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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

IMIDOCARB

SUMMARY REPORT (1)

1. Imidocarb is a carbanilide derivative with antiprotozoal activity. It is usually administered as the dipropionate salt. In veterinary medicine it is administered by subcutaneous or intramuscular injection to horses (3.4 mg/kg bw) and cattle (2.1 mg/kg bw). In horses, up to 4 doses may be given at 72-hour intervals. A second dose may be given 2 weeks after the first. The substance is also used in sheep (1.2mg/kg bw) for the treatment of babesiosis and anapolasmosis.

2. The mode of action of imidocarb is uncertain though two mechanisms have been proposed: interference with the production and/or utilisation of polyamines, or prevention of entry of inositol into the erythrocyte containing the parasite. In a general pharmacological screen, mice were given single subcutaneous doses of imidocarb dihydrochloride; myosis was observed at 50 mg/kg bw but mydriasis at 150 mg/kg bw. In another study, cardiovascular and neuromuscular effects, attributable in part to the anticholinesterase effect of imidocarb, were observed following intravenous administration of dihydrochloride to cats and dogs. No overall conclusions could be drawn regarding a pharmacological NOEL.

3. The absorption, distribution and excretion of imidocarb were investigated in mice, rats, dogs, monkeys and in target species. Autoradiography studies in rats using ^{14}C-imidocarb indicated that both the dipropionate and the dihydriochloride salt were poorly absorbed after oral administration to rats but it was not possible to estimate of the extent of the oral bioavailability. Following daily oral administration of 5 mg/kg bw/day imidocarb to dogs and Patas monkeys, residues in muscle and brain were undetectable (below 500 µg/kg) 24 hours after the last dose but significant residues levels were found in liver and kidney.

4. Calves were given a single subcutaneous dose of 3 mg/kg bw ^{14}C-imidocarb dipropionate. Absorption of the dose was rapid with mean peak blood concentrations of 1316 µg equivalent/kg occurring 1 hour after dosing. The concentration of radioactivity remained constant for up to 4 hours after dosing. Thereafter, concentrations declined to 279 µg equivalent/kg, 24 hours after dosing. More than 70% of the radioactivity was bound to plasma proteins. Most of the administered radioactivity was excreted in faeces (39% over 28 days) with smaller amounts in the urine (15%). Renal excretion was essentially complete in about 4 days whereas significant amounts were excreted in faeces for up to 10 days after dosing. The major component of both urine and faeces was unmetabolised imidocarb.

5. In mice dosed intravenously with ^{14}C-imidocarb and killed 3.5 hours later, over 90% of the residues present in liver and kidney was unmetabolised imidocarb. Over 95% of the radiolabelled material in mouse urine was unmetabolised imidocarb. An *in vitro* study using bovine liver slices, isolated hepatocytes and microsomal fractions found no evidence for the metabolism of imidocarb.

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6. The acute oral LD_{50} values of imidocarb dipropionate were in the range of 646 to 723 mg/kg bw in mice and 454 to 1251 mg/kg bw (expressed as imidocarb base) in rats. The dihydrochloride and diacetate salts were of similar acute toxicity. The signs of acute toxicity were generally consistent with anticholinesterase activity and included lethargy, salivation, lacrimation, ataxia, tremors and convulsions.

7. Groups of Wistar rats were given daily oral doses of 0, 125, 250, 750 or 1500 mg/kg bw/day of imidocarb dihydrochloride for 3 months. All rats given 1500 mg/kg bw died. Pathological changes, described as cloudy swelling, were observed in the livers of rats given 125 and 250 mg/kg bw; no histopathology was carried out at higher doses. No NOEL was established. In another study which was designed to identify dose levels for a chronic toxicity study, groups of CR rats were fed diets calculated to provide the equivalent of 0, 26.0, 75.5 or 415.2 mg/kg bw and 0, 32.3, 101.4 or 554.3 mg/kg bw of imidocarb as dipropionate in males and females, respectively. Body weight gain was reduced in animals of both sexes given the top dose but there were no significant effects on haematology, clinical chemistry or urinalysis values. During weeks 7 and 13, groups of 5 rats/sex/dose from the top dose and control groups were killed and acetylcholinesterase activity was measured in one-half of the brains. No effects of treatment were observed. At termination, pathological changes were found in the livers of rats given the top dose; these consisted of mild stasis of the bile in the canaliculi of the liver in 1 male and 4 females. On the basis of reduced body weight gain and liver toxicity, the NOEL was 75.5 and 101.4 mg/kg bw/day in males and females, respectively.

8. Beagle dogs were given daily oral doses of 0, 5, 20 or 80 mg/kg bw/day imidocarb as dipropionate in gelatin capsules for 90 days. All males and 2 out of 4 females given 80 mg/kg bw died or were euthanased. Signs of toxicity in this group included recumbency, salivation, muscle fasciculation, ataxia and splayed legs. Eosinophilia was also observed at 80 mg/kg bw/day, together with increased serum alanine aminotransferase, aspartate animotransferase and bilirubin. Similar but less severe changes were observed in the 20 mg/kg bw group. Kidney, thyroid and adrenal weights were increased at 80 mg/kg bw and pathological changes were found in a range of tissues. In the kidney, fatty changes were observed in the thick section of the loop of Henle and the distal convoluted tubules. In the liver, haemorrhagic necrosis, fatty change and micro-granularity or vacuolation of the hepatocytes were observed. Similar though less severe changes were found in the livers of dogs given 20 mg/kg bw/day. The NOEL was 5 mg/kg bw/day, based in minor changes in haematology and clinical chemistry values and hepatocellular changes. There was no information concerning potential anticholinesterase effects in dogs.

9. Imidocarb had a small therapeutic index in the target species. It was contraindicated for intravenous administration. Signs of toxicity were attributed to the anticholinesterase effect of the substance. In calves, whole blood cholinesterase activities were significantly reduced following an intramuscular injection of 3.3 mg/kg bw imidocarb dipropionate. Maximum depression occurred around 30 minutes after dosing and significant recovery was observed within 6 hours. The cholinesterase depression did not correlate with the intensity of the clinical response.

10. A multigeneration study was initiated as part of a larger study designed to investigate all aspects of reproduction including teratogenicity and to provide weanlings for a 2-year study. Groups of Sprague-Dawley rats were fed diets calculated to provide the equivalent of 0, 45, 128 or 760 mg/kg bw/day for 60 days prior to mating. The numbers of pups born in the F1a and F1b litters of the 760 mg/kg bw group were significantly reduced and there was a similar reduction in the F1b litters receiving the mid-dose. The study was terminated early due to failure of the temperature controls and so no firm conclusions can be drawn. In another study, groups of 20/sex/dose Wistar rats were fed diets calculated to provide intakes of 0, 15, 45 or 135 mg/kg bw/day of imidocarb as dipropionate. Dosing commenced 60 days prior to the first mating and continued throughout the breeding of 3 successive generations. Maternal body weight gain was reduced at 135 mg/kg bw.

The numbers of live births were reduced at this dose level following the first mating of the F0 generation and there was an increase in the number of dead or missing foetuses. A similar trend was observed following the first mating of the F1 generation. The NOEL was 45 mg/kg bw/day.

11. In a teratogenicity study carried out as part of a multigeneration study, rats were fed imidocarb dipropionate continuously in the diet for 60 days prior to mating. In female animals the achieved drug intakes were 0, 47, 138 and 760 mg/kg bw/day of imidocarb. The dams were killed on day 19 of gestation. Maternal body weight gain was reduced in the 138 and 760 mg/kg bw groups and resorptions were increased in a dose-related manner at these doses. Foetal body weight and length were significantly reduced in the 760 mg/kg bw group. There was no evidence of teratogenicity at any dose level. In another study, groups of mated female Wistar rats were given daily by oral gavage doses of 0, 19, 76 or 304 mg/kg bw/day of imidocarb as dipropionate from days 6 to 16 of gestation. Twenty to 25 dams from each group were killed on day 19 and the uterine contents examined; 6 dams from each group were allowed to deliver naturally and rear the offspring. Maternal body weight gain was significantly reduced in the 304 mg/kg bw group. There was no evidence of teratogenicity at any dose level. The numbers of foetuses with bifid or H-shaped sternebrae were increased in the 76 and 304 mg/kg bw groups. There were no treatment-related effects on the growth or survival of the offspring post-partum. The NOELs for maternal toxicity and foetotoxicity were 76 and 19 mg/kg bw/day, respectively.

12. Groups of female Dutch rabbits were given daily oral gavage doses of 0, 20, 80 or 320 mg/kg bw/day from day 8 to 19 of gestation. All does given 320 mg/kg bw and one given 80 mg/kg bw died. The remaining does showed signs of stress (nervous behaviour, diarrhoea and weight loss) which was attributed to their close proximity to a dog colony. Five to six does in each group were allowed to litter naturally but most killed their offspring. There was no evidence of teratogenicity at any dose level. No conclusions could be drawn regarding NOELs for maternal toxicity or foetotoxicity. In another study, groups of female New Zealand White rabbits were given by oral gavage daily doses of 0, 20, 60 or 180 mg/kg bw/day of imidocarb as dipropionate from day 6 to 18 of gestation. All does given 180 mg/kg bw and 12 out of 15 dams given 60 mg/kg bw died. Post-implantation losses were increased, foetal weights were reduced, and there was an increased incidence of foetal delayed ossification in the group given 60 mg/kg bw. There was no evidence of teratogenicity at any dose level. The dose of 20 mg/kg bw/day was the NOEL for both maternal toxicity and foetotoxicity.

13. Negative results were obtained in 3 different *in vitro* assays for gene mutation in *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100 in either the presence or absence of metabolic activation. No increase in revertants was observed in a host mediated assay in which male ICR mice were given 5 daily oral doses of 15, 45 or 150 mg/kg bw/day and the test organism (*Salmonella typhimurium* TA1530 and G-46) was given by intraperitoneal injection 30 minutes after the last dose. Negative results were also obtained in an *in vitro* assay for gene mutation at the HGPRT locus of L5178Y mouse lymphoma cells. Three chromosomal aberration assays were carried out in human peripheral blood lymphocytes. The first study carried out in 1983 gave negative results. In the second study performed according to GLP requirements, treatment in the presence of metabolic activation resulted in significant increases in the frequencies of cells with numerical aberrations. The third study was designed specifically to test for aneuploidy and tested the ability of imidocarb to induce micronuclei in human peripheral blood lymphocytes; there was no increase in the incidence of micronuclei at any concentration tested (range 367 to 1120 µg/ml). In this study slides from a single dose level of 895.5 µg/ml were examined by metaphase analysis for polyploidy; the induction of polyploidy was confirmed at this dose level.

    All *in vivo* assays gave negative results. Negative results were obtained in an *in vivo* cytogenetics assay (bone marrow metaphase analysis) following the administration of 5 consecutive daily oral doses of 10, 30 or 100 mg/kg bw imidocarb to Sprague-Dawley rats. No increase in micronuclei was observed in an *in vivo* micronucleus test in mouse bone marrow in which CD-1 mice were given a single intraperitoneal injection of 8.5, 17 or 34 mg/kg bw imidocarb and the bone marrow harvested 24, 48 and 72 hours after dosing. No evidence of a dominant lethal effect was found in an *in vivo*

dominant lethal assay in which male Evans rats were given 5 daily intramuscular injections of 4 or 16 mg/kg bw/day of imidocarb followed by a 6-week mating schedule. It was concluded that imidocarb was not genotoxic.

14. In a combined chronic toxicity and carcinogenicity study, groups of 50/sex/dose Wistar rats were fed diets calculated to provide intakes of 0, 15, 60 or 240 mg/kg bw/day of imidocarb as dipropionate for 104 weeks. Satellite groups of 15/sex/dose were used for the collection of blood and urine samples. Survival was adversely affected at the top dose with only 9 out of 65 males surviving to termination. Rats given this dose level became emaciated, body weight gain and food consumption were significantly reduced and there were changes in haematology values indicative of anaemia. There were transient changes in clinical chemistry values indicative of a toxic effect on the liver and kidneys. Rats given 60 and 240 mg/kg bw consumed considerable more water than the controls and polyuria was observed in males given 240 mg/kg bw. At termination, kidney weights of males given 240 mg/kg bw were significantly increased. Dose-related histopathological changes were found in the 60 and 240 mg/kg bw groups and included cystic distension of the renal tubules and glomeruli and dystrophic mineralisation of the renal medulla. The NOEL based on chronic toxicity was 15 mg/kg bw/day. In the 240 mg/kg bw group, there were increases in the incidences of multiple fibroadenomas of the mammary gland in females and multiple subcutaneous fibromas in males. Because of the excessive toxicity at this dose level, the poor animal survival and the inadequate histopathology, the significance of these findings was doubtful. There were no significant increases in any malignant tumours.

15 No specific studies were provided concerning potential immuntoxicity if imidocarb. The results of haematology and pathological investigations in the repeated-dose toxicity studies were not indicative of any effect on the immune system.

16. *In vitro* MIC values were determined for 20 bacterial isolates representing 7 different genera. For 18 isolates, MIC values of greater than 16 µg/ml were obtained. The results indicated that the isolates were not sensitive to imidocarb.

17. It was reported that imidocarb had been used occasionally in human medicine but no details concerning doses or adverse effects were available.

18. An ADI of 0.010 mg/kg bw (0.6 mg/person) was established by applying a safety factor of 500 to the NOEL of 5 mg/kg bw/day which was established in the 90-day repeated-dose toxicity study in dogs. The safety factor of 500 was used to compensate for the limited pathological and clinical chemistry investigations in this study, including the absence of investigations into possible effects on cholinesterase activity and potential neurotoxicity. The large safety factor also compensated for the limited data on carcinogenic potential. It was noted that the ADI was the same as that proposed by the Joint WHO/FAO Expert Committee on Food Additives (JECFA).

19. Studies were carried out in which sheep were administered 4.5 mg/kg bw imidocarb or 50 µg/kg ^{14}C-imidocarb by intramuscular injection. The animals were killed at intervals up to 32 days after dosing (1 or 2 per time-point). The substance was widely distributed to all tissues and residues were still detectable in most tissues 32 days after dosing. There was no evidence for the formation of imidocarb metabolites in urine, bile, liver or kidney. In another study, sheep were given two intramuscular doses of 1.2 mg/kg bw imidocarb, 7 days apart. The sheep were killed (3 per time-point) 7, 14 or 28 days after the last dose. Residues in tissues were determined using a non-specific spectrophotometric method with a claimed limit of quantification of 100 µg/kg. Residues in kidney were in the range 22600 to 121200 µg/kg 7 days after the last dose and declined to 5600 to 9600 µg/kg 28 days after the last dose. Residues in liver were in the range 5700 to 14300 µg/kg 7 days after the last dose and declined to 900 to 3100 µg/kg 28 days after the last dose. In muscle, residues were in the range 1100 to 1200 µg/kg 7 days after the last dose but only less than 100 to 400 µg/kg 28 days after the last dose. In fat, imidocarb concentrations ranged from less than 100 to 100 µg/kg at 7 days to below 100 µg/kg in all samples at days 14 and 28. Residues in injection site muscle were

higher than in normal muscle but were lower than in liver and kidney they were 700 to 2300 µg/kg at 7 days, less than 100 to 900 µg/kg at 14 days and < 100 µg/kg on day 28.

20. In a published study, 5 lactating sheep were given an intramuscular injection of imidocarb dipropionate as a dose equivalent to 4.5 mg imidocarb base per kg bw (more than 3 times the recommended dose). Samples of milk were taken from ewes at 4 and 6 hours (one ewe/time-point), 24 hours (2 ewes) and 32 days (one ewe). The samples were analysed using a spectrophotometric method with a claimed limit of detection of 1000 µg/kg. Residues of imidocarb in the milk taken from the ewes 4 and 6 hours after dosing were 4500 µg/kg and 5300 µg/kg respectively. Mean residues at 24 hours were 5600 µg/kg. Residues were undetectable in milk taken 32 days after dosing.

21. Calves and lactating dairy cows (3 in early and 3 in late lactation) were given a single subcutaneous dose of 3 mg/kg bw ^{14}C-imidocarb dipropionate. The animals were killed (4 or 6 per time point) at 28, 56 or 90 days after dosing. Mean total residues in liver depleted from 8240 µg equivalent/kg 28 days after dosing, to 4010 µg equivalent/kg 56 days after dosing and 2190 µg equivalent/kg 90 days after dosing. Over the same time-period, mean total residues in kidney depleted from 12810 µg equivalent/kg to 3770 µg equivalent/kg to 1400 µg equivalent/kg. Mean total residues in muscle depleted from 680 µg equivalent/kg 28 days after dosing, to 410 µg equivalent/kg 56 days after dosing and 308 µg equivalent/kg 90 days after dosing. Over the same time-period, mean total residues in fat depleted from 130 µg equivalent/kg to 100 µg equivalent/kg to 30 µg equivalent/kg. 78 to 84% of the residues in liver, 95% of the residues in kidney and 80 to 96% of the residues in muscle were extractable. HPLC analysis indicated that 66%, 69% and 67% of the total residues in liver on days 28, 56 and 90 were imidocarb. In kidney, 82%, 92% and 91% of the residues on days 28, 56 and 90 were imidocarb. In muscle samples, 79% of the total residues on day 28, 89% on day 56 and 95% on day 90 were identified as imidocarb. At the first milking after treatment, mean residues in milk were 102 µg equivalent/kg. Peak milk residues were found at the second milking (374 µg equivalent/kg) and declined to 31 µg equivalent/kg at the 12th milking after dosing. Seventy seven to 95% of the milk samples were extractable. Seventy to 80% of the total residues in milk for the first 3 days after dosing consisted of imidocarb.

22. Cattle were given a single intramuscular injection of 3 mg/kg bw imidocarb. Groups of 3 animals were killed 7, 14 and 28 days after dosing. Residues in tissues were determined using a non-specific spectrophotometric method with a claimed limit of quantification of 100 µg/kg. Residues in most samples of fat were around or below the limit of quantification. Mean residues in kidney depleted from 13600 µg/kg 7 days after dosing to 3200 µg/kg 28 days after dosing. Mean residues in liver depleted from 16300 µg/kg 7 days after dosing to 3700 µg/kg 28 days after dosing. Mean residues in muscle depleted from 1500 µg/kg 7 days after dosing to 500 µg/kg 28 days after dosing. Residues at the injection site depleted from 4200 µg/kg 7 days after dosing to 1700 µg/kg 28 days after dosing. In a more recent study, residues of imidocarb in cattle tissues were determined using HPLC with UV detection. Mean residues of imidocarb in muscle depleted from 1070 µg/kg 14 days after dosing to 312 µg/kg 70 days after dosing. Over the same time-period, mean residues in liver depleted from 5400 µg/kg to 2070 µg/kg. Samples of kidney, fat and injection site were taken for analysis but no results were reported.

23. Three lactating cows were given two injection of 3 mg/kg bw imidocarb, 28 days apart. Residues of imidocarb in milk were determined using GC/MS with a claimed limit of quantification of 10 µg/kg. One day after the first treatment, residues in milk were in the range 604 to 793 µg/kg. Seven days after the first dose, residues in milk from 2 cows were below the limit of quantification. A similar depletion of residues was observed following the second dose.

24. The proposed routine analytical method was based on HPLC with UV detection. The method was not described in a suitable international format and was not fully validated for edible tissues and milk, in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community. Based on the limited data for recovery and precision, the limits of quantification appeared to be 50 µg/kg for muscle and fat, 100 µg/kg for liver and kidney and 10 µg/kg for milk. There was no information concerning possible interference from residues of other substances used in veterinary medicine.

25. There were no pharmacokinetic or residues depletion data for equidae and no information concerning the likely distribution of residues in this species. Consequently no MRLs could be proposed for equidae.

Conclusions and recommendation

Having considered:

- an ADI of 0.010 mg/kg bw (0.6 mg/person) was established for imidocarb,
- the average (on days 28, 56 and 90) percentage of total residues present as imidocarb in bovine tissues, after subcutaneously administering 3 mg/ kg bw ^{14}C-imidocarb dipropionate, was as follows: liver 68%; kidney 88%; muscle 88%; milk 77%; data were not available for fat and so a factor based on the lowest ratio in liver was applied (68%);
- the concentration of imidocarb in the relevant tissues of cattle 90 days after subcutaneously administering 3 mg/ kg bw ^{14}C-imidocarb dipropionate,
- imidocarb was also the major component of the residues in ovine liver and kidney; the data suggested that the relationship between marker and total residues would be the same as for bovines,
- the proposed routine analytical method was not satisfactorily validated;

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

Pharmacologically active substance(s)	Marker residue	Target species	MRLs	Target tissues	Other provisions
Imidocarb	Imidocarb	Bovine, ovine	300 µg/kg 50 µg/kg 2000 µg/kg 1500 µg/kg 50 µg/kg	Muscle Fat Liver Kidney Milk	Provisional MRLs expire on 1.1.2002

It was noted that JECFA had proposed the same MRL values for bovines but had not made any proposals in respect of other species. Based on these MRLs, the theoretical maximum daily intake of total residues was calculated to be 583 µg/person.

Before the Committee can consider the inclusion of imidocarb in Annex I to Council Regulation (EEC) No 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The proposed routine analytical method should be fully validated for edible tissues and milk of cattle and sheep, in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community and presented in a standard international format (e.g. ISO 78/2).