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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ACETYLSALICYLIC ACID, SODIUM ACETYLSALICYLATE, ACETYLSALICYLIC ACID DL-LYSINE AND CARBASALATE CALCIUM

SUMMARY REPORT (1)

1. Acetylsalicylic acid (2-(acetyloxy)benzoic acid; CAS Number 50-78-2) is an anti-inflammatory, antipyretic and analgesic agent. It is the standard to which all the other non-steroidal anti-inflammatory drugs (NSAIDs) are compared. This group of drugs is mainly used for treatment of inflammatory musculoskeletal disorders.

   Acetylsalicylic acid, mainly as its sodium salt is used in veterinary medicine orally in water and feed in pigs, calves and chickens at doses of 5 to 300 mg/kg bw/day for periods not exceeding 10 days.

   Acetylsalicylic acid DL-lysine (DL-lysine mono[2-(acetyloxy)benzoate]; CAS Number 62952-06-1) is used in cattle (25 to 100 mg/kg bw/day) and pigs (25 to 50 mg/kg bw/day) twice a day intravenously, subcutaneously or intramuscularly and in calves (200 mg/kg bw/day for 5 to 7 days) orally, twice a day.

   Carbasalate calcium (CAS No 5749-67-7, acetylsalicylic acid calcium-urea) is administered orally in water in pigs, calves and chickens at doses of 40 to 130 mg/kg bw/day for 5 days. It is also administered in milk to calves.

   Acetylsalicylic acid is widely used in human medicine. The dose depends on the condition to be treated: the therapeutic dose for anti-inflammatory, antipyretic and analgesic indications is 300 to 900 mg/person every 4 to 6 hours, with a maximum of 4000 mg/person/day. Several long-term (sometimes life-long) low dose regimes beginning at doses of 50 mg/person are also used for several cardiovascular diseases.

2. The pharmacological activity of acetylsalicylic acid is mainly due to salicylic acid, a metabolite formed after hydrolysis. The effects are linked to the inhibition of the cyclo-oxygenase and the synthesis of the prostanoids from arachidonic acid. They also inhibit the release of PGF_{2a} and PGE_2 from thrombin-stimulated platelets as well as the synthesis of thromboxanes and favour the production of prostacyclin PGI_2. As a result platelet aggregation is inhibited and bleeding time is prolonged. In a placebo-controlled cross-over trial in 19 subjects a daily dose of 10 mg acetylsalicylic acid sodium given for 3 weeks did not increase the bleeding time, although significant inhibition (by 61%) of thromboxane B2-production by platelets was observed. The higher dose of 30 mg/person given daily for 3 weeks resulted in significant prolongation of bleeding time (1.6 times the control values) and inhibition of thromboxane B2-production by 94%. Thus a daily dose of 10 mg/person, equivalent to 0.167 mg/kg bw/day, can be retained as a pharmacological LOEL.

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3. Acetylsalicylic acid is readily absorbed from the gastro-intestinal tract in dogs, cats and pigs (half-life of absorption about 0.6 hours). Absorption is slower in cattle (half-life of absorption 2.9 hours after oral administration of doses between 20 and 100 mg/kg bw) and in horses. After oral administration of 90 mg/kg bw of acetylsalicylic acid DL-lysine to dogs and cattle and of 20 grams of acetylsalicylic acid to horses, t_{max} was between 2 and 4 hours with a C_{max} in dogs of approximately twice that in cows (104 versus 64 µg/ml). The area under the curve (AUC) was 4837 µg/min/ml for cattle and 65 639 µg/min/ml for dogs. The plasma half-life varies from 0.8 hours in the ruminant to 37.5 hours in the cat. After a single intravenous dose of 90 mg/kg bw of acetylsalicylic acid DL-lysine to dogs and cattle the half-life of elimination in cattle was approximately 10 times shorter (36.5 minutes versus 368 minutes in dogs). This is due partly to the low distribution volume in cattle (0.35 l/kg versus 0.64 l/kg in dogs). Serum albumin binding ranges between 50 and 70% in common domestic animals. Thus for cattle the pharmacokinetic parameters are marked by a slower and more incomplete absorption, a low area under the curve and a low volume of distribution, a very quick elimination and thus body clearance.

   In 6 pigs (body weight 25 kg) after a single oral administration of a commercial medicated premix at a dose of 50 mg acetylsalicylic acid/kg bw, absorption was rapid (half-life of absorption 0.9 hours) with a lag time of 0.2 hours. A C_{max} of 64 µg/ml was observed after 3.9 hours. The half-life of elimination was 4.2 hours. After 12 hours the plasma concentration was still 21 µg/ml and after 24 hours the concentrations were below the limit of detection (0.08 µg/ml). The volume of distribution was 0.5 l/kg, and the body clearance was 1.25 ml/min/kg. No pharmacokinetic data are provided on acetylsalicylic acid DL-lysine to be administered by parenteral route in pigs.

   The qualitative metabolism is similar in all animal species studied involving hydrolysis of the parent compound in plasma, liver and some other organs to salicylic acid followed by the formation of salicyluric acid, salicyluric glucuronide, salicyl ester glucuronide, salicyl phenol glucuronide, gentisic acid and gentisuric acid. Few quantitative data on metabolites in plasma and tissues of animals were provided. After intravenous administration of sodium salicylate to goats the excretion of parent compound and salicyluric acid amounted to 67.9% and 34.6%, respectively. After oral dosing the respective figures were 30.2% and 71.7%. In cattle intravenous treatment with sodium salicylate resulted in a lower excretion of parent compound (54%), but a higher fraction of salicyluric acid (49.9%). The same pattern was found after oral administration. In both species almost 90% of the drug excreted as sodium salicylate was found in the urine.

4. Single dose toxicity studies revealed LD_{50} values in mice and rats for acetylsalicylic acid DL-lysine in the range of 2200 to 2600 mg/kg bw by oral route. The clinical signs of toxicity are non-specific: nausea, restlessness, seizures, coma, stimulation of respiration with respiratory alkalosis.

5. Short term repeated dose toxicity studies with sodium acetylsalicylate were carried out in dogs and cats. Gastrointestinal toxicity was studied in cats for 4 weeks with a dose of 25 mg/kg bw without adverse effects. Although no adverse effects were observed, this value could not be accepted as a NOEL due to the poor study design.

   Gastrointestinal toxicity was studied in dogs at doses of 32.5, 65 and 97.5 mg/kg bw acetylsalycilic acid administered twice a day for 7 days; the severity of gastro-intestinal bleeding was dose-related. Nephrotoxicity studies were carried out in the same animal species. In 9 dogs, doses of 7 mg/kg bw acetylsalicylic acid followed by 70 mg/kg bw and 200 mg/kg bw were given intravenously over 4 treatment periods depending on the plasma salicylic acid values. With all the doses there were significant influences on some of the urinary parameters, including decreased sodium excretion, notwithstanding these may be classified as adverse effects of treatment. In dogs no doses were found without side or toxic effects, therefore no NOEL could be derived.

6. Repeated dose toxicity studies were provided in rats and dogs. In rats, equivalent doses of 0, 50, 150 and 500 mg/kg bw of acetylsalicylic acid were given daily as acetylsalicylic acid DL-lysine and 500 mg/kg bw as sodium acetylsalicylate. The highest dose induced severe clinical abnormalities and mortality. No clinical signs were observed at 150 mg/kg bw. Congestion, petechiae, haemorrhages, and punctiform lesions were observed in the stomach at 150 and 500 mg/kg bw. Necropsy examination revealed a dose related hepatomegaly, with no histological expression. Kidney weights were increased in males at all dose levels. A dose related decrease in serum globulins was recorded. This effect was still significant at the lowest dose tested (50 mg/kg bw) in females.

   In dogs, acetylsalicylic acid DL-lysine was administered at doses of 0, 50, 150, 250 and 500 mg acetylsalicylic acid equivalents/kg bw/day for 3 months and sodium acetylsalicylate was administered at doses of 0, 250 and 500 mg/kg bw/day. The highest dose induced rapid mortality and all animals died within 2 to 7 days post treatment initiation. Doses of 150 and 250 mg/kg induced vomiting and mortality. Vomiting were still observed at the dose of 50 mg/kg but at a lower frequency. In this group (n=6), one dog presented gastric striae and two dogs presented focal atrophy of the mucosa with dedifferenciation of the epithelial lining and glandular epithelium. A slight decrease in the heart rate in all of the treated animals was recorded.

   From these repeated dose toxicity studies, no NOEL could be established.

7. Tolerance studies in target animal species were not submitted.

8. Embryotoxicity and foetotoxicity studies in dogs (days 15 to 21, days 23 to 30), mice (treatment period not stated) and rats (days 6 to 15) with doses of 500 to 1200 mg/kg bw orally resulted in a high incidence of stillborns in dogs and of resorption in mice and rats. In the rabbit, inseminated does given 7 doses (150 mg/kg bw) of acetylsalicylic acid prior to implantation showed reduced fertility and abnormal blastocysts. Doses of 40 mg/kg bw given to pregnant Rhesus monkeys (days 25 to term) did not induce anomalies. No fertility studies in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community were provided.

9. In an oral teratology study, rats were treated on days 6 to 15 of pregnancy with doses of 30, 90 and 180 mg sodium salicylate/kg bw. A significant dose-related reduction in foetal weight and significant increases in delayed ossification of the limbs and vertebrae were observed at 90 and 180 mg/kg bw. In the 90 mg/kg bw group, one foetus had anophthalmia and another had generalised oedema together with a malformed tail. 30% of the foetuses in the 180 mg/kg bw group were malformed; the predominant malformation was craniorachischisis affecting 22.7% of the foetuses in this group. The dose of 30 mg/kg bw was without foetotoxicity or teratological effects. In rat *in vitro* studies (embryo mid-brain cells) level lower than 50 µg/ml plasma did not provoke teratogenic effects.

   In rat *in vitro* studies (whole embryos) with levels of 100 µg salicylic acid/ml plasma did not cause teratogenic effects.

   In dogs no teratogenic nor embryotoxic effects were seen with doses of 100 mg acetylsalicylic acid/kg bw; by contrast maternotoxicity, increase in the number of resorption and malformations (including cleft palate, micrognathia, anasarca, cardiovascular malformations and tail malformations) were observed with doses of 400 mg/kg bw.

   Although teratogenic effects were observed in rats and dogs studies, an oral dose of 30 mg sodium salicylate/kg bw could be accepted as a NOEL for teratogenicity.

10. Negative results were obtained in 5 independent bacterial gene mutation assays in both the presence and absence of metabolic activation with sodium salicylate. *In vitro* DNA-repair tests in bacteria and in primary rat hepatocytes also gave negative results. Acetylsalicylic acid did not induce recessive lethal mutations in *Drosophila melanogaster*. Inconsistent results were obtained in *in vitro* metaphase analyses: positive results were obtained in fibroblast and lymphocyte cultures but negative results were obtained in V79 cells both with and without metabolic activation. Negative results were also obtained in a cell transformation assay in mouse embryo cells. Positive results were reported in an *in vivo* metaphase analysis in rat bone marrow. *In vivo* chromosomal aberration assays in rat embryos and *in vivo* micronucleus tests in bone marrow from rats and mice also gave negative results. No evidence of chromosomal damage was found in the lymphocytes of human volunteers administered oral doses of 2.4 g per person per day of acetylsalicylic acid for 1 month. The Committee concluded that the balance of the evidence indicated that acetylsalicylic acid was not genotoxic.

11. No carcinogenicity studies on acetylsalicylic acid were provided. However, its lack of genotoxicity suggests that it would not be active as an initiator of neoplasms.

    Some studies have been performed on the potential of acetylsalicylic acid to promote tumours initiated by other agents. The results of a study of the promotional activity of chemicals on tumours of the rat glandular stomach showed a lower incidence of hyperplasia and tumours of the glandular stomach and duodenal tumors in animals given acetylsalicylic acid (1% in diet for 32 weeks) than in control animals.

    Similarly 0.05 and 1% acetylsalicylic acid in the diet for 58 weeks did not increase the incidence of liver tumours in rats pre-treated with a potent initiator of liver cancer. In contrast, 0.5% acetylsalicylate in the diet for 12 weeks after initiation with a potent inducer of bladder cancer caused an increased incidence of bladder carcinoma.

12. In humans, the reported adverse effects include nausea, vomiting and dyspepsia and more rarely, blood dyscrasias such as thrombocytopenia, aplastic anaemia and agranulocytosis. Acetylsalicylic acid has been associated with haemolytic anaemia in individuals with glucose-6P-dehydrogenase deficiency. Even doses of 300 mg per person (5 mg/kg bw) daily or less carry a risk of peptic ulcer bleeding. Hypersensitivity reactions have also been reported. A dose-dependent tinnitus and hearing loss has been reported with serum acetylsalicylic acid concentrations higher than 110 µg/ml. Reversible hepatotoxicity has been associated with serum concentrations higher than 150 µg/ml. Reye's syndrome (acute encephalopathy and hepatic fatty degeneration, occurring mostly in children) has been associated with ingestion of acetylsalicylic acid in children suffering from viral infection such as varicella or influenza. For this reason the substance is no longer indicated in children, except in cases of juvenile rheumatoid arthritis, in some countries. The occurrence and severity of the disease are linked to the salicylate concentration, which shows a dose-related effect. Data collected in the USA showed that patient with Reye's syndrome were found to have received greater average daily and maximum daily doses of sodium acetylsalicylate and greater doses of sodium acetylsalicylate for the first 4 days preceeding illness (median 25.1 mg/kg bw; 33.0 mg/kg bw; and 65.4 mg/kg bw, respectively) than did controls (median 14.5 mg/kg bw; 19.0 mg/kg bw; and 27.0 mg/kg bw, respectively). The incidence of Reye's syndrome in USA declined sharply after the association of Reye's syndrome with sodium acetylsalicylate was reported. In countries where sodium acetylsalicylate and salicylates are widely used in animals, no increase of incidence has been reported. Reye's syndrome seems therefore to be linked with ingestion of high doses of sodium acetylsalicylate or other salicylates and should be regarded as a dose-dependent phenomenon which is very unlikely to be observed after ingestion of minute doses as residues in food.

    In humans, the widespread use of acetylsalicylic acid for treatment of hypertension during pregnancy at a dose of 150 to 325 mg/person has not been associated with teratogenic effects in the offspring. Acetylsalicylic acid is contraindicated at term because of its inhibition effect on platelet function and its uterine relaxant properties.

The results of epidemological studies are inconsistent with regard to the cancer risk to humans. There was some evidence of an increased risk of renal cancer in people on long-term therapy with acetylsalicylic acid, but a large cohort study of more than 620 000 persons showed only a decreased incidence of colon cancer. There is no information to suggest that the concentrations of acetylsalicylic acid likely to occur as food residues will cause any tumour promotion.

13. In absence of a NOEL from the repeated dose toxicity studies no toxicological ADI could be established for acetylsalicylic acid. A pharmacological ADI of 0.0083 mg/kg bw (i.e. 0.5 mg/person) can be established on the LOEL of 0.167 mg/kg bw/day observed in humans (based on effects on bleeding time and thromboxane B2-production), using a safety factor of 20. This factor resulted from the standard safety factor of 10, when human data form the basis of the ADI, multiplied by a factor 2, which was considered necessary since a LOEL rather than a NOEL was used as basis to derive the ADI.

14. No radiolabelled residue depletion studies in the target species were provided.

15. In 12 pigs (body weight 23 kg), acetylsalicylic acid in a premix was administered orally at the dose of 50 mg/kg bw/day for 10 consecutive days as two daily administrations. Four animals were sacrificed 24 hours, 3 days and 7 days after the end of administration. Tissue were assayed for acetylsalicylic acid and salicylic acid residues. Acetylsalicylic acid was converted to salicylic acid and the results were expressed as total salicylic acid. The mean residue values at 24 hours were 167 µg/kg in muscle, 387µg/kg in liver, 363 µg/kg in kidney and 816 µg/kg in skin and fat. At 3 days, the values dropped below the limit of quantification in muscle. In the liver, the values were 164 and 78 µg/kg in 2 animals and below the limit of quantification for 2 animals. The mean value in kidney was 72 µg/kg in kidney. In skin and fat, the values were 200 and 156 µg/kg in 2 animals and below the limit of quantification in 2 other animals. At 7 days, the values were 58 and 210 µg/kg in 2 animals in muscle and below the limit of quantification in the 2 remaining animals. In the liver, the values were 81 and 51 µg/kg in 2 animals and below the limit of quantification in 2 other animals. In kidney the values ranged from 80 to 100 µg/kg and were below the limit of quantification in one animal. In skin and fat, the values were 194 and 124 µg/kg in 2 animals and below the limit of quantification in 2 other animals. From these data, the daily intake from pig edible tissues would be 160 µg at 24 hours, 25 µg at 3 days and 40 µg at 7 days.

    Residue depletion studies following parenteral administration of acetylsalicylic acid DL-lysine were not performed in pigs.

    In 18 chickens, a water-soluble powder containing carbasalate calcium was administered orally in drinking water at a dose equivalent to 50 mg of acetylsalicylic acid/kg bw/day for 5 consecutive days. Animals were sacrified 24 hours, 3 days and 7 days after the end of administration. Tissue samples were assayed for acetylsalicylic acid and salicylic acid residues. Acetylsalicylic acid was converted to salicylic acid and the results were expressed as total salicylic acid. At 24 hours, the values were below the limit of quantification in muscle (50 µg/kg), liver and kidney, whereas in skin and fat the mean value was 206 µg/kg. At 3 days, the muscle residues were below the limit of quantification in 2 animals and ranged from 83 to 614 µg/kg in 4 animals. In liver, the residue values were 91 and 271 µg/kg in 2 animals and less than limit of quantification in 4 animals. In kidney, residues were only detectable in one animal (399 µg/kg). In skin and fat, the values ranged from 203 to 2468 µg/kg. At 7 days, residue in muscle, liver and kidney were only detectable once in each tissue, whereas values ranged from 82 to 2444 µg/kg in skin and fat. Taking into account the mean values recorded, the daily intake from chicken edible tissues would be 18 µg at 24 hours, 175 µg at 3 days and 52 µg at 7 days.

Twenty cattle aged 8.8 months on average were administered an intramuscular injection of acetylsalicylic acid DL-lysine at the dose of 50 mg acetylsalicylic acid equivalents/kg bw/day, for 2 consecutive days as tw2o daily administrations. Four animals in each group were sacrificed 12, 24, 48, 96 and 240 hours after the last dose. Tissues were assayed for acetylsalicylic acid and salicylicacid residues. Acetylsalicylic acid was converted to salicylicacid and the results were expressed as total salicylic acid. Kidney was the target tissue. Total salicylic acid residues were 765 µg/kg at 12 hours in kidney and dropped to 186 µg/kg at 24 hours. Residues were still detectable at 240 hours (132 µg/kg). In liver, residues were detectable in 2 animals at 12 hours (76 and 217 µg/kg) and were close to the limit of quantification (50 µg/kg) or lower than the limit of quantification at the other sampling times. In muscle and fat, the residues were lower than the limit of quantification (100 and 50 µg/kg respectively) at each time sampling. At the injection site, the mean residues were 659 µg/kg (n=4) at 12 hours and 452 µg/kg (n=3) at 24 hours. An outlier value of 7506 µg/kg was recorded in the remaining animal. At 240 hours, salicylic acid was still detectable in 2 animals (142 and 180 µg/kg). The daily intake from cattle tissues taking into account mean values would be 53 µg and 15 µg at 12 and 24 hours respectively.

Residue depletion study following administration via oral route was not performed in cattle.

16. No residue depletion studies were performed in lactating cows.

17. No residue depletion studies in laying hens were performed.

18. An HPLC analytical method with spectrofluorimetric detection for the determination of residues of acetylsalicylic acid and salicylic acid in tissues is available. Validation data were provided for edible tissues in cattle, pigs and chickens. The limit of quantification was 50 µg/kg for all tissues, excepted for cattle muscle (100 µg/kg). The method was described in the ISO 78/2 format. However no methods for the determination of residues in eggs and milk were provided.

Conclusions and recommendation

Having considered that:

- a pharmacological ADI of 0.0083 mg/kg bw (i.e. 0.5 mg/person) has been established for acetylsalicylic acid,
- the depletion of residues of acetylsalicylic acid, acetylsalicylic acid DL-lysine and carbasalate calcium in cattle, pigs and chicken tissues is rapid,
- residues likely to be ingested by the consumer are below the ADI at all time points, the maximum amounts being 11% from bovine tissues 12 hours after treatment, 33% of the ADI from porcine tissues 24 hours after treatment, 36% from chicken tissues 3 days after treatment,
- no information on residue depletion was available for bovine milk and chicken's eggs;

the Committee for Veterinary Medicinal Products recommends the inclusion of acetylsalicylic acid, sodium acetylsalicylate, acetylsalicylate DL-lysine and carbasalate calcium in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Acetylsalicylic acid	Bovine	Not for use in animals from which milk is produced for human consumption
	Porcine	
	Chicken	Not for use in animals from which eggs are produced for human consumption
Sodium acetylsalicylate	Bovine	Not for use in animals from which milk is produced for human consumption
	Porcine	
	Chicken	Not for use in animals from which eggs are produced for human consumption
Acetylsalicylic acid DL-lysine	Bovine	Not for use in animals from which milk is produced for human consumption
	Porcine	
	Chicken	Not for use in animals from which eggs are produced for human consumption
Carbasalate calcium	Bovine	Not for use in animals from which milk is produced for human consumption
	Porcine	
	Chicken	Not for use in animals from which eggs are produced for human consumption