



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### CLAVULANIC ACID

#### SUMMARY REPORT (1)

1. Clavulanic acid (3 (2-hydroxyethylidene)-7-oxo-4-oxo-1-azabicyclo-(3.2.0)heptane-2-carboxylic acid) is a compound structurally related to the penicillins. It is a product of the fermentation of *Streptomyces clavugiferus*.

Clavulanic acid has been used in human and veterinary medicine for several years in combination with amoxicillin.

In animals the formulations contain a ratio of 1:4 clavulanic acid (usually as potassium clavulanate) to amoxicillin trihydrate and are intended for use in cattle, pigs and sheep as intramuscular injectable suspensions (1.75 mg/kg bw once daily for 5 days), in lactating cows for intramammary infusion (50 mg/quarter, 0.4 mg/kg bw, twice daily, i.e. 0.8 mg/kg bw/day, for 3 days) and for oral treatment in preruminant calves (2.5 mg/kg bw, twice daily, i.e. 5 mg/kg bw/day, for 3 days).

2. Clavulanic acid is a specific and irreversible inhibitor of a wide range of bacterial beta-lactamases.

No alterations were elicited in the rat by oral single doses of 80 mg/kg or less on body temperature and on digestive, excretory and neurological/neuromuscular functions.

Marked alterations of heart rate, blood pressure and electrocardiogram were observed in dogs after a single intravenous injection of 125 mg/kg bw potassium clavulanate or greater.

3. Pharmacokinetic studies were carried out *in vivo* with <sup>14</sup>C-clavulanate on rats, dogs and humans following oral administration, and biotransformation assays *in vitro* on liver homogenates of rat, dog and calf.

Oral absorption was 34% in the rat, 44% in the dog and 73% in man. Urine was the main excretion route in all species, accounting for 42%, 52% and 75% of the absorbed dose, in the rat, dog and in man, respectively. The main identifiable compounds in the urine of rats and dogs were the parent compound (16-23% and 14-38% of the urinary recovery in the rat and the dog, respectively) and the metabolite 1-amino-4-hydroxybutan-2-one (21-35% and 10-20% of the urinary recovery in the rat and the dog, respectively). The latter was identified as the main metabolite also *in vitro*.

The substance is widely used in human medicine.

4. Clavulanic acid has a low oral acute toxicity in both adult rats and mice, with an LD<sub>50</sub> greater than 2000 mg/kg bw. However, the single-dose toxicity was higher in a study on pre-weaning rats, with gastrointestinal signs and deaths occurring even at the lowest dose level tested (125 mg/kg bw).
5. Several toxicity studies were performed on laboratory species both with potassium clavulanate and with 1:2 clavulanic acid:amoxicillin.

In general clavulanic acid:amoxicillin appeared slightly more toxic than potassium clavulanate. In the repeated dose toxicity studies, gastrointestinal irritancy in clavulanic acid:amoxicillin treated rats and dogs was observed even at the lowest dose levels tested (30 and 15 mg/kg bw, respectively); renal tubular vacuolation in dogs was the most sensitive systemic effect (LOEL and NOEL 30 and 15 mg/kg bw i.e., 10 and 5 mg/kg bw clavulanic acid). However, since a contribution from amoxicillin cannot be ruled out, the clavulanic acid:amoxicillin studies were not used to derive a ADI.

6. A 28-day and a 90-day oral toxicity study with potassium clavulanate were performed both in the rat and in the dog. Clinical, biochemical and haematological effects, reduced bodyweight gain, gastrointestinal irritation and liver toxicity were observed.

In the rat the most sensitive indicators of potassium clavulanate effects were decreased urine output and increased osmolarity and increased white blood cell count; in the dog, clinical signs (emesis, salivation) and hepatocyte hydropic changes were observed. In both species the LOEL and NOEL in 90-day studies were 50 and 20 mg/kg bw. Caecal enlargement was observed in the rat at lower dose levels (LOEL and NOEL 20 and 10 mg/kg bw in the 90-day study).

7. The following oral reproductive and developmental toxicity studies are available on clavulanic acid: 1-generation, rat; segment-II, rat; segment-II, mouse (2 studies); segment-III, rat. The studies were adequately designed. Overall, a moderate reduction of female fertility and/or growth and survival of the foetus were seen at dose levels eliciting slight systemic or maternal toxicity.

Although a statistically non significant, but dose-related, reduction in the mean number of *corpora lutea* per dam was detected in both the rat F0 and the mouse F1 at the lowest dose level in the testing programme, 10 mg/kg bw, this dose level could be retained as a NOEL.

8. Clavulanic acid was tested in an adequate set of *in vitro* and *in vivo* genotoxicity studies.

Positive results were obtained in one forward mutation assay in mouse lymphoma cells. Significant increases in mutation rate were obtained in the absence and to the lesser extent in the presence of metabolic activation: however, effective concentrations were high (4000 µg/ml and 8000 µg/ml with and without metabolic activation, respectively) and cytotoxicity was concurrently present.

Negative results were obtained in the following *in vitro* and *in vivo* tests: gene conversion in *S. cerevisiae*; Ames test, with and without metabolic activation, dominant lethal in mouse, orally at dose levels up to 4500 mg/kg bw and micronucleus test in mouse at oral dose levels of up to 9000 mg/kg bw.

The available data indicate that clavulanic acid is not a genotoxic agent.

9. No carcinogenicity studies were performed. They may not be required, according to available genotoxicity studies on clavulanic acid .

10. Clavulanic acid did not induce skin sensitization in guinea pig when tested by Magnusson-Kligman method.

The subcutaneous administration of clavulanic acid:amoxicillin (greater than or equal to 25.5 mg clavulanic acid) slightly reduced antibody titres in the rabbit.

The effects on several bacteria isolated from the human gastrointestinal tract were assayed *in vitro*. *Eubacterium aerofaciens* was identified as the most sensitive species (MIC<sub>50</sub>: 8 µg/ml).

The assay was performed on a lower (5 or less) number of isolates per species than currently required; several relevant bacterial species, in particular *Streptococcus* spp., were not tested.

11. The effects on bacteria relevant for the food industry were assessed; *Lactobacillus delbrueckii* was identified as the most sensitive species (MIC<sub>50</sub>: 4 µg/ml and 8 µg/ml, in agar and in milk, respectively).

A no-effect concentration for bacteria used in food industry was not determined.

12. Clavulanic acid:amoxicillin has a history of widespread use in human therapy. Hypersensitivity reactions and adverse, mostly gastrointestinal, side effects have been reported to occur with a rate and severity comparable to that of other beta-lactams.

However, no detailed data have been provided to determine a level without adverse effects in humans.

A 60% oral bioavailability was assessed in healthy human volunteers.

13. In the absence of a dose level without adverse effects in human patients only a provisional toxicological ADI of 0.05 mg/kg bw (3 mg/person of 60 kg bodyweight) can be established based on the NOEL of 10 mg/kg bw observed in the reproductive toxicity studies in rats and mice, with a 200 uncertainty factor to take into account the effects on reproductive function induced in rats and mice.
14. For the assessment of the microbiological risk, use was made of the formula recommended by the CVMP:

$$\text{ADI} = \frac{\frac{\text{geometric mean MIC}_{50} \times \text{CF2}}{\text{CF1}} (\mu\text{g/ml}) \times \text{daily faecal bolus (0.15 l)}}{\frac{\text{fraction of an oral dose available for microorganisms}}{\text{weight of human (60 kg)}}} (\mu\text{g/kg bw})$$

Based on the above formula, a MIC<sub>50</sub> of 8 µg/ml assessed on the most sensitive tested species (*Eubacterium aerofaciens*) and as several relevant bacteria were not assayed, only a provisional microbiological ADI can be established as follows :

$$\frac{8 \times 1/3 (\mu\text{g/ml}) \times 150 \text{ ml}}{0.4 \times 60} = 16.67 \mu\text{g/kg bw (1 mg/person of 60 kg bodyweight)}$$

where :

- 8 = most sensitive MIC<sub>50</sub> in the species assayed
- 150 (g) = faecal bolus
- 0.4 = fraction of the total dose which is available in the intestinal tract, according to studies on healthy human volunteers
- CF1 = 3: arbitrary value to correct for the lack of information on several relevant bacteria of the human intestinal tract.
- CF2 = 1: conservative value as no information was provided to allow correction for concentration and pH of the gastrointestinal tract

As the provisional microbiological ADI is lower than the toxicological one the MRLs are to be based on the microbiological ADI.

16. Serum pharmacokinetics were determined in target species (dairy cows, calves, young pigs, sheep) following a 5-day intramuscular injection treatment with 1.75 mg/kg bw.

In dairy cows, mean terminal half-life of approximately 2 hours and peak serum concentrations of less than 2 µg/ml at 1 hour after dosing are reported.

In calves and young pigs peak serum concentrations of approximately 2-3 µg/ml at less than 30 minutes after dosing and mean terminal half-life of approximately 1 hour were observed in both species.

In adult sheep, peak serum concentrations ranging from 4 to 8 µg/ml were detected 30 minutes after dosing and the mean terminal half-life was 0.76 hour.

Following a single 2.5 mg/kg bw oral treatment of preruminant calves, 34% of the dose is absorbed and peak concentrations of 0.8 µg/ml occurred within 3-4 hours after dosing; mean terminal half-life was 2.0 hours.

The main excretion pathway is through the urine in both calves and pigs.

17. Residue studies did not show detectable (less than 10 µg/kg) residues in the tissues of young calves treated orally with 8 mg/kg bw clavulanic acid for 3 days and killed after more than 3 days withdrawal, nor in the tissues, including injection sites, of fattening calves, pigs and sheep treated intramuscularly with 1.75 mg/kg bw for 5 days and killed after more than 10, 7 and 14 days after the end of administration, respectively.

Residues were detected by means of a microbiological assay using *Klebsiella aerogenes* NCTC 11228.

However the prolonged periods between treatment and killing of the animals did not allow the extrapolation of proper residue depletion curves in the target species.

18. Excretion in milk occurs to a limited extent, the concentrations being lower than those detected in the serum.

Detectable residues were found in cow's milk following intramammary administration of 125 mg/quarter in all four quarters. Residue depletion was as follows (mean concentration, µg/l) : 30000 (8 h); 3500 (24 h); 860 (32 h); 70 (48 h); 20 (56 h). No detectable (less than 4 µg/l) residues were observed 72 hours after the administration.

After a 5-day, 1.75 mg/kg bw intramuscular treatment of lactating cows, 20-40 µg/l were observed after 8 hours; no detectable residues were found after 24 hours.

In dairy ewes treated intramuscular with 5 daily injections of 1.75 mg/kg bw, 20 µg/l were found in milk 8 hours after the end of treatment; no detectable residues were found after 24 hours.

19. In metabolic studies on rats, dogs and humans, the urinary recovery of the parent compound was in a variable, but approximately 1:1 ratio to that of the main metabolite 1-amino-4-hydroxybutan-2-one, which is a substance without β-lactamic structure. This was the main metabolite also in calf liver homogenates assayed *in vitro*.

In calves treated intravenously and in pigs treated intramuscularly with clavulanic acid: amoxicillin, the parent compound, accounted for 11-33 % and 45 % of the dose excreted in urine respectively.

The parent compound can be provisionally proposed as marker residue. However, no data are available concerning the ratio of parent compound:main metabolite in relevant edible tissues.

20. A sensitive microbiological assay is reported using *Klebsiella aerogenes* NCTC 11228. Owing to the combined presence of amoxicillin, the method employs additional organisms (*M. luteus* 9341, *B. stearothermophilus*, *B. subtilis* ATCC6633) for the selective detection of the latter drug. Limits of detection (LOD) are: 4 µg/l for milk and 10 µg/kg for liver, kidney, muscle, fat, skin.

21. An HPLC confirmatory method is provided for the determination of clavulanic acid in bovine tissues and milk. The possible interferences by natural compounds or xenobiotics were assessed, and no likely interference was found. Linearity of the method was 0.1-100 µg/ml. The coefficient of variation was between 0.031-0.32 (mean 0.13).

Limit of quantification (LOQ) was 0.1 µg/ml in milk and bovine tissues.

The method was presented in an internationally recognised format (in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community, Chapter IVB, section II3).

However, this method has not been validated for pig and sheep edible tissues, including ewe's milk.

## Recommendations and conclusion

Having considered :

- the provisional microbiological ADI of 0.016 mg/kg bw,
- clavulanic acid as the provisional marker residue,
- that the analytical method proposed for residues monitoring purposes is not validated;

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

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Clavulanic acid	Clavulanic acid	Bovine, ovine, porcine	200 µg/kg	Muscle, liver, kidney, fat	Provisional MRLs expire on 1.7.1999
		Bovine, ovine	200 µg/kg	Milk	

Based on these MRL values, the daily intake will represent about 40% of the provisional microbiological ADI.

## LIST OF QUESTIONS

### SAFETY FILE

1. The applicant should provide data on the effects on all main bacteria of human intestinal flora, with special regard to *Streptococcus* spp.  
Moreover, it would be desirable to have information about the effects on antibacterial action of pH of the gastrointestinal tract and bacterial density.
2. The applicant should provide a no-effect concentration for bacteria used in food industry.
3. The applicant should provide the available information on side-effects in humans, with regard to both the type of effects and the dose-response relationship.

### RESIDUE FILE

4. The applicant should provide adequate data in order to assess the residue depletion profiles in all relevant edible tissues, including injection sites, of target species (cattle, pigs, sheep) following treatment by relevant routes (oral, intramuscular, intramammary).
5. The applicant should provide additional data about the ratio of parent compound:main microbiological active metabolite in relevant edible tissues.
6. The proposed confirmatory HPLC method should be properly validated for edible tissues of pigs and sheep, including ewes' milk.  
Moreover, the method should be properly validated with regard to possible interference by amoxicillin or other beta-lactams.