this study the evidence for carcinogenic activity of hydrochlorothiazide was judged negative for female mice and equivocal for male mice. The low dose (280 mg/kg bw) may be seen as threshold level in mice.

11. In human medicine, hydrochlorothiazide has been one of the most widely used oral diuretic and anti-hypertensive agents. Side effects include hypokalaemia with muscle cramps, cardiac arrhythmia, hyperglycaemia, and hyperlipidaemia. Electrolyte imbalances may be involved in sudden death of patients suffering electrocardiographic abnormalities. High doses (100 mg/day) of hydrochlorothiazide were associated with greater incidences of sudden death in patients with both high blood pressure and electrocardiographic abnormalities. However, the involvement of hypokalaemia and hypomagnesaemia remains a point of controversy.

    It has been suggested that thiazides can cause primary hyperparathyroidism, which is supported by the parathyroid hyperplasia observed in dogs and rats. Therefore, the reduced calcium ion excretion and the increased potassium ion loss may be in part secondary to the increased parathyroid hormone secretion under therapy.

    Immunological reactions, including severe allergic pneumonitis, photoallergic dermatitis and several types of haematology dyscrasias have been observed in humans treated with hydrochlorothiazide. Neutropenia was reported in several patients with a pattern of onset, which suggested a toxic depression of the bone marrow. Thrombocytopenia, that appears to be immunologically mediated, was also seen after hydrochlorothiazide therapy. In one person, a specific IgM antibody was identified as an antiplatelet factor associated with hydrochlorothiazide-induced thrombocytopenia.

12. Available toxicity studies suggested that a dose-related incidence in kidney mineralisation in rats together with body weight reduction were the most sensitive compound related effects. Both, loss of body weight and organ mineralisation, as a result of increased calcium retention, were considered as being connected with the pharmacological, i.e. diuretic, activity of the compound and a dose of 12.5 mg/kg bw was considered the LOEL for these effect. By using an increased safety factor of 500 an ADI of 0.025 mg/kg bw or 1.5 mg/person was established.

13. Residue depletion studies in cattle tissues were not available. Residues in milk were investigated in a cow receiving an intramuscular dose of approximately 0.5 mg/kg bw on 3 consecutive days. The highest concentration of hydrochlorothiazide in milk was observed at 8 hours post dose with 0.047 mg/l. The proportion of the hydrochlorothiazide dose excreted in milk (milk production of 20 l/day) may be estimated at 0.3%, which is considered very low. In a worst case calculation, based on the volume of distribution, it may be estimated that residue concentrations in edible tissues and milk would be lower then the ADI within 24 hours after treatment.

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 0.025 mg/kg bw or 1.5 mg/person has been established,
- the substance is used in a small number of individual animals, for infrequent and non-regular treatment,
- the animals are unlikely to be sent for slaughter during or immediately after treatment,
- pharmacokinetic data indicate rapid elimination of unchanged hydrochlorothiazide,
- worst case estimations indicate that residues would be lower than the ADI within 24 hours after treatment;

the Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for hydrochlorothiazide 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
Hydrochlorothiazide	Bovine	